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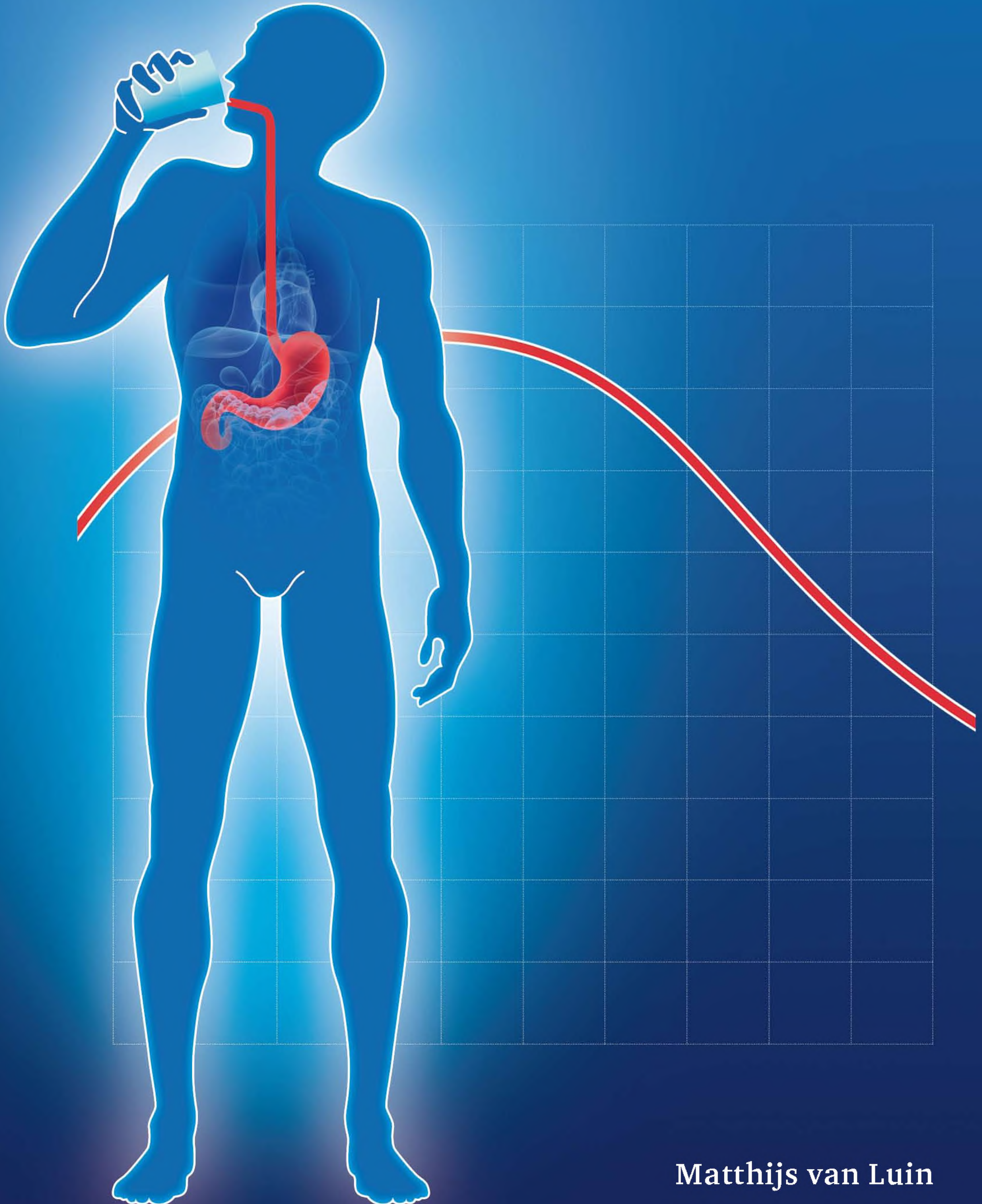
HIV Treatment: A Clinical Pharmacology Perspective



Matthijs van Luin

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HIV Treatment:
A Clinical Pharmacology Perspective

Een wetenschappelijke proeve
op het gebied van de Medische Wetenschappen

Proefschrift

Ter verkrijging van de graad van doctor
aan de Radboud Universiteit Nijmegen
op gezag van de rector magnificus prof. mr. S.C.J.J. Kortmann,
volgens besluit van het college van decanen,
in het openbaar te verdedigen op vrijdag 26 november 2010
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Introduction

Human immunodeficiency virus (HIV)

In 1981, a sudden increase of Kaposi's sarcoma was reported amongst young homosexual men (1). In that same year, there was an outbreak of the rare lung infection Pneumocystis carinii pneumonia (PCP) in homosexual men and drug abusers (2).

Both problems reflected a severe deficiency in the immune system, which was named 'Acquired Immune Deficiency Syndrome' (AIDS). The causative agent of AIDS turned out to be a retrovirus; which was called 'human immunodeficiency virus' (HIV) (3;4). HIV enters predominantly helper T cells of the human immune system by binding to CD4 receptors. HIV kills the CD4 positive immune cells that it infects, thereby crippling the immune system. A distinction is made between HIV-1 and HIV-2, the latter being less virulent and prevalent than HIV-1. HIV-2 is mostly prevalent in West-Africa (5).
HIV can be transmitted through unprotected sexual intercourse, intravenous drug use with contaminated injection needles, transfusion of HIV-infected blood, and by mother-to-child-transmission during pregnancy, delivery and breast feeding.

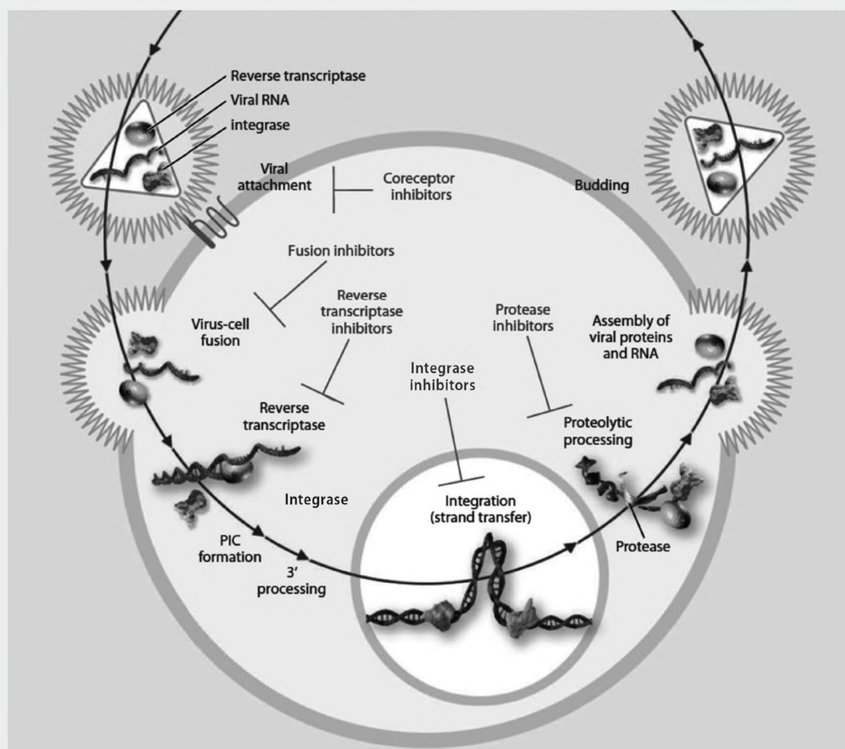
HIV is still a major global health problem. In 2008, AIDS killed approximately 2.0 million people while in the same year, 2.7 million people were newly infected with HIV. By the end of 2008, 33.4 million people were living with the virus, more people than ever before (6). Nonetheless, there are promising results in the fight against HIV. First, the continuing rise in the number of HIV-infected patients is not only the result of continued high rates of new HIV-infections. It is also a reflection of the beneficial impact of increased global access to antiretroviral therapy (6). Second, the number of new HIV infections has decreased from a peak of 3.6 million in 1996 to 2.7 million in 2008 (6). Finally, the number of AIDS-related deaths has declined from 2.2 million in 2004 to 2.0 million in 2008.

Antiretroviral drugs

In 1987, zidovudine was introduced, the first drug for the treatment of HIV and AIDS, which is still part of the current antiretroviral armamentarium. Unfortunately, it took approximately another 10 years before sustained suppression of HIV replication became achievable. Combination antiretroviral therapy (cART), consisting of at least three drugs, coming from at least two drug classes, appeared to be essential for controlling the virus. Since 1996, the introduction of cART has led to a sustained, well-documented reduction in AIDS-related mortality and morbidity among those who had access to cART (7).

Current HIV treatment guidelines still recommend the use of cART (8). At this moment, drugs from six different classes are available (figure 1, table 1).

Figure 1 Targets of the six different classes of antiretroviral drugs.



Adapted from (50).

Nucleoside and nucleotide analogue reverse transcriptase inhibitors

Nucleoside analogue reverse transcriptase inhibitors are pro-drugs that require intracellular phosphorylation to their active tri-phosphate metabolites to become pharmacologically active. The triphosphate metabolites compete with the cell's endogenous deoxynucleotide triphosphates for incorporation into the nucleic acid chain and, after incorporation, terminate the DNA chain by preventing addition of new bases (9). Tenofovir is a nucleo<u>t</u>ide analogue, because it already contains a phosphate group itself, in contrast to the nucleoside analogue reverse transcriptase inhibitors (10).

Table 1 Overview of available antiretroviral drug classes in The Netherlands.

NRTIs	NNRTIs	Protease inhibitors	Fusion inhibitors	CCR5 receptor antagonists	Integrase inhibitors
Abacavir	Efavirenz	Atazanavir	Enfuvirtide	Maraviroc	Raltegravir
Didanosine	Etravirine	Darunavir			
Emtricitabine	Nevirapine	Fosamprenavir			
Lamivudine		Indinavir			
Tenofovir		Lopinavir			
Stavudine		Nelfinavir			
Zidovudine		Ritonavir			
		Saquinavir			
		Tipranavir			

NRTIs, nucleoside analogue reverse transcriptase inhibitors,
NNRTIs, non-nucleoside reverse transcriptase inhibitors

Non-nucleoside reverse transcriptase inhibitors

Non-nucleoside reverse transcriptase inhibitors (NNRTIs) decrease HIV-1 reverse transcriptase activity by allosteric inhibition but, unlike nucleoside analogue reverse transcriptase inhibitors, do not require intracellular phosphorylation to become pharmacologically active (11). Nevirapine and efavirenz were the first NNRTIs on the market. They are still used by many HIV-infected patients, both as part of the initial regimen or as part of a maintenance regimen after starting with a protease inhibitor-based cART regimen. Recently, a new NNRTI, called etravirine, was approved for the treatment of HIV-1 infection in treatment-experienced patients. Etravirine has a higher genetic barrier to the development of resistance than efavirenz and nevirapine have (12).

Protease inhibitors

Protease inhibitors (PIs) inhibit the HIV protease enzyme and prevent cleavage of the gag-pol polyprotein, thus preventing nascent viral particles from reaching a mature, infectious state (13). A major advance in PI-based antiretroviral therapy has been the co-administration of PIs with a low 'boosting' dose of ritonavir, which increases PI drug exposure. This reduces the risk of emergence of resistance and has allowed twice-daily and, for some PIs, once-daily dosing (14;15).

CCR5 antagonists

Interaction of the HIV envelope with CD4 is followed by binding to an additional co-receptor, either the chemokine receptor CCR5 or the chemokine receptor CXCR4. In 2007, the first CCR5 co-receptor antagonist, maraviroc, was approved for the treatment of HIV-1 infection in treatment-experienced patients. Maraviroc is only useful in the treatment of patients who have HIV strains that utilize the CCR5 co-receptor for cell-entry ('R5-tropic HIV-1 virus') (16).

Fusion inhibitors

The first and still only available fusion inhibitor, enfuvirtide, was approved for the treatment of HIV-1 infection in treatment-experienced patients in 2003. Enfuvirtide is a synthetic peptide which binds to the HIV envelop glycoprotein 41, thereby preventing the fusion of viral and cellular membranes. Enfuvirtide has to be administered subcutaneously; local injection site reactions are common (17).

Integrase inhibitors

In 2007, the first HIV integrase inhibitor, raltegravir, was approved for the treatment of HIV-1 infection. Raltegravir acts by targeting the HIV integrase, thereby preventing the integration of HIV DNA into the genome of the human host-cell (18). In part II of this thesis, two drug-drug interaction studies with raltegravir are presented.

Clinical pharmacology

Clinical pharmacology is a biomedical science which focuses on pharmacodynamics and pharmacokinetics of drugs in humans. Pharmacodynamics deals with the effect of drugs on the human body, while pharmacokinetics describes the effects of the human body on drugs, such as drug metabolism. For a number of antiretroviral drugs, relationships have been established between drug plasma concentrations (pharmacokinetics) and antiretroviral efficacy (pharmacodynamics) (19). Specifically for most PIs and NNRTIs, there is international consensus on concentration-based target concentrations for efficacy (8;19). Plasma concentrations below the lower threshold for efficacy may lead to higher rates of virologic failure and the development of drug resistance (20;21).

Adequate drug exposure is thus essential in the treatment of HIV. Nevertheless, obtaining adequate drug exposure in an individual can be challenging because of the considerable interindividual variability in plasma concentrations among patients taking the same dose (22).

There are numerous factors which may lead to interindividual variations in pharmacokinetics, such as genetic constitution, gender, age, body weight, noncompliance or the occurrence of drug-drug interactions (23-29).

Therapeutic drug monitoring

One tool to obtain optimal drug exposure in an individual is therapeutic drug monitoring (TDM). TDM pursues tailor-made antiretroviral therapy by using the individual's plasma concentrations to select the optimal dose for that individual. The first part of this thesis focuses on the application of TDM in current clinical practice.

At the end of the 20th century, there were many problems with the early cART regimens, such as frequent inadequate absorption, large interpatient variability with frequent suboptimal or toxic exposure to antiretroviral agents, many drug-drug interactions and a high and frequent daily intake of pills. These problems were the incentive for introducing TDM into the HIV field. Ever since, many drugs that were used in the early cART regimens, such as indinavir and nelfinavir, have been replaced by drugs with better pharmacokinetic profiles. Consequently, the role of TDM has evolved. **Chapter 1** provides a review of the current evidence for TDM, focusing on arguments which are in favor and arguments that refute the use of TDM in current clinical practice.

There are only two antiretroviral drugs for which a well-defined upper threshold plasma concentration for toxicity has been established, namely indinavir for renal toxicity and efavirenz for central nervous system (CNS) toxicity (19). For efavirenz, however, conflicting data exist. Some studies have reported an association between elevated efavirenz plasma concentrations and CNS disturbances (21;30-32), but other studies reported a lack of such an association (33-36). To provide more insight into this matter, we undertook the retrospective analysis described in **chapter 2,** which aimed to determine whether patients in the EuroSIDA study with high efavirenz plasma concentrations had an increased likelihood of toxicity-driven discontinuations of efavirenz.

Despite the conflicting data described above, pharmacists at our TDM practice advise dose reduction of efavirenz in patients with high plasma concentrations who suffer from persistent CNS disturbances. Anecdotally, this has been reported to be an effective intervention, but the outcome of this intervention had never been formally evaluated. The retrospective analysis with data from the ATHENA cohort

study, described in **chapter 3,** evaluates whether dose reduction in patients with high efavirenz plasma is safe with regards to virologic efficacy and whether dose reduction reduces the risk of toxicity-driven efavirenz discontinuations. **Chapter 4** describes an HIV-tuberculosis co-infected patient who had unexpectedly high efavirenz plasma concentrations and concomitant CNS toxicities, despite co-administration of the strong enzyme inducer rifampicin. This chapter illustrates the value that TDM can have in individualized patient management.

In 2005, the Dutch Association of AIDS Physicians (NVAB) issued guidelines for the treatment and management of HIV-infected patients, including recommendations for Therapeutic Drug Monitoring (TDM) (37). **Chapter 5** provides an evaluation of the uptake of these recommendations in the Dutch HIV treatment centres.

Drug-drug interactions

As depicted above, drug-drug interactions may lead to undesirable low or high plasma concentrations of antiretroviral drugs. In addition, drug-drug interactions may lead to clinically significant changes in the pharmacokinetics of drugs that patients receive for the treatment of co-existing medical conditions (38). It is therefore important to study potentially relevant drug-drug interactions. The second part of this thesis contains four drug-drug interaction studies between antiretroviral drugs and drugs being used for the prevention and treatment of co-existing medical conditions.

The mechanism of many of the potential drug-drug interactions between antiretroviral drugs and concomitant drugs involves Cytochrome P_{450} (CYP_{450})-mediated metabolism (39). HIV-protease inhibitors may be both substrates, inducers and inhibitors of several CYP_{450} subtypes, whereas most NNRTIs are both substrates and inducers of this system.

Less attention has been paid to other types of drug-drug interactions, for instance via mediation of glucuronidation of drugs. Yet, PIs and NNRTIs may induce glucuronidation of concomitantly administered drugs and this may lead to clinically significant reductions in plasma concentrations of UDP-glucuronosyltransferase (UGT) substrates (40;41).

Atovaquone co-formulated with proguanil is frequently used by western HIV-infected patients who travel to malaria-endemic destinations. Atovaquone is considered a substrate for glucuronidation (42). Chronic use of PIs or NNRTIs may hence lead to

diminished atovaquone plasma concentrations and possibly suboptimal prophylaxis of malaria.
To study this potential problem, we designed the study described in **chapter 6**, which compared atovaquone/proguanil plasma concentrations between healthy volunteers and HIV-infected patients who were treated with efavirenz, lopinavir/ritonavir, or atazanavir/ritonavir.

Chapters 7 and 8 describe drug-drug interaction studies of the recently approved HIV-1 integrase inhibitor raltegravir with lamotrigine and pravastatin, respectively. Raltegravir is metabolized by UGT1A1 and hence its pharmacokinetics can be influenced by inhibitors (e.g., atazanavir) or inducers (e.g., etravirine, tipranavir, rifampicin) of UGT1A1 (43-46). However, the influence of raltegravir itself on UGT substrates had not been evaluated in clinical studies. Therefore, we undertook the study described in **chapter 7** to investigate the influence of raltegravir on the UGT substrate lamotrigine.
Dyslipidemia is a common complication during chronic HIV infection. One strategy to manage dyslipidemia is the use of lipid-lowering drugs. Pravastatin is considered a preferred lipid-lowering drug for HIV-infected patients (47;48) and frequent combined use of raltegravir and pravastatin can be expected in the ageing HIV-infected population (49). Because both drugs share a common metabolic pathway, we studied the effect of the new HIV-integrase inhibitor raltegravir on pravastatin pharmacokinetics and vice-versa **(Chapter 8)**.

Fungal infections are among the most prevalent opportunistic infections in HIV-infected patients. It is thus important to study potential drug-drug interactions between antiretroviral drugs and antifungal drugs. **Chapter 9** describes the drug-drug interaction study that we performed between the second generation triazole posaconazole and the protease inhibitor fosamprenavir. In this study we investigated whether ritonavir could be replaced by posaconazole as an alternative booster of the pharmacokinetics of fosamprenavir.

Objectives of this thesis

All studies in this thesis focus on clinical pharmacology issues in HIV treatment. Part I of the thesis presents studies that were performed to obtain more insight into the use of TDM in current clinical practice. Part II of this thesis presents pharmacokinetic drug-drug interaction studies between antiretroviral drugs and drugs being used for the prevention and treatment of co-existing medical conditions. Finally, a general discussion is presented.

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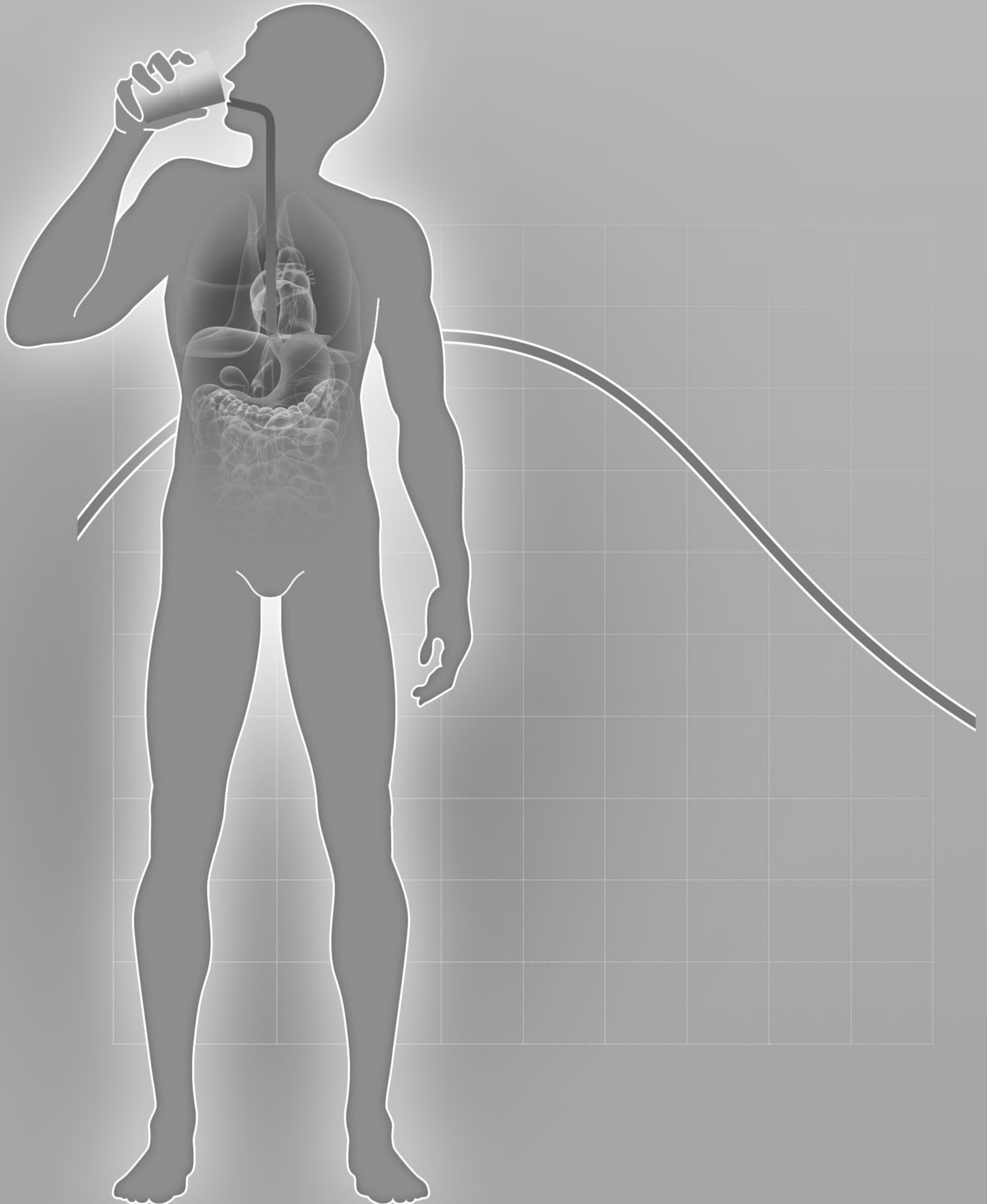

1

Part

Therapeutic drug monitoring of antiretroviral drugs

1

Chapter

Use of therapeutic drug monitoring in HIV disease

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Current Opinion in HIV and AIDS 2008; 3: 266-271

Abstract

Purpose of review

Therapeutic drug monitoring is frequently used in several European countries, and international guidelines recommend it in selected cases. We discusses the main arguments for and against therapeutic drug monitoring in HIV infection.

Recent findings

Accumulating evidence favors the use of therapeutic drug monitoring in the management of drug concentration-related toxicities. Interindividual variability in the pharmacokinetics of antiretroviral drugs is at least partially caused by genetic polymorphisms. Additionally, body weight, sex and ethnicity have been identified as independent predictors of pharmacokinetics. Several studies have revealed subtherapeutic drug concentrations in children who were treated in accordance with the label information, which is in favor of therapeutic drug monitoring in children. The inhibitory quotient concept has been further explored, but more work is needed to justify full implementation into routine clinical practice. A limitation of therapeutic drug monitoring is the significant intraindividual variability in protease inhibitor concentrations. Furthermore, there is a lack of sufficiently powered randomized controlled trials that assess the use of routine therapeutic drug monitoring for current first-line antiretroviral drugs.

Summary

Although routine therapeutic drug monitoring cannot be recommended for current first-line antiretroviral drugs, there are many frequently encountered clinical situations in which therapeutic drug monitoring provides valuable information.

Introduction

Therapeutic drug monitoring (TDM), namely use of drug concentrations to optimize antiretroviral therapy, is frequently used in some European countries, such as the UK, France and the Netherlands. In addition, the US department of Health and Human Services guidelines (1) and the British HIV Association guidelines (2) recommend the use of TDM for several categories of patients, such as those with suspected drug interactions, pregnant women and patients with hepatic dysfunction.

This review discusses relevant publications (published from January 2005 to November 2007), that favor or refute the use of TDM. Finally, the pros and cons are weighed in order to draw conclusions regarding the clinical utility of TDM in HIV disease.

Pro TDM: Drug concentrations correlate with virological response

A prerequisite for the use of TDM is that antiretroviral drug concentrations correlate with virological response. Indeed, there is extensive evidence on concentration-response relationships for both protease inhibitors and non-nucleoside reverse transcriptase inhibitors (NNRTIs), which has led to international consensus on the concentration-based cutoff values for performing TDM in antiretroviral therapy naïve patients (table 1) (3).

These cutoff values are not applicable to protease inhibitor experienced patients, who may need higher plasma concentrations because of the emergence of protease inhibitor-related mutations. Furthermore, concentration-based cutoff values have not been established for the fusion inhibitor enfuvirtide or for the nucleoside reverse transcriptase inhibitors (NRTIs). NRTIs are pro-drugs that require intracellular phosphorylation to become pharmacologically active and therefore plasma concentrations do not necessarily correlate with efficacy (3).

For enfuvirtide, there seems to be no correlation between plasma concentrations and virological response (4).

Pro TDM: The IQ concept might benefit treatment-experienced patients

NNRTIs have a limited genetic barrier to resistance, because just one mutation can render them therapeutically ineffective. Consequently, it is generally thought that it is futile to try to overcome NNRTI resistance by increasing plasma concentrations. In contrast, for protease inhibitors the extent of a patient's resistance is a result of the cumulative number of relevant protease inhibitor mutations, and there is

Table 1 Cutoff concentrations (mg/L) for performing TDM of antiretroviral agents in therapy-naïve patients. Adapted with permission from (3).

	Efficacy (C_{trough})	Toxicity
Atazanavir	0.15	
Fosamprenavir	0.40	
Indinavir	0.10	C_{max} 10.0
Lopinavir	1.0	
Nelfinavir	0.80	
Ritonavir[&]	2.1	
Saquinavir	0.10	
Tipranavir[#]	20.5	
Efavirenz	1.0[†]	4.0[†]
Nevirapine	3.0[†]	

[&] as a single PI
[#] in therapy-experienced patients
[†] for efavirenz and nevirapine, plasma concentrations can be randomly taken during the dosage interval

increasing evidence that raising plasma protease inhibitor concentrations to a level that exceeds the degree of resistance of a particular virus strain may help to overcome its reduced susceptibility (5).

To define an individualized target protease inhibitor plasma concentration based on the susceptibility of the virus, the inhibitory quotient concept has been introduced into HIV therapy, which combines the results of TDM and resistance testing (5).

Elsewhere, La Porte *et al.* (6) discuss the inhibitory quotient concept in greater detail. In summary, a number of recent retrospective observational studies have demonstrated that the genotypic inhibitory quotient (GIQ), which is defined as the ratio of the protease inhibitor trough concentration to the number of primary protease inhibitor-associated genotypic mutations in the HIV RNA (5), is significantly correlated with virological response (7-9;9-13). Moreover, the general picture is that the integration of resistance data with pharmacokinetics provides equal or better prediction of virological response than resistance data or pharmacokinetic data

alone. Notwithstanding this benefit, some limitations apply to the GIQ-concept. First, there is a need for standardization: different studies still use different lists of mutations to calculate the GIQ for the same protease inhibitor (9;14-16). Second, all mutations are weighed equally, although it is well established that not every mutation contributes equally to the degree of resistance. Finally, none of the proposed GIQ cut off values has been validated in prospective studies.

Pro TDM: TDM is a tool to manage concentration-related toxicities
There are relatively few data that relate plasma concentrations to toxicity. To date, the evidence is the strongest for indinavir, atazanavir and efavirenz.

Recent studies reported improved clinical outcomes in patients with high indinavir or efavirenz concentrations that received dose adjustments under the guidance of TDM. Two studies demonstrated improved renal function (17) and overall tolerability (18) in patients who underwent TDM-guided indinavir dose reduction. In both studies, virological suppression was maintained, which demonstrates the safety of this strategy.

High efavirenz plasma concentrations have been linked to central nervous system (CNS) toxicity and the study of Gutierrez *et al.* confirmed previous findings (19). Two other recent publications, however, have challenged the concept of there being a relation between efavirenz plasma concentrations and CNS toxicity (20) (21). Nevertheless, successful efavirenz dose reduction in terms of diminished drug toxicity has been described in patients with high efavirenz plasma concentrations, both in Japanese (22) and in Dutch HIV-infected patients (23). The Japanese study prospectively selected patients who had high (> 6.0 mg/L) efavirenz plasma concentrations because of single nucleotide polymorphisms of the CYP2B6 enzyme and observed an improvement of CNS-related adverse effects after dose reduction (22). The Dutch study retrospectively compared patients with high (>4.0 mg/L) efavirenz plasma concentrations who did or did not undergo dose reduction in routine clinical practice. After 1 year, there was a trend towards a decrease of toxicity-related efavirenz discontinuations in patients in whom the dosage was reduced (23). Both studies established the safety of TDM guided dose reduction with regards to virological suppression.

Several studies highlighted the existence of a relation between atazanavir plasma concentrations and its main adverse effect, namely increased serum bilirubin concentrations (13;24-27). Nevertheless, this side effect is usually asymptomatic

and it may have to be accepted in return for efficacy in protease inhibitor-pretreated patients. Although there is a lack of formal evidence, TDM might be applied to reduce the dose of atazanavir in protease inhibitor-naïve patients with high atazanavir plasma concentrations (e.g., C_{trough} > 0.63 mg/L (27)) who experience toxicity.

Pro TDM: There is large interindividual variability in pharmacokinetics

One of the main incentives to perform TDM in HIV disease management has been the marked interindividual variability in the pharmacokinetics of antiretroviral drugs. This was once more illustrated by Molto *et al.* who reported large inter individual variability for both protease inhibitors and NNRTIs in a routine outpatient setting (28).

New information was published on the patient characteristics that determine interpatient variability of antiretroviral drugs. The pharmacokinetics of atazanavir appeared significantly influenced by the 3435C>T polymorphism of the gene that encodes p-glycoprotein. Patients with two wild-type alleles had significantly higher plasma concentrations of atazanavir compared to patients with at least one mutant allele, both in boosted (25) and unboosted regimens (26).
The pharmacokinetics of efavirenz are influenced by gender and ethnicity, with higher efavirenz plasma concentrations in females and non-Caucasian patients (29-31), and there is evidence that interracial differences in CYP2B6 activity (32) play a role in the observed differences between races. Furthermore, female gender and positive hepatitis B status have been related to diminished nevirapine clearance (29), and body weight appeared to be inversely related to plasma concentrations of lopinavir (33).

Pro TDM: TDM is a tool for managing drug-drug interactions

Numerous potential drug interactions are yet to be formally studied, and the outcomes of such studies are sometimes unexpected (34). Repeated measurement of drug concentrations is advisable if a (potentially) interacting agent is started or withdrawn in order to prevent reduced efficacy or increased toxicity of antiretroviral treatment (35). Park-Willie *et al.* recently showed the importance of the appropriate handling of drug interactions, as patients with efavirenz-based interactions who received dosage adjustments had a significantly greater mean reduction in viral load than did patients with unadjusted dosages (36).

Pro TDM: TDM may reveal non-adherence

Adherence to antiretroviral therapy is a key determinant of virological response. Unfortunately, there is no 'gold standard' with which to assess adherence. One of the tools used to measure adherence is TDM, which provides objective and direct proof of the presence of a drug in the patient's body (37). Once one uses TDM to investigate adherence, it is important to recognize that a therapeutic drug concentration does not necessarily reflect good adherence, because it only reflects recent drug intake. On the other hand, an extremely low plasma concentration is an indication of poor adherence.

Pro TDM: Special patient populations may benefit from TDM

Patients with renal dysfunction or elevated liver enzymes are frequently excluded from clinical trials, and so there is relatively little knowledge on the behavior of antiretroviral drugs in these patient categories. Consequently, dose recommendations are frequently not available, especially in liver impairment (1). Nevertheless, liver impairment may have significant influence on antiretroviral drug pharmacokinetics. For example, Barreiro *et al.* showed that efavirenz plasma concentrations above the toxic threshold (>4.0 mg/L) were significantly more common among patients with liver cirrhosis. They observed a similar trend for nevirapine, but not for lopinavir and atazanavir (38). Another study, however, did demonstrate altered lopinavir pharmacokinetics in patients with moderate liver impairment (39). Clearly, there is a rationale for performing TDM in patients with moderate to severe hepatic impairment in order to prevent them from exposure to unnecessary high plasma concentrations.

Recent publications demonstrated a high prevalence of subtherapeutic efavirenz and lopinavir plasma concentrations among children who were dosed in accordance with current guidelines (40-42). These findings are in favor of routine TDM in children in order to prevent them from being underdosed. Moreover, TDM is an objective method for detecting non-adherence, which is a substantial problem in HIV-infected children.

A report on a pregnant woman who had a virological relapse associated with low nelfinavir plasma concentrations provided an important incentive to recommend use of TDM during pregnancy to prevent such episodes (43). Lowered exposure during the third trimester has also been demonstrated for lopinavir (44). As a result of the altered pharmacokinetics during pregnancy, frequent measurement of protease inhibitor plasma concentrations is advisable.

Contra TDM: Only one component of the antiretroviral regimen is measured

Current treatment regimens exist of two NRTIs plus either a protease inhibitor or a NNRTI, but in TDM, plasma concentrations of only the protease inhibitor or the NNRTI are measured. It may appear inadequate to measure only one component of a regimen in an evaluation of virological response. Nonetheless, a number of studies have demonstrated a relation between the single NNRTI or protease inhibitor component and therapeutic response (3;45). TDM of a single component, therefore, may still contribute to improved response.

Contra TDM: Large intra-individual variability in antiretroviral TDM results

Large intra-individual variability limits the value of a single drug concentration measurement. Nettles *et al.* used frequent sampling in 10 HIV infected patients to obtain a total of 36 plasma samples per patient and reported modest and considerable intra-individual variability for the NNRTIs (25%) and the protease inhibitors (44%), respectively (46). The POPIN trial yielded similar results (47).

The main reason for significant intra-individual variability is probably the variation in compliance with regular drug intake, which has been reported to account for 55% of intra-individual variability (48). Noncompliance with food instructions and inaccurate reporting of the time of drug intake may also contribute to intraindividual variations in plasma protease inhibitor concentrations.

Given the considerable intraindividual variability, one must interpret with caution a single concentration measurement of an antiretroviral drug, particularly in the case of protease inhibitors. On the other hand, outlying plasma concentrations or highly fluctuating concentrations within an individual may indicate poor adherence. Suspicion of poor adherence is one of the indications for TDM (2).
Another more general conclusion is that important clinical decisions, such as dose adjustments, must not be made solely on the basis of a single blood concentration measurement. TDM will only benefit patient outcome if it is used as one the input factors in a decision-making process, along with other essential patient-related data, such as adherence history or viral load data. If the outcome of the decision making process is a dose adjustment for a protease inhibitor, it may be wise to repeat a concentration measurement if only one recent measurement is available.

Contra TDM: TDM does not measure unbound drug concentrations

Most currently used protease inhibitors and NNRTIs are highly bound to the plasma proteins alpha-1-acid glycoprotein (AAG) and/or albumin. Nevertheless, it is the

free fraction of the drug that exerts its pharmacological effect. AAG is an acute phase protein and AAG concentrations may fluctuate during acute and chronic infections. In addition, the concentrations of albumin are prone to major decreases in patients with severe states of disease.

Little is known regarding the clinical relevance of this limitation of TDM. Nevertheless, it appears appropriate to interpret plasma concentrations with caution in unstable clinical conditions.

Contra TDM: Absence of evidence from Randomized Controlled Trials

In 2003, the results of randomized controlled trials provided indisputable evidence on the benefits of routine TDM in therapy-naïve patients who started on indinavir-containing or nelfinavir-containing antiretroviral regimens (45;49). At present however, we have entered a new era in which indinavir and nelfinavir have largely been replaced by drugs with better pharmacokinetic profiles.
The drugs that are currently in use as first-line agents (lopinavir/ritonavir, efavirenz, and nevirapine) provide sufficiently high plasma concentrations in the great majority of antiretroviral therapy naïve patients (31). The improved pharmacokinetic characteristics of these drugs is reflected in the large numbers of participants that would be needed in prospective randomized controlled trials to obtain enough statistical power to judge the potential benefits of routine TDM. According to Khoo *et al,* approximately 1,000 to 2,000 patients would be required (47). For most clinical research groups it is not possible to conduct such a large and expensive trial.

In view of this, it is not surprising that both the POPIN trial and the recent study of Best *et al.* were unable to detect significant differences in virological suppression or toxicity in the TDM arms compared to the standard of care (SOC) arms in their studies (47) (50). The POPIN trial compared adherence support combined with TDM to SOC and included 122 patients; Best *et al.* obtained data from 190 patients. Clearly, both trials were statistically underpowered to detect a difference in virological suppression between the TDM and the SOC arms. It must be noted that Best *et al.* acknowledged this in advance. Their trial was designed to identify those patients who were most likely to achieve concentrations outside the therapeutic range, and they identified use of efavirenz, lopinavir/ritonavir and a high body weight as independent predictors of non-target concentrations (50).

Conclusion on the clinical utility of TDM

Although both protease inhibitors and NNRTIs meet most of the requirements of candidacy for TDM, the currently available evidence does not allow one to recommend routine use of TDM in antiretroviral-naïve patients. For treatment-experienced patients, the inhibitory quotient concept still is promising but its use urgently requires validation in prospective studies to clarify its role in routine clinical practice.

TDM may be recommended in those specific situations in which it is proven to be or likely to be beneficial. These situations include suspected non-adherence, manifestations of concentration-dependent toxicities, and all situations in which patients are more likely to achieve concentrations outside the therapeutic range, for instance use of antiretroviral drugs in children and pregnant women, and in patients taking drugs that may influence the pharmacokinetics of antiretroviral drugs.

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2

Chapter

Absence of a relation between efavirenz plasma concentrations and toxicity-driven efavirenz discontinuations in the EuroSIDA study

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Abstract

Background: Conflicting data exist regarding the effect of efavirenz (EFV) plasma concentrations on central nervous system (CNS) toxicity. We aimed to determine whether patients with high EFV plasma concentrations have an increased likelihood of toxicity-driven EFV discontinuations.

Methods: EFV plasma concentrations were measured from patients in the EuroSIDA study starting EFV after 1 January 1999. Patients with a plasma concentration available were divided into those that discontinued EFV due to any toxicity or by the choice of the patient or physician within 2 years (TOXPC group) and those that continued EFV for ≥ 2 years (no toxicity group). Multivariable logistic regression modeling was used to investigate the effect of the EFV plasma concentration and those of other potentially relevant factors on the risk of toxicity-induced EFV discontinuations.

Results: A total of 843 patients were included. Of these patients, 138 patients (16.4%) discontinued EFV due to TOXPC and 705 (83.6%) patients continued EFV for ≥ 2 years. A total of 20 (14.5%) patients in the TOXPC group had high EFV plasma concentrations (>4.0 mg/L) compared to 99 (14.0%) of the patients in the no toxicity group, p=0.89. A positive hepatitis C status (p=0.026), but not the EFV plasma concentration, was an independent predictor of toxicity-driven EFV discontinuations.

Conclusions: No association was found between EFV plasma concentrations and the risk of EFV discontinuations because of (CNS) toxicity. This result questions the designation of EFV plasma concentrations >4.0 mg/L as being 'toxic', at least when defined by treatment discontinuation.

Introduction

Efavirenz (EFV) is a non-nucleoside reverse transcriptase inhibitor (NNRTI) that is used as a first line agent in the treatment of HIV infection. It combines patient-comfort (one pill; once-daily dosing and no food restrictions) with potent antiretroviral activity and favorable safety properties (1;2).

A well-known disadvantage of EFV is its central nervous system (CNS) side effects, such as insomnia, dizziness and headache. In a large clinical trial, >50% of the patients treated with EFV-containing regimens experienced CNS adverse effects (3). However, CNS toxicity is usually transient and pooled data from controlled clinical trials show that only 2.7% of EFV-treated patients discontinued EFV because of CNS toxicity (4). Nonetheless, discontinuation might occur more frequently in clinical practice given the high discontinuation rates (13% and 24%) reported by two small observational studies (5;6).

A number of studies (6-9) have described a relationship between CNS toxicity and higher EFV plasma concentrations. This has led to an international consensus on a toxic threshold (that is, 4.0 mg/L) of EFV plasma concentrations in guidelines for therapeutic drug monitoring (TDM) (10). However, most of these studies had small sample sizes and routine TDM is currently not recommended for EFV because of a lack of data from large prospective trials (1;2). Moreover, several studies did not establish an increased risk of CNS toxicity in patients with EFV plasma concentrations >4.0 mg/L (11-14). Clearly, there still is a need for large studies on the relation between EFV pharmacokinetics and pharmacodynamics to provide more insight into the potential advantages of TDM of EFV.

An important outstanding issue is the consequence of interindividual differences in EFV pharmacokinetics in terms of treatment discontinuation. It is important to know whether patients with high EFV plasma concentrations are at increased risk of toxicity-induced EFV discontinuations. If so, routine application of TDM for EFV might prevent (unnecessary) discontinuations from this potent antiretroviral drug.

In the present study, we used the EuroSIDA database to study whether patients with high EFV plasma concentrations had an increased likelihood of toxicity-driven EFV discontinuations. Furthermore, we evaluated the influence of other potentially relevant factors, such as the hepatitis status of the patients, on the risk of EFV discontinuations because of toxicity.

Methods

Patients

EuroSIDA is a prospective pan-European cohort study of >14,000 HIV type-1 (HIV-1) infected patients in 93 centres from 31 countries across Europe (also including Israel and Argentina). Details have been previously published (15). At 6 monthly intervals, a blood sample is taken and stored for each patient, leading to the accumulation of a large sample repository. To date, no pharmacokinetic analyses have been performed on these samples. Although time between last intake and time of sampling is not noted, these samples could still be suitable for pharmacokinetic analyses of EFV because the long elimination half-life (40-55 h) of the drug results in minimal variability of plasma concentrations during a dose interval (7;16).

The EuroSIDA sample repository was searched for patients with a plasma sample collected after having started EFV treatment. Only patients that had started EFV after 1 January 1999 were included; reasons for discontinuation of antiretroviral agents were collected thereafter.
As only 30 patients with an available sample had discontinued EFV because of CNS toxicity, and to ensure that we were not missing any CNS-associated toxicity that might have been recorded as physician's/patient's choice, we divided patients into those that discontinued EFV because of any toxicity (including CNS toxicity) or patient's/physician's choice within 2 years (TOXPC group) and those that continued on EFV for ≥ 2 years (no toxicity group). Patients that had discontinued EFV because of other reasons (for example, virologic failure) within 2 years were excluded.
For each included patient, one sample was analyzed to determine the EFV plasma concentration. Patients with an undetectable plasma concentration (<0.20 mg/L) were excluded to prevent bias caused by non-adherence.

EFV analyses

Plasma samples were analyzed at the laboratory of the Department of Clinical Pharmacy of the Radboud University Nijmegen Medical Centre (Nijmegen, The Netherlands) by a previously described validated reversed-phase HPLC method (17).

Statistical methods

Patient characteristics at the time of starting EFV were compared between patients who continued EFV for ≥ 2 years, those who discontinued because of CNS toxicity and those who discontinued because of another toxicity or patient's/physician's

choice. EFV plasma concentrations and proportions with high concentrations (>4.0 mg/L) were compared in the TOXPC group versus the no toxicity group and in Caucasians versus non-Caucasian patients. They were also compared in the no toxicity group versus patients who discontinued because of CNS toxicity only. Chi-squared tests were used for categorical data and Kruskal-Wallis tests for continuous data. All tests were two-sided and a p-value of <0.05 was taken to be statistically significant.

Multivariable logistic regression modeling was used to investigate the effects of EFV plasma concentration on whether EFV was discontinued because of toxicity, after adjustment for potentially confounding variables. Factors that were significant in univariable analyses (p<0.10) were included in multivariable analyses and a stepwise selection method was used to confirm final model selection. The factors investigated included gender, ethnicity, HIV exposure group, region of Europe in which patients visited the clinical centre, prior diagnosis of any AIDS defining illness, hepatitis B virus (HBV) and hepatitis C virus (HCV) status, whether patients had previously received antiretroviral therapy (ART), number of drugs in regimen, calendar year of starting EFV, age, baseline CD4 count, nadir CD4 count, viral load, time from HIV positive diagnosis and type of nucleoside analogue reverse transcriptase inhibitor (NRTI) backbone. Analyses were repeated in the subset of patients with baseline weight and height available, adjusting for body mass index (BMI; weight in kg/ height in m^2). Sensitivity analyses were carried out with the outcomes: whether patients discontinued EFV because of CNS toxicity, whether patients discontinued any drug in the EFV-based regimen and whether patients discontinued EFV within 6 months because of toxicity. SAS software version 9.1 (SAS institute, Cary, North Carolina, USA, 2002-2003) was used for all statistical analyses.

Results

A total of 872 patients met the inclusion criteria and had plasma samples collected after having started EFV. Of these patients, 29 (3.3%) were excluded from further analysis because their plasma samples did not contain EFV; thus, 843 patients were included in the study.

In Table 1, patient characteristics are compared between 705 (83.6%) patients in the no toxicity group and 138 (16.4%) patients in the TOXPC group, which is split into those who discontinued because of CNS toxicity (n=30, 3.6%) and those who discontinued because of another toxicity or patient's/physician's choice (n=108, 12.8%).

Table 1 Patient characteristics at start of EFV treatment.

Characteristic		Total		Continued on EFV	
		n	%	n	%
All patients		843	100.0	705	83.6
Male		677	80.3	565	80.1
Exposure group					
	IDU	119	14.1	97	13.8
	Other	724	85.9	608	86.2
Ethnicity					
	Caucasian	776	92.1	643	91.2
	Non-Caucasian	67	7.9	62	8.8
Region of Europe					
	North	413	49.0	333	47.2
	South	99	11.7	77	10.9
	Central West	171	20.3	153	21.7
	East	160	19.0	142	20.1
Previous AIDS		267	31.7	226	32.1
Hepatitis B status					
	Negative	644	76.4	533	75.6
	Positive	55	6.5	45	6.4
	Unknown	144	17.1	127	18.0
Hepatitis C status					
	Negative	445	52.8	383	54.3
	Positive	131	15.5	103	14.6
	Unknown	267	31.7	219	31.1

Discontinued EFV because of CNS toxicity		Discontinued EFV because of other toxicities[a]		P-value
n	%	n	%	
30	3.6	108	12.8	-
28	93.3	84	77.8	0.16[b]
				0.27[b]
4	13.3	18	16.7	-
26	86.7	90	83.3	-
				0.12[b]
29	96.7	104	96.3	-
1	3.3	4	3.7	-
				0.004[b]
23	76.7	57	52.8	-
2	6.7	20	18.5	-
3	10.0	15	13.9	-
2	6.7	16	14.8	
9	30.0	32	29.6	0.86[b]
				0.42[b]
25	83.3	86	79.6	-
3	10.0	7	6.5	-
2	6.7	15	13.9	
				0.25[b]
12	40.0	50	46.3	-
6	20.0	22	20.4	-
12	40.0	36	33.3	-

Previous ART		670	79.5	552	78.3	24	80.0	94	87.0	0.11[b]
Number of ART drugs in regimen										0.42[b]
	≤ 3	588	69.8	497	70.5	20	66.7	71	65.7	-
	4	161	19.1	136	19.3	5	16.7	20	18.5	-
	≥ 5	94	11.2	72	10.2	5	16.7	17	15.7	-
		Median	**IQR**	**Median**	**IQR**	**Median**	**IQR**	**Median**	**IQR**	
EFV plasma concentration (mg/L)		2.2	1.6-3.2	2.2	1.6-3.3	2.6	1.9-3.9	2.3	1.6-3.0	0.51[c]
Date of starting EFV		4/01	1/00-9/02	4/01	2/00-10/02	1/02	1/00-6/03	2/01	12/99-2/02	0.12[c]
Date of enrolment		06/97	11/96-11/01	07/97	01/97-11/01	02/97	07/94-03/99	04/97	02/97-05/99	0.033[c]
Age (yrs)		41	35-49	41	35-49	41	35-49	42	36-48	0.99[c]
CD4 count (/mm^3)										
	Baseline[d]	359	205-538	353	204-529	353	250-590	403	207-573	0.56[c]
	Nadir[e]	131	50-220	129	49-214	141	47-249	161	67-237	0.18[c]
Time from nadir (months)		37	6-60	37	5-59	49	18-70	36	11-57	0.37[c]
Viral load (log_{10} copies/mL)										
	Baseline[f]	3.2	1.7-4.7	3.1	1.7-4.7	3.9	1.7-4.8	3.5	1.7-4.8	0.62[c]
	Maximum[g]	4.9	4.2-5.4	4.9	4.2-5.4	5.0	4.2-5.3	5.0	4.0-5.5	0.98[c]
Time from HIV-1 diagnosis (months)[h]		92.1	48-141	91.0	46-139	116.0	75-176	97.1	51-154	0.014[c]
Baseline weight (kg)[i]		70.4	62-77	70.1	63-77	76.0	65-80	71.2	61-77	0.34[c]
Height (cm)[j]		176.0	170-181	176.0	170-181	178.0	172-182	175.0	170-180	0.51[c]

[a]Including patients who discontinued efavirenz (EFV) because of patient's/physician's choice. [b]Chi-squared tests and [c]Kruskal-Wallis tests were used. [d]Baseline CD4 count was available for 819 patients. [e]Nadir CD4 count was available for 839 patients. [f]Baseline viral load was available for 803 patients. [g]Maximum viral load was available for 826 patients. [h]Date of HIV type-1 (HIV-1) diagnosis was available for 831 patients. [i]Baseline weight was available for 484 patients. [j]Height was available for 772 patients.
CNS, central nervous system; IDU, intravenous drugs use; IQR, interquartile range.

Other toxicities included 9 patients with clinical fat abnormalities, 4 with dyslipidemia, 1 with a hypersensitivity reaction, 11 with toxicity in the abdomen/gastrointestinal tract, 1 with toxicity in the endocrine system and 23 with any other toxicity. A total of 33 patients discontinued because of their own choice and 26 because of the physician's choice. The median (interquartile range (IQR)) duration of EFV treatment before EFV discontinuation was 12 (7-17) months and 10 (6-19) months in the TOXPC and CNS toxicity groups, respectively.

Characteristics were mostly similar between all three groups; however, the distribution of patients across the EuroSIDA geographical regions differed significantly with 23 out of the 30 (76.7%) patients who discontinued because of CNS toxicity coming from the north of Europe. The majority of patients (79.5%) were ART-experienced when they started EFV. These patients had a median (IQR) duration of previous treatment of 67 (43-92) months.

EFV plasma concentrations

In the 843 plasma samples with detectable EFV plasma concentrations, the median (range) EFV plasma concentration was 2.2 (0.2-25.5) mg/L. A total of 50 samples (5.9%) contained a subtherapeutic EFV plasma concentration (<1.0 mg/L). By contrast, a total of 119 samples (14.1%) contained a high EFV plasma concentration (>4.0 mg/L).

TOXPC group versus no toxicity group

No significant difference was found in the EFV plasma concentrations between the TOXPC group and the no toxicity group (median [range] 2.3 [0.6-12.3] mg/L versus 2.2 [0.2-25.5] mg/L, respectively, $p=0.68$). A total of 20 (14.5%) patients in the TOXPC group had EFV concentrations > 4.0 mg/L compared with 99 (14.0%) of the patients in the no toxicity group ($p=0.89$).

From the TOXPC group, 30 patients discontinued because of CNS toxicity. Again there was no significant difference in median concentrations between the CNS toxicity group and the no toxicity group (median [range] 2.6 [0.9-11.3] mg/L versus 2.2 [0.2-25.5] mg/L, respectively, $p=0.24$). There was also no significant difference between the proportions of patients with high concentrations in the CNS toxicity group compared with the no toxicity group (16.7% versus 14.0%, respectively ($p=0.60$)).

Caucasians versus non-Caucasians

A total of 776 Caucasian patients and 67 non-Caucasian patients were included in the analyses, of whom 16 (23.9%) were Asian and 51 (76.1%) were Black. There was

a borderline significant difference in EFV plasma concentrations between Caucasians and non-Caucasians (median [range] 2.2 [0.2-25.5] mg/L versus 2.6 [0.4-21.9] mg/L, respectively, p=0.081).
A significantly higher proportion of non-Caucasian patients were found to have high concentrations of EFV (18 [26.9%] non-Caucasians versus 101 [13.0%] Caucasians had EFV plasma concentrations >4.0 mg/L, p=0.002).

Factors affecting discontinuation of EFV because of TOXPC

Univariable logistic regression models showed that factors such as the region of Europe in which patients visited a clinical centre, HCV status, whether patients had previously received ART, the type of NRTI backbone started as part of the regimen and nadir CD4 count significantly affected whether patients discontinued EFV because of toxicity. A stepwise selection method also identified baseline viral load as significant after adjustment. In a multivariable model containing all these variables, there was no significant difference in the odds of discontinuation between patients with an EFV plasma concentration of <1.5 mg/L compared with 1.5-1.9 mg/L (p=0.90), 2.0-2.9 mg/L (p=0.73) or ≥ 3.0 mg/L (p=0.77). Patients with a positive HCV status had an 87% increased odds of discontinuation when compared with patients with a negative HCV status (p=0.026) and there was a borderline significant difference for ethnicity with non-Caucasians having a 59% reduced odds of discontinuation compared with Caucasians (p=0.072; Table 2).
In a subset of 449 patients with baseline weight and height data available, BMI was also entered as a categorical covariate in the model, with the categories BMI <18.5, 18.5-25 and >25 kg/m^2. There was a borderline significant difference in median (IQR) EFV plasma concentrations between these three BMI groups: 3.4 (2.0-4.1) mg/L, 2.4 (1.6-3.4) mg/L and 2.2 (1.6-2.9) mg/L, respectively, for <18.5, 18.5-25 and > 25 kg/m^2 (p=0.056). In the multivariable model, BMI was not found to be a significant predictor for toxicity-induced EFV discontinuations, p=0.71.

Sensitivity analyses

A total of 30 patients discontinued EFV because of CNS toxicity only. After adjustment for the variables as in the main analysis, no significant differences were found in the odds of discontinuation because of CNS toxicity between patients with different EFV plasma concentrations (Table 3).

A total of 246 patients discontinued ≥ 1 drug because of toxicity within 2 years of starting the EFV-based regimen. Of these patients, 40 (16.3%) discontinued their first drug because of CNS toxicity.

Table 2 Odds ratios and 95% confidence intervals for discontinuation of EFV due to TOXPC.

		Univariable			**Multivariable**		
		OR	95% CI	P	OR	95% CI	P
EFV plasma concentration							
	<1.5 mg/L	1.00	-	-	1.00	-	-
	1.5-1.9 mg/L	1.01	(0.56, 1.82)	0.97	1.04	(0.56, 1.93)	0.90
	2.0-2.9 mg/L	1.17	(0.70, 1.94)	0.56	1.10	(0.64, 1.88)	0.73
	≥ 3.0 mg/L	1.02	(0.61, 1.72)	0.93	0.92	(0.52, 1.61)	0.77
Ethnicity							
	Caucasian	1.00	-	-	1.00	-	-
	Non-Caucasian	0.39	(0.15, 0.99)	0.047	0.41	(0.16, 1.08)	0.072
Region							
	North	1.00	-	-	1.00	-	-
	South	1.19	(0.70, 2.03)	0.52	0.96	(0.53, 1.75)	0.90
	Central West	0.49	(0.28, 0.85)	0.010	0.43	(0.24, 0.77)	0.004
	East	0.53	(0.31, 0.91)	0.022	0.33	(0.17, 0.64)	0.001
Hepatitis C status							
	Negative	1.00	-	-	1.00	-	-
	Positive	1.68	(1.02, 2.76)	0.041	1.87	(1.08, 3.23)	0.026
	Unknown	1.35	(0.90, 2.04)	0.15	1.19	(0.76, 1.85)	0.45
Previous ART		1.64	(0.99, 2.71)	0.057	2.34	(1.21, 4.53)	0.012
Type of NRTI backbone							
	ZDV/3TC	1.00			1.00		
	ddI/d4T	2.40	(1.26, 4.55)	0.008	2.61	(1.28, 5.31)	0.009
	ddI/3TC	0.21	(0.03, 1.58)	0.13	0.21	(0.03, 1.60)	0.13
	ddI/ABC	1.21	(0.39, 3.76)	0.75	0.99	(0.29, 3.38)	0.98

	d4T/3TC	1.05	(0.60, 1.84)	0.87	1.24	(0.68, 2.25)	0.48
	d4T/ABC	0.52	(0.12, 2.29)	0.39	0.44	(0.09, 2.07)	0.30
	3TC/ABC	1.06	(0.46, 2.43)	0.90	1.01	(0.42, 2.41)	0.99
	Other dual NRTI	0.46	(0.16, 1.36)	0.16	0.41	(0.13, 1.27)	0.12
	> 2 NRTIs	1.23	(0.65, 2.31)	0.52	1.00	(0.51, 1.98)	0.99
	< 2 NRTIs	1.09	(0.62, 1.94)	0.76	1.19	(0.62, 2.28)	0.60
Baseline viral load (copies/mL)							
	< 500	1.00			1.00		
	500-10,000	1.08	(0.63, 1.85)	0.77	1.24	(0.69, 2.25)	0.47
	>10,000	1.38	(0.92, 2.09)	0.12	2.24	(1.35, 3.72)	0.002
	Missing	1.26	(0.53, 3.00)	0.60	3.22	(1.21, 8.61)	0.020
Nadir CD4 count per 100 cells/mm^3 increase		1.13	(0.98, 1.30)	0.085	1.27	(1.08, 1.49)	0.005

OR, Odds ratio; CI, confidence interval; ART, antiretroviral therapy; EFV, efavirenz; NRTI, nucleoside analogue reverse transcriptase inhibitor; ABC, abacavir; AZT, zidovudine; ddI, didanosine; d4T, stavudine; 3TC, lamivudine; TOXPC, discontinuation because of toxicity or by choice of the patient or physician.

Table 3 Odds ratios and 95% confidence intervals for discontinuation of EFV because of CNS toxicity.

		Univariable			Multivariable		
		OR	95% CI	P	OR	95% CI	P
EFV plasma concentration							
	< 1.5 mg/L	1.00			1.00		
	1.5-1.9 mg/L	0.63	(0.16, 2.58)	0.52	0.63	(0.15, 2.65)	0.53
	2.0-2.9 mg/L	1.32	(0.47, 3.72)	0.60	1.38	(0.48, 3.99)	0.55
	≥ 3.0 mg/L	1.41	(0.51, 3.89)	0.51	1.59	(0.55, 4.62)	0.40
Ethnicity							
	Caucasian	1.00			1.00		
	Non-Caucasian	0.36	(0.05, 2.67)	0.32	0.28	(0.04, 2.16)	0.22
Region							
	North	1.00			1.00		
	South	0.38	(0.09, 1.63)	0.19	0.26	(0.06, 1.18)	0.081
	Central West	0.28	(0.08, 0.96)	0.043	0.24	(0.07, 0.84)	0.026
	East	0.20	(0.05, 0.88)	0.033	0.11	(0.02, 0.57)	0.008
Hepatitis C status							
	Negative	1.00			1.00		
	Positive	1.86	(0.68, 5.07)	0.23	2.39	(0.82, 6.94)	0.11
	Unknown	1.75	(0.77, 3.96)	0.18	1.47	(0.63, 3.44)	0.38
Previous ART		0.90	(0.36, 2.25)	0.83	1.00	(0.32, 3.14)	1.00
Type of NRTI backbone							
	ddI/d4T	1.00			1.00		
	Other	0.91	(0.21, 3.94)	0.90	0.59	(0.13, 2.70)	0.49

Baseline viral load (copies/mL)							
	< 500	1.00			1.00		
	500-10,000	0.86	(0.27, 2.74)	0.80	0.98	(0.30, 3.25)	0.98
	> 10,000	1.52	(0.68, 3.40)	0.31	1.97	(0.78, 4.97)	0.15
	Missing	0.82	(0.10, 6.54)	0.85	3.10	(0.33, 29.2)	0.32
Nadir CD4 count (per 100 cells/mm^3 increase)		1.05	(0.79, 1.40)	0.75	1.09	(0.78, 1.53)	0.61

OR, Odds ratio; CI, confidence interval; ART, antiretroviral therapy; CNS, central nervous system; ddI, didanosine; d4T, stavudine; EFV, efavirenz; NRTI, nucleoside analogue reverse transcriptase inhibitor.

Factors found to significantly affect whether patients discontinued ≥ 1 drug were gender, region of Europe, HBV and HCV status, whether patients had previously received ART, the number of drugs in the regimen, the type of NRTI backbone and nadir CD4 counts; therefore, these were adjusted for in the multivariable model. No significant differences were found in the odds of discontinuation of any of the drugs in the regimen between patients with an EFV plasma concentration of <1.5 mg/L compared with 1.5-1.9 mg/L (adjusted odds ratio [OR] 1.10, 95% CI: 0.65-1.88, p=0.73), 2.0-2.9 mg/L (adjusted OR: 1.17, 95% CI: 0.74-1.85, p=0.51) or ≥ 3.0 mg/L (adjusted OR: 1.09, 95% CI: 0.69-1.74, p=0.70).

A total of 22 patients discontinued EFV within 6 months of starting treatment, 1 patient (4.5%) because of toxicity in the gastrointestinal tract, 8 (36.4%) because of CNS toxicity, 5 (22.7%) with any other toxicity, 4 (18.2%) discontinued as their own choice and 4 (18.2%) because of physician's decision. Factors found to significantly affect this were year of starting EFV, age and baseline viral load; therefore, these were adjusted for in the multivariable model.
No significant differences were found in the likelihood of discontinuation within 6 months between patients with an EFV plasma concentration of <1.5 mg/L compared with 1.5-1.9 mg/L (adjusted OR: 0.25, 95% CI: 0.03-2.12, p=0.20), 2.0-2.9 mg/L (adjusted OR: 0.81, 95% CI: 0.23-2.79, p=0.74) or ≥ 3.0 mg/L (adjusted OR: 1.90, 95% CI: 0.62-5.79, p=0.26).

Discussion

The results of our study show no apparent association between EFV plasma concentrations and drug discontinuations because of toxicity. Some other factors, however, were identified as independent predictors for toxicity-related EFV discontinuations.

Patients with an HCV-positive status had an 87% increased odds for toxicity-induced EFV discontinuations, which is probably caused by increased hepatotoxicity of EFV in patients with HCV co-infection (18-20). The mechanism behind this is not fully understood, but several factors might play a role (18;21). One mechanism is immune reconstitution. Liver damage in patients with chronic hepatitis C infection is predominantly immune-mediated and highly active antiretroviral therapy-induced immune restoration could thus result in hypertransaminasemia or even exacerbation of chronic hepatitis (21) (22). Another hypothesis is that HCV- coinfected patients

have impaired drug metabolism, leading to increased drug exposure and consequently increased drug toxicity (18). Nonetheless, recent data showed that chronic hepatitis C in itself does not modify EFV plasma exposure (23); only patients with liver cirrhosis have increased EFV plasma concentrations (24). Because the present study did not find a relation between drug exposure and toxicity-related EFV discontinuations, this mechanism seems less relevant, at least in this cohort of patients.

Another factor that was positively correlated with EFV discontinuations because of toxicity was the use of didanosine/stavudine as an NRTI backbone. This result should be attributed to the toxicity of this backbone (for example, peripheral neuropathy) (25) and is probably not directly related to EFV toxicity.

Because previous reports showed increased EFV plasma concentrations in non-Caucasian patients (26-30), we compared EFV plasma concentrations between Caucasians and non-Caucasian patients. Indeed, a significantly higher proportion of non-Caucasian patients had high EFV plasma concentrations. Median EFV plasma concentrations were only borderline statistically different, which is probably caused by the low number of non-Caucasian patients included and the consequently limited power for this analysis.
EFV is predominantly metabolized by the polymorphic Cytochrome P450 2B6 (CYP2B6) enzyme (31) (32). Increased EFV plasma exposure in non-Caucasian patients is at least partially explained by a higher frequency of certain single nucleotide polymorphisms of CYP2B6 (30;32;33). The 516 G>T and the 785 A>G polymorphisms, which are both more common among non-Caucasians, together characterize the CYP2B6*6 allele (32). Individuals homozygote for CYP2B6*6 were shown to have on average 4 times higher plasma exposure to EFV (32) and lowering the EFV dose or even starting on a lower dose appeared feasible in *6/*6 carriers (34). In addition, 983T>C polymorphism, which has been only observed in Hispanic and African populations, is strongly associated with increased EFV exposure (33).

The clinical consequences of ethnic differences in EFV pharmacokinetics are largely unknown. In the large clinical trial ACTG A5095, Gulick *et al.* observed an increased risk of virologic failure among non-Hispanic Black patients. The authors speculated that increased EFV-plasma exposure in Black patients might have resulted in less tolerability and compromised adherence and thus leading to higher rates of virologic failure (35). In the current study, there was no significant difference of toxicity-induced EFV discontinuations among patients of non-Caucasian or

Caucasian ancestry. Given the low number of non-Caucasians included (7.9%), this result should be interpreted with caution.
It is important that additional data from other large and diverse cohorts are forthcoming to further address the consequence of ethnic differences in EFV pharmacokinetics in terms of (CNS) toxicity, adherence and efficacy.

Regardless of race, this analysis did not demonstrate any significant relationship between EFV pharmacokinetics and (CNS) toxicity-induced discontinuations. There are several explanations for this result.

The first explanation could be that there is no strong relationship between CNS side effects and EFV plasma concentrations. Although a number of other studies did establish such a relationship (6-9), several other studies did not, including the 2NN study (11), the Swiss HIV cohort study (13) and two smaller prospective studies that were performed in Sweden (12) and Japan (14). An explanation for the latter results might be found in the development of tolerance to CNS toxicity. This phenomenon was extensively described in the ACTG A5097s study (36), in which the presence of the CYP2B6 516 T/T genotype was associated with EFV CNS toxicity at week 1 after initiation of EFV therapy, but not at week 24 despite persistently higher EFV plasma exposure (30).

Part of the explanation for the observed absence of a relationship between efavirenz plasma concentrations and toxicity-driven discontinuations might also be that this analysis had limited power because of the low number of patients that were reported to have discontinued EFV as a result of CNS toxicity. Of the 3,238 patients who started EFV after 1 January 1999, 256 (7.7%) discontinued EFV because of CNS toxicity; however, only 30 of those 256 patients had a plasma sample available.
A comparison between these 30 patients and the 226 patients who did not have a plasma sample available showed that their characteristics were mostly similar, including the proportion of Caucasians in each group. However, patients with a sample available were enrolled earlier into the EuroSIDA study ($p<0.001$) and more of these patients were from the north of Europe than patients without a sample ($p<0.001$).

Remarkably, a strong association was found between the geographical EuroSIDA region and the risk for CNS toxicity-induced discontinuations, with 23 out of the 30 patients that discontinued EFV because of CNS toxicity coming from the north of Europe. This result suggests that some centres are more aware or more active in

reporting CNS toxicity as a reason for discontinuation and this phenomenon might have also limited the power of this analysis. In an attempt to overcome this lack of power, and to ensure that we were not missing any CNS-associated toxicities that might have been recorded as physician's/patient's choice, we compared patients that discontinued EFV within 2 years because of any toxicity (including patient's/ physician's choice) to those who did not discontinue EFV in our statistical analyses.

A third explanation for our results might be found in the population that we have been investigating. The majority of our cohort (670 patients, 79.5%) was treatment-experienced at the moment of EFV initiation with a median treatment duration of 5-6 years. Many of these patients might have been using protease inhibitor-based (for example, indinavir and nelfinavir) regimens with difficult dosing schedules and tedious side effects before initiating EFV. It is imaginable that some of these patients, despite CNS toxicity, were motivated to continue EFV therapy.

In conclusion, we observed no apparent association between EFV plasma concentrations and toxicity-driven discontinuations among patients in the EuroSIDA study. This result questions the designation of EFV concentrations ≥ 4.0 mg/L as being 'toxic', at least when defined by treatment discontinuation.

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3

Chapter

Efavirenz dose reduction is safe in patients with high plasma concentrations and may prevent efavirenz discontinuations

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Abstract

Objective: To establish whether efavirenz dose reduction in patients with high plasma concentrations prevents toxicity-induced efavirenz discontinuations.
Methods: HIV-infected patients with a high efavirenz plasma concentration (≥ 4.0 mg/L) while using efavirenz 600 mg once-daily as part of their HAART regimen were selected from the ATHENA cohort study. These patients were classified into two groups. The reduced dose group contained all patients who underwent dose reduction following the high plasma concentration measurement; the standard dose group consisted of patients who had no dose reduction. Kaplan-Meier and Cox proportional hazards analysis were used to assess the impact of dose reduction on toxicity-induced efavirenz discontinuations.
Results: 180 Patients with high efavirenz plasma concentrations were included, 47 of which subsequently had their efavirenz dose reduced from 600 mg to 400 mg once-daily, which resulted in a 41% decrease in the median efavirenz plasma concentration. At week 48, the Kaplan-Meier estimated cumulative incidence of toxicity-induced efavirenz discontinuations was 11.5% in patients who continued the standard dose versus 2.3% in patients who had a dose reduction; $p=0.066$ (log-rank test). Dose reduction was not associated with loss of virologic suppression.
Conclusion: Dose reduction may prevent toxicity-induced discontinuations in patients with high efavirenz plasma concentrations, while not compromising virologic efficacy.

Introduction

Therapeutic drug monitoring (TDM) has been advocated as a means to optimize the safety and efficacy of antiretroviral therapy. Nevertheless, apart from indinavir and nelfinavir in treatment-naïve HIV-infected patients (1;2), there is no evidence from randomized controlled trials that TDM improves therapeutic outcome (3).

Standard dosing of efavirenz (EFV), a currently preferred first-line antiretroviral agent, leads to therapeutic plasma concentrations in at least 80% of the HIV infected individuals (4)(5), compared to just 40% for older agents such as nelfinavir (1). As a consequence, large and expensive trials, with more than 500 patients per treatment arm (6), are required to obtain adequate statistical power to judge the potential benefits of the application of TDM as a routine measurement in all patients taking EFV. Therefore, we agree with Khoo *et al.* that in the present situation, the value of TDM is best assessed by performing 'utilitarian' studies (6). The goal of these studies is not to provide evidence for routine use of TDM, but to explore the use of TDM in specific clinical situations. These studies should for instance focus on the use of TDM during pregnancy or the use of TDM in patients with severe liver impairment. In this paper, we describe the use of TDM to manage EFV-related toxicity.

Central nervous system (CNS) side effects are a well-known and frequently occurring complication of EFV therapy (7). Several reports have demonstrated the relationship of these side effects to high EFV plasma concentrations (4;8-11). In addition, there is international consensus on a therapeutic window for EFV plasma concentrations: 1.0-4.0 mg/L (12).
At our TDM practice, we regularly receive requests for TDM in patients using EFV who suffer from CNS side effects. In case these patients are found to have high EFV plasma concentrations (≥ 4.0 mg/L), our advice to the clinicians is to reduce the dose of EFV to 400 mg once-daily (QD) under the guidance of TDM. However, we have no formal evidence that this intervention improves the clinical outcome of these patients.

The objective of the present study was to establish whether EFV dose reduction prevents toxicity-induced EFV discontinuations in patients with high plasma concentrations. In addition, we aimed to evaluate whether dose reduction affects virologic efficacy.

Methods

Patients

All 25 Dutch hospitals that provide antiretroviral treatment participate in the AIDS Therapy Evaluation in The Netherlands (ATHENA) observational cohort study. Currently, data from over 15,000 patients have been anonymously recorded in a central database that is maintained by the HIV Monitoring Foundation (13).

We selected all patients in ATHENA who had a high EFV plasma concentration (i.e., ≥ 4.0 mg/L) recorded within 48 weeks after commencing EFV-based antiretroviral combination therapy. This cohort was subsequently classified into two groups. The Reduced Dose (RD) group consisted of those who underwent dose reduction following the high plasma concentration determination. The date of dose reduction was considered baseline. The Standard Dose (SD) group consisted of patients who continued the standard EFV dosage (i.e., 600 mg QD). For them, baseline was the first documented clinic visit following the high EFV plasma concentration measurement.

Statistics

Patient characteristics at the time of starting EFV were tabulated for patients in the RD group and the SD group. Differences between groups were compared using Chi-squared or Fisher's exact tests for categorical data and Mann-Whitney tests for continuous data. All tests were two-sided and a p-value of less than 0.05 was considered statistically significant.

Pharmacokinetics

EFV plasma concentrations before and after baseline were compared by using the Wilcoxon matched pairs signed rank sum test. For patients that underwent dose reduction, the EFV plasma concentration after baseline had to be taken at least 10 days after the date of dose reduction in order to have achieved new steady state conditions.

Toxicity-induced discontinuations

Reasons for discontinuation of antiretroviral agents are collected in the ATHENA database. Kaplan-Meier and Cox proportional hazards analysis were used to assess the impact of dose reduction on toxicity-induced EFV discontinuations. Patients who discontinued EFV for reasons other than toxicity were censored from the moment of discontinuation. Possible effect-measure modification and

confounding were assessed for the following parameters: gender, region of origin (as a surrogate for ethnicity), age, body mass index (calculated as the weight in kilograms divided by the square of the height in meters), hepatitis B and C status, HIV transmission risk group, CD4 count at the start of EFV, specific NRTI backbone, the EFV concentration before baseline and pre-treated status at the start of EFV. Pre-treated status was categorized as follows: i) treatment naïve patients that started EFV; ii) treatment experienced patients with an undetectectable viral load (<50 copies/mL) at the start of EFV; iii) treatment experienced patients with a detectectable viral load at the start of EFV.

Virologic response

Multivariable logistic regression modeling was used to investigate the effect of dose reduction on virologic response, which was defined as having a viral load below 50 copies/mL at week 24 after baseline.

We used an observed-failure approach in which patients who discontinued EFV due to virologic failure were considered failures at subsequent time points, whereas patients who discontinued EFV due to other reasons (e.g., pregnancy wish) were censored from that moment onwards. The same factors that we used as independent covariables in the Cox regression analysis for toxicity-related discontinuations were investigated (see above). In addition, plasma viral load at baseline was used as an independent covariable. We used a stepwise selection procedure to identify parameters that were significantly ($p<0.10$) associated with virologic response. The EFV dose (reduced dose or standard dose) was a fixed parameter in all models.

Apart from the primary analysis of the virologic response 24 weeks after baseline, several sensitivity analyses were carried out to see whether dose reduction affected virologic suppression at week 48 after baseline and at week 24 and week 48 after starting EFV. In addition, the analyses for the above mentioned four virologic efficacy endpoints were repeated stratified for pre-treated status at the start of EFV-based antiretroviral therapy. All data were analyzed with SPSS for MS Windows, version 16.0.1.

Results

Baseline characteristics

We identified 180 subjects who had high EFV plasma concentrations following the start of an EFV containing antiretroviral regimen. The date of starting EFV ranged from July 1998 to February 2007. Forty-nine patients out of the 180 patients (27.2%) underwent a dose reduction (RD group) and 131 patients (72.8%) continued the standard dosage regimen (SD group).

The patient characteristics between these groups differed significantly with regard to gender, HIV transmission risk group, region of origin and EFV plasma concentration. The median (interquartile range (IQR)) EFV plasma concentration was significantly higher in patients who underwent dose reduction (6.8 (5.7-9.6) mg/L), compared to patients that continued the standard dose (5.1 (4.3-6.4) mg/L), $p<0.001$). Furthermore, there were more females, heterosexually-infected patients and patients originating from Sub-Saharan Africa in the RD group (see Table 1).

Magnitude of Dose Reduction and pharmacokinetic outcome

The EFV dose was reduced from 600 to 400 mg in 47 out of the 49 patients in the RD group. As a result, the median (IQR) EFV plasma concentration decreased from 6.8 (5.6-9.5) to 4.0 (3.0-5.6) mg/L ($p<0.001$) in these 47 patients.

Two patients underwent a dose reduction directly from 600 mg QD to 200 mg QD. In one of these, the plasma concentration decreased from 6.4 mg/L to 2.7 mg/L. In the other patient, the EFV plasma concentration decreased from 27.7 mg/L to 11.4 mg/L. The dosage was further reduced in this latter patient to 100 mg QD which resulted in a therapeutic concentration of 2.7 mg/L. Both patients had viral loads below 50 copies/mL at all subsequent time points and were included in all further analyses.

The EFV plasma concentration remained above the threshold for efficacy (i.e., 1.0 mg/L) in all 42 patients who had a second plasma concentration available after dose reduction. In spite of the significant reduction in EFV plasma concentrations in the RD group, 22 patients still had EFV plasma concentrations above 4.0 mg/L. Seven of these had their dosage subsequently further reduced to 200 mg QD, which resulted in a therapeutic EFV concentration in three patients. Two patients still had an elevated EFV plasma concentration of 5.1 mg/L following the dose reduction to 200 mg QD and for two patients no measured EFV concentrations were available.

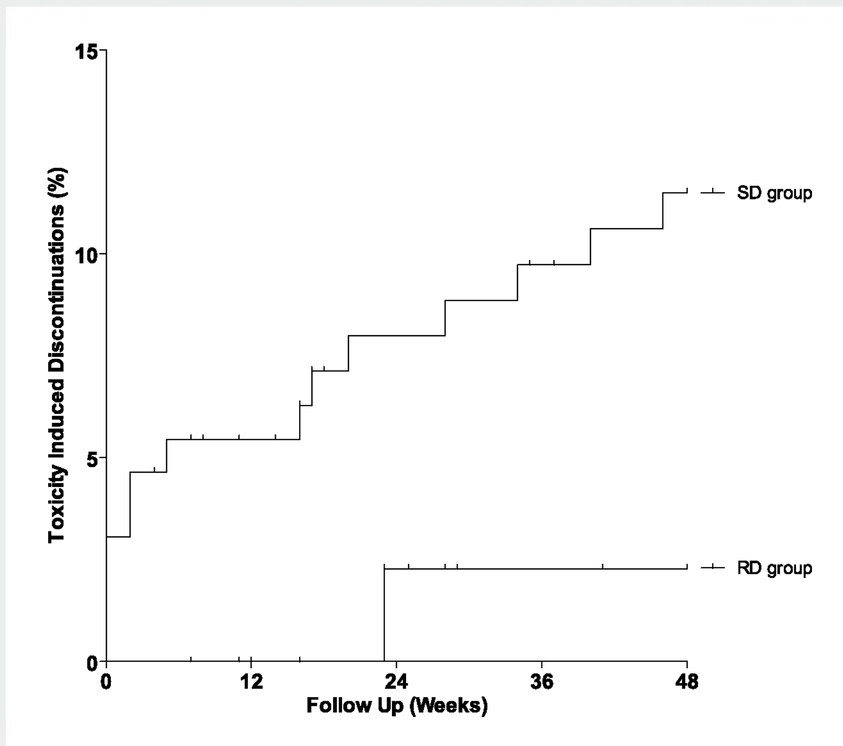
Half of the patients (n=68, 52%) that remained on the standard dose had no EFV plasma concentration measured after baseline. In the 63 patients who had a second

EFV plasma concentration available the median (IQR) EFV plasma concentrations were 5.3 (4.3-6.4) and 4.6 (3.5-7.3) mg/L before and after baseline, respectively (p=0.12).

Toxicity induced discontinuations of efavirenz

At week 48, fourteen patients from the SD group had discontinued EFV due to toxicity, compared to 1 patient in the RD group; Figure 1 shows the Kaplan-Meier curves for toxicity-induced discontinuations in both groups. At week 48, the estimated cumulative incidence of toxicity-induced discontinuations was 11.5% for patients in the SD group compared to 2.3% for patients in the RD group; p=0.066 (log-rank test).

Figure 1 Kaplan-Meier curves for toxicity-induced efavirenz discontinuations.



RD group, reduced dose group; SD group, standard dose group.

Table 1 Patient characteristics at start of efavirenz treatment.

		Total	
		n	%
All		180	100.0
Gender			
	Male	111	61.7
	Female	69	38.3
HIV transmission risk group			
	IDU[1]	5	2.8
	Homosexual	57	31.7
	Heterosexual	102	56.7
	Other	16	8.9
Region of origin			
	Western Europe	72	40.0
	Sub-Saharan Africa	54	30.0
	Caribbean / Latin America	37	20.6
	Other	17	9.4
Pre-treated status			
	Treatment naive	80	44.4
	Treatment experienced, VL < 50 copies/mL	42	23.3
	Treatment experienced, VL > 50 copies/mL	58	32.2
Viral load status at baseline			
	VL < 50 copies/mL	118	65.6
	VL > 50 copies/mL	62	34.4

Reduced dose		Standard dose		P-value
n	%	n	%	
49	27.2	131	72.8	
				0.032†
24	49.0	87	66.4	
25	51.0	44	33.6	
				0.044††
1	2.0	4	3.1	
9	18.4	48	36.6	
36	73.5	66	50.4	
3	6.1	13	9.9	
				0.022†
13	26.5	59	45.0	
20	40.8	34	26.0	
8	16.3	29	22.1	
8	16.3	9	6.9	
				0.82†
21	42.9	59	45.0	
13	26.5	29	22.1	
15	30.6	43	32.8	
				0.76†
33	67.3	85	64.9	
16	32.7	46	35.1	

NRTI backbone							0.62[†]
3TC + AZT	31	17.2	10	20.4	21	16.0	
TDF + 3TC	61	33.9	18	36.7	43	32.8	
TDF + FTC	19	10.6	6	12.2	13	9.9	
Other	69	38.3	15	30.6	54	41.2	
Hepatitis B status							0.48[†]
Negative	111	61.7	28	57.1	83	63.4	
Positive	15	8.3	6	12.2	9	6.9	
Unknown	54	30.0	15	30.6	39	29.8	
Hepatitis C status							0.21[††]
Negative	110	61.1	26	53.1	84	64.1	
Positive	6	3.3	3	6.1	3	2.3	
Unknown	64	35.6	20	40.8	44	33.6	
	Median	**IQR[2]**	**Median**	**IQR[2]**	**Median**	**IQR[2]**	
Age (yrs)	37	32-46	36	33-44	38	32-47	0.63[*]
Date of starting EFV	4/04	2/03-9/05	6/04	10/03-7/05	1/04	12/02-9/05	0.41[*]
Time between start EFV and baseline (days)	92	56-174	106	66-191	91	53-172	0.35[*]
Body mass index (kg/m^2)[3]	23.2	21-25	23.0	22-26	23.2	20-25	0.84[*]
Viral load at baseline ($\log_{10}$ copies/ml)	1.70	1.7-2.3	1.70	1.7-2.1	1.70	1.7-2.3	0.62[*]
CD4 count ($/mm^3$)[4]	220	93-410	230	110-415	220	77-400	0.45[*]
EFV plasma concentration (mg/L)	5.5	4.4-7.2	6.8	5.7-9.6	5.1	4.3-6.4	<0.001[*]
Time between sampling and latest EFV dose (hours)[5]	13.2	11.1-15.5	13.3	12.0-16.3	13.2	10.4-15.5	0.17[*]

[†] Chi-squared test; [††] Fisher's exact test; * Mann-Whitney test; [1] IDU = intravenous drugs use; [2] IQR = interquartile range;
[3] Body mass index available for 168 patients; [4] CD4 count available for 176 patients; [5] Time between sampling and latest EFV dose available for 144 patients.

Table 2 Odds ratios (ORs) and 95% confidence intervals (CIs) for patients in the reduced dose group achieving virological suppression (VL<50 copies/mL) at week 24 & 48 after baseline and at week 24 & 48 after starting EFV.

		Univariable			**Multivariable**		
		OR	95% CI	P	OR	95% CI	P
Week 24 after baseline							
	Standard dose	1.00			1.00		
	Reduced dose	3.23	0.71-14.77	0.13	3.76[a]	0.69-20.49	0.13
Week 48 after baseline							
	Standard dose	1.00			1.00		
	Reduced dose	5.09	0.64-40.48	0.12	6.88[b]	0.67-70.43	0.10
Week 24 after starting EFV							
	Standard dose	1.00			1.00		
	Reduced dose	1.24	0.49-3.14	0.65	0.43[c]	0.12-1.50	0.19
Week 48 after starting EFV							
	Standard dose	1.00			1.00		
	Reduced dose	2.21	0.47-10.44	0.32	2.28[d]	0.38-13.89	0.37

[a] corrected for pre-treated status and plasma viral load at baseline
[b] corrected for pre-treated status and plasma viral load at baseline
[c] corrected for pre-treated status, CD4 count, efavirenz concentration before baseline and plasma viral load at baseline
[d] corrected for pre-treated status and plasma viral load at baseline

In a Cox proportional hazards model, patients from the RD group had a lower risk (hazard ratio 0.18, 95% CI 0.02-1.40) of toxicity-related EFV discontinuations compared to patients from the SD group. Further explorations using multivariable Cox proportional hazards models showed that no other parameter was significantly associated with the risk of discontinuation of EFV due to toxicity, nor significantly (>10%) modified the observed effect of the dose reduction.

Virologic response

Twenty-four weeks after baseline, 95.2% of the patients in the RD group had a viral load below 50 copies/mL, compared to 86.1% of the patients who continued the standard dose (p=0.15). Univariable logistic regression models showed that the pre-treated status, the plasma viral load at baseline, the HIV transmission risk group

and the EFV concentration before baseline significantly affected whether or not patients had virologic response. In the multivariable analysis, pre-treated status and plasma viral load at baseline remained significantly associated with virologic outcome. Patients who had undergone dose reduction had an adjusted OR of 3.76 (95% CI: 0.69-20.49, $p=0.13$) for virologic response when compared to patients that continued the standard dose.

Sensitivity analyses

Table 2 shows the adjusted ORs for achieving virologic response at week 48 after baseline and at week 24 and week 48 after starting EFV. At most time points, patients in the RD group trended towards better virologic response, although statistically significant differences were not observed. After stratification for pre-treated status, dose reduction still had no negative impact on virologic response (data not shown).

Discussion

This study demonstrates that TDM guided EFV dose reduction may prevent toxicity-induced discontinuations in patients with high plasma concentrations. This result is conforming our expectations. We hypothesized that by reducing EFV plasma concentrations, CNS toxicity would diminish, which would thereupon prevent toxicity-induced EFV discontinuations.

In addition, we did not observe any detrimental effect of dose reduction on virologic response (see Table 2). Pre-treated status appeared to be the most important factor predicting virologic response. Treatment-experienced patients with a detectable viral load (>50 copies/mL) performed significantly worse in all virologic analyses, compared to either treatment naïve patients or treatment experienced patients that had no detectable viral load when switching to EFV. Therefore, we repeated all virologic analyses stratifying for pre-treated status at the start of EFV-based antiretroviral therapy. Again, dose reduction was not associated with virologic response in any of these strata. These results demonstrate that dose reduction is safe in patients with high EFV plasma concentrations, regardless of the pre-treated status at the start of EFV.

Of importance, no patient decreased to subtherapeutic EFV plasma concentrations (i.e., <1.0 mg/L) following dose reduction, which also confirms the safety of the

dose reduction strategy. It is often stated that EFV *trough* levels should be at least 1.0 mg/L, but this is in fact not in line with the work of Marzolini *et al.*, who established the therapeutic window of EFV based on mid-dose interval plasma levels which were taken during the day, 8 to 20 hours after EFV administration at bedtime (4).
In this study we also used mid-dose interval plasma levels, which were taken on average 13 hours post-dose, both before and after baseline.

Patients who underwent dose reduction had higher baseline EFV plasma concentrations (median 6.8 mg/L) than patients that continued the standard dose (median 5.1 mg/L). This difference may be caused by a tendency of physicians to decrease the dose with increasing plasma concentrations, but it may also be explained by increased EFV-toxicity at higher plasma concentrations, resulting in a higher clinical necessity to adjust the dose. Despite this unbalance at baseline favoring the patients who continued the standard dose, the proportion of patients who stopped EFV because of toxicity was still higher in the latter group, demonstrating the effectiveness of the dose reduction strategy in those who are most in need of it.

Women and patients originating from Sub-Saharan Africa were over-represented in the RD group. Previous studies indeed demonstrated higher EFV plasma concentrations in women (5) and black African patients (5;14;15). EFV is metabolized by the polymorphic Cytochrome P450 2B6 (CYP2B6) enzyme (16)(17) and black patients are known to have higher frequencies of certain CYP2B6 polymorphisms (e.g., 516 G>T and 983T>C), which are clearly associated with elevated EFV plasma concentrations (11;17;18). Possible causes for higher plasma concentrations in women are differences in body weight (in this cohort, female patients had an average weight of 63 kg compared to 73 kg for men), hormonal influences and body composition.

The ATHENA cohort study does not collect data on the seriousness of drug toxicity. Thus, the only reliable endpoint available to evaluate the pharmacodynamic consequence of dose reduction was discontinuation of EFV due to toxicity. Because there were only 15 toxicity-induced discontinuations, we had limited statistical power, which is a limitation of our analysis. A study in which CNS toxicity had been scored systematically before and after dose reduction could have been more powerful. Nonetheless, discontinuation of a drug due to toxicity is the ultimate consequence of drug-toxicity and we consider this a clinically relevant endpoint.

Another limitation is the retrospective design of this analysis. The best evidence for the dose reduction strategy would come from a controlled trial with a prospective design, in which one would randomly assign subjects to a TDM group in which the results of EFV concentration measurements plus advice (e.g., dose reduction) were reported to the treating physician, or to a control group for whom TDM results were not reported. Because it is quite improbable that such a trial will be ever organized, we must rely on alternative evaluations of the potential benefits of TDM in HIV-disease management.

In conclusion, our study demonstrates that TDM guided dose reduction can be considered in patients who have high EFV plasma concentrations. Dose reduction does not negatively affect virologic efficacy and may prevent toxicity-induced discontinuations.

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4

Chapter

Efavirenz dose reduction to 200 mg once-daily in a patient treated with rifampicin

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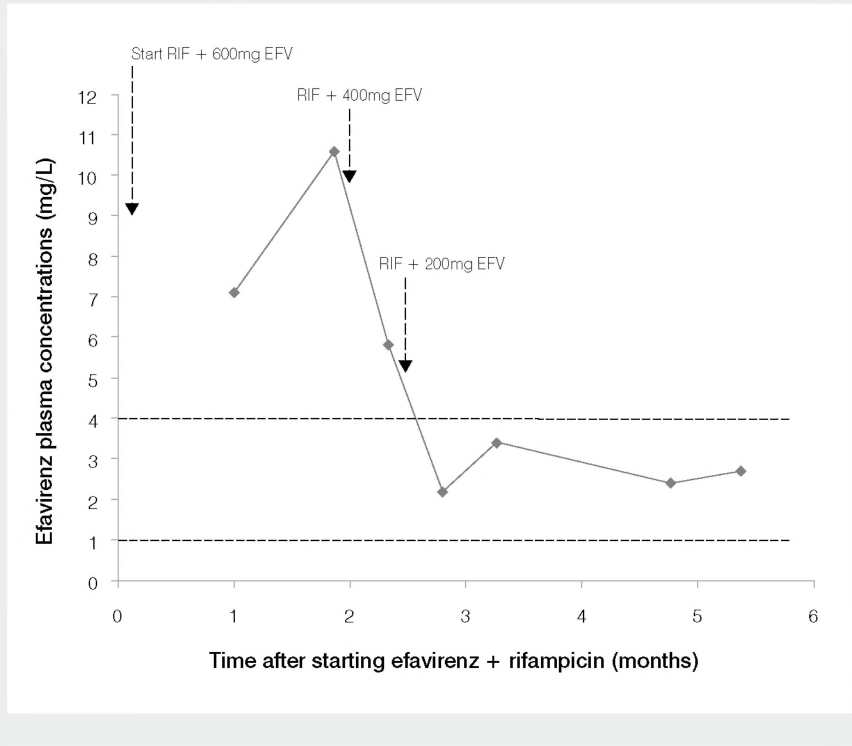
International guidelines suggest that HIV-tuberculosis (TB) co-infected patients are treated with efavirenz (EFV)-based antiretroviral regimens, because rifampicin decreases EFV plasma concentrations only modestly (22-35%) (1-4). It is recommended to use the standard EFV dose in patients weighing <50 kg and to consider an increase of the dose to 800 mg in patients weighing >50 kg (1) or >60 kg (2). In addition, therapeutic drug monitoring (TDM) may aid to ensure adequate EFV plasma exposure.
We describe a rifampicin-treated patient who was severely overdosed by using the standard EFV dose.

A 46-year-old man from Mauretania, known with Takayashu arteriitis, chronic hepatitis C and HIV commenced antiretroviral treatment with nevirapine (NVP), emtricitabine and tenofovir in 2004. NVP was used in a dose of 400 mg once-daily (QD) and this resulted in average NVP plasma exposure (i.e., 6.6 mg/L four hours after the latest drug intake). In March 2007, the 50 kg weighing patient was admitted in our hospital because of chest pain. At that time, the HIV viral load was below the limit of detection (<40 copies/mL) and the CD4 count was 190 cells/mm^3. The patient was diagnosed with tuberculosis and treatment was initiated containing rifampicin, isoniazid, ethambutol and pyrazinamide. Simultaneously, NVP was replaced by EFV in a dose of 600 mg QD. After switching to EFV, our patient had complaints such as drowsiness and weariness and was by times very agitated or even aggressive. At one and two months after switching therapy, the EFV mid-dose levels were measured and both appeared unexpectedly high (7.1 mg/L and 10.6 mg/L, respectively). Because the patient kept on being agitated, the EFV dose was lowered to 400 mg QD, resulting in a plasma level of 5.8 mg/L. Signs of agitation were still present and the dose was further decreased to 200 mg QD, which resulted in an EFV concentration of 2.2 mg/L (Fig. 1).
After the second dose reduction, patient's complaints decreased considerably and his behaviour became relaxed again. The HIV viral load remained undetectable and the patient completed TB treatment without further complications. Afterwards, the patient was switched back to his original HAART regimen. Again, the NVP plasma concentration was of average value.

In this patient, concomitant use of EFV and rifampicin resulted in unexpectedly high EFV plasma exposure and persisting neuropsychiatric side effects. Indeed, our patient had some characteristic features that have been related to high EFV plasma exposure, such as low body weight and black ethnicity (4). However, these factors have also been related to higher NVP exposure (4) (5) and therefore such

Figure 1 Efavirenz plasma concentrations during concomitant treatment with rifampicin. The therapeutic window of efavirenz plasma concentrations is 1.0 to 4.0 mg/L (12). EFV denotes efavirenz, RIF denotes rifampicin



high EFV plasma exposure was not expected, especially given the presence of rifampicin. In an attempt to understand this, we performed pharmacogenetic testing of the Cytochrome P450 2B6 (CYP2B6) enzyme, which plays a major role in both EFV and NVP metabolism, although NVP is also metabolised via CYP3A4 (6;7).

At first, the three most frequently observed polymorphisms related to high EFV plasma concentrations were investigated, being the 516 G>T exchange, the 785 A>G exchange (together characterizing the *CYP2B6*6* allele) and the 1459 C>T exchange (8). To our surprise, none of these three single nucleotide polymorphisms (SNPs) were found. Subsequently, all CYP2B6 exons were sequenced and the sample proved homozygote for the 1172 T>A polymorphism in exon 8, which characterizes the *CYP2B6*15* allele (9).

Until today, this polymorphism had been only described in 5 patients by Lang and colleagues, who demonstrated that 1172 T>A polymorphism results in undetectable CYP2B6 enzyme activity (9). Whereas their study comprised Caucasian patients heterozygote for 1172 T>A, we describe a black patient with *CYP2B6*15* homozygosity and the phenotypic consequences for NVP and EFV plasma concentrations.

The explanation for the observed unexpected high EFV exposure in the presence of rifampicin may be as follows. Rifampicin induces CYP2B6 activity by increasing CYP2B6 gene transcription (10). Nonetheless, in a patient homozygote for 1172 T>A, increased formation of CYP2B6 mRNA will merely lead to enhanced production of poor-functioning CYP2B6 enzyme, which apparently does not reverse a poor metabolizer phenotype into a normal metabolizer phenotype (11).

In spite of poor-functioning CYP2B6, our patient had no high NVP plasma levels. However, NVP is also metabolised via CYP3A4 which may serve as an escape route for NVP metabolism in patients with poor-functioning CYP2B6 (7).

In conclusion, physicians should be aware of the possibility of high EFV plasma exposure and persisting neuropsychiatric side effects in patients that start EFV in conjunction with rifampicin, even if former NVP plasma exposure did not indicate reduced CYP2B6 metabolism. TDM and pharmacogenetic testing can be valuable in such cases.

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5

Chapter

Adherence to therapeutic drug monitoring guidelines in The Netherlands

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Submitted

Abstract

Several international HIV treatment guidelines recommend therapeutic drug monitoring (TDM) for specific clinical scenarios, such as drug interactions or pregnancy. The adherence to these recommendations is unknown. We evaluated the adherence to the Dutch TDM guideline of 2005. From the ATHENA observational cohort study, we selected three scenarios for which the guideline recommended TDM: i) start of a combination of lopinavir/ritonavir + efavirenz or nevirapine (drug-drug interaction); ii) start of efavirenz (routine TDM), iii) use of nelfinavir during pregnancy. The adherence to the TDM guideline was 46.7% in patients who started lopinavir/ritonavir plus efavirenz or nevirapine; 9.5% for patients who started efavirenz; and 58.5% for patients who used nelfinavir during pregnancy. Patients treated in clinics that had a TDM assay locally available and patients treated in academic clinics were more likely to receive TDM. A higher baseline HIV viral load was another significant predictor for the utilization of TDM.

Introduction

Therapeutic drug monitoring (TDM), meaning the use of drug plasma concentrations in the management of antiretroviral therapy, is frequently utilized in some European countries such as The Netherlands, Spain and France. In addition, several international treatment guidelines, including the US Department of Health and Human Services (DHSS) guidelines, recommend TDM for specific clinical scenarios, such as in patients with drug-drug interactions, with drug concentration-dependent toxicities or in patients with lack of virologic response (1).

The scientific basis for TDM consists of three observations. First, the importance of sufficiently high plasma drug concentrations for adequate suppression of HIV replication (2-6). Second, the large inter-individual variability in plasma concentrations of protease inhibitors (PIs) and non-nucleoside transcriptase inhibitors (NNRTIs) among patients taking the same dose (3;7-9). Third, data from two randomized controlled trials which demonstrated better therapeutic outcome in treatment-naive patients who received routine TDM of nelfinavir or indinavir (10;11).

Little is known about the adherence of clinicians to TDM recommendations in HIV treatment guidelines. In addition, the determinants of adherence to TDM recommendations are unknown. For instance, a potential determinant might be the local availability of a TDM assay in the hospital of an HIV outpatient clinic. By understanding which factors are associated with adherence to TDM guidelines, strategies can be developed to improve adherence of clinicians to the guidelines as well as to improve the TDM guidelines themselves.

To obtain more insight into the use of TDM and the determinants of its use, we evaluated the use of TDM in The Netherlands from January 2004 to December 2008. Within this period, in 2005, the Dutch Association of AIDS Physicians (NVAB) issued evidence-based guidelines for the treatment of HIV-infected patients, including recommendations for TDM (12;13). The guidelines were published (13), they were discussed at plenary NVAB meetings and they were distributed by mail to all Dutch HIV-physicians. We studied the utilization of TDM in three specific clinical scenarios for which TDM was recommended by the Dutch guideline (12;13). In addition, we studied whether TDM use was associated with therapeutic outcome.

The first scenario was the use of TDM in the setting of a drug-drug interaction, namely patients who started with the combination of the HIV-1 protease inhibitor

lopinavir/ritonavir plus a non-nucleoside reverse transcriptase inhibitor (NNRTI), either efavirenz or nevirapine. NNRTIs induce CYP3A-mediated metabolism of lopinavir, and it is therefore recommended to increase the dose of lopinavir/ritonavir when concomitant use is indicated (14). TDM of lopinavir may be helpful to achieve optimal lopinavir plasma exposure in this situation.

The second scenario was the use of routine TDM in patients starting efavirenz in combination with two nucleoside analogue reverse transcriptase inhibitors (NRTIs). Although there was no formal evidence that routine TDM would benefit patient outcome for efavirenz, the scientific committee recommended it because efavirenz had similar pharmacological characteristics as indinavir and nelfinavir (i.e. large inter-individual variability in pharmacokinetics; and an established relationship between the plasma concentration of efavirenz and antiretroviral efficacy and toxicity (3)).

The third scenario was in patients who used a nelfinavir-containing regimen during pregnancy. At the moment of the introduction of the guideline in 2005, nelfinavir in combination with lamivudine and zidovudine was a commonly used regimen for HIV-infected pregnant women in the Netherlands (12). Nelfinavir plasma concentrations may be decreased during pregnancy (15;16), which may in turn lead to an increased risk of virologic failure (17). In order to ensure adequate nelfinavir plasma concentrations during pregnancy, the guideline recommended TDM of nelfinavir during pregnancy.

Methods

Patients

All Dutch hospitals that provide antiretroviral treatment participate in the AIDS Therapy Evaluation in The Netherlands (ATHENA) observational cohort study. Currently, data from over 16,000 patients have been anonymously recorded in a central database that is maintained by the HIV Monitoring Foundation (18).

Scenario 1: Drug interaction lopinavir + efavirenz/nevirapine

From the ATHENA observational cohort study, we selected all adult patients who started lopinavir/ritonavir + efavirenz or nevirapine between 1 January 2004 and 31 December 2008. Adherence to the guideline was defined as the presence of a lopinavir plasma concentration in the ATHENA database between week 1 and 12.

Patients who discontinued the combination of lopinavir + efavirenz or nevirapine within three weeks were excluded.

Scenario 2: Routine TDM of efavirenz

We selected all adult patients who started for the first time efavirenz in combination with two NRTIs between 1 January 2004 and 31 December 2008. For efavirenz, the guideline recommended to determine plasma concentrations at week 4 and week 24 after commencing therapy. Therefore, we defined full adherence to the guideline as the presence of plasma concentrations of efavirenz in the ATHENA database between week 2 and 8, and between week 16 and 32. Patients who discontinued efavirenz within 32 weeks were excluded. In addition, we defined 'partial adherence', defined as having at least a measurement at week 4. For this analysis, we excluded patients who had discontinued efavirenz within 8 weeks.

Scenario 3: TDM of nelfinavir during pregnancy

For scenario 3, we selected all adult female patients who started nelfinavir during pregnancy or who became pregnant while using nelfinavir between 1 January 2004 and 31 December 2008. We defined adherence to the guideline as the availability of at least one plasma concentration of nelfinavir during pregnancy. Patients who used nelfinavir for less than 8 weeks during pregnancy were excluded.

Statistics

Baseline characteristics

Patient characteristics at the start of one of the three scenarios were tabulated for patients who received TDM according to the guideline and those who did not. Differences between groups were compared using Chi-squared or Fisher's exact tests for categorical data and Mann-Whitney U tests for continuous data. All tests were two-sided and a p-value of less than 0.05 was considered statistically significant.

Adherence to the guidelines

We used multivariable logistic regression modeling to investigate the relationship of the following factors with the utilization of TDM.

1. Time period of starting therapy i.e.,
   i) pre-introduction of guideline (2004);
   ii) introduction of guideline (2005-2006);
   iii) post-introduction of guideline (2007-2008).

2. Context of outpatient clinic. Outpatient clinics were categorized as follows:
   i) small academic HIV outpatient clinics,
   ii) large academic outpatient clinics;
   iii) small non-academic outpatient clinics; and
   iiii) large non-academic outpatient clinics.
   Outpatient clinics were classified as large or small if they had greater or fewer than the median number of patients for academic (n=354) and non-academic HIV outpatient clinics (n=221), respectively.
3. Regular presence of a clinical pharmacist at multidisciplinary HIV treatment-team meetings.
4. Presence of a TDM assay in the hospital laboratory of the outpatient clinic.

In addition, we evaluated the influence of several patient-related factors, namely gender, country of birth (as a surrogate for ethnicity), age, body mass index (calculated as the weight in kilograms divided by the square of the height in meters), hepatitis B and C status, HIV transmission risk group, CD4 count, HIV viral load at the start of the regimen of interest, specific NRTI backbone, and pre-treated status at the start of therapy. Pre-treated status was categorized as follows: i) treatment naïve patients; ii) treatment experienced patients with an undetectable viral load (< 50 copies/mL) at the start of the regimen of interest iii) treatment experienced patients with a detectable viral load at the start of the regimen of interest.

Effect of adherence to the guidelines on virologic response and toxicity-driven drug discontinuations

To assess whether adherence to the TDM guideline affected therapeutic outcome, we investigated the effect of adherence to the TDM guideline on virologic response and toxicity-driven drug discontinuations.

For scenarios 1 and 2, virologic response was defined as a viral load below 50 copies/mL at week 48. For scenario 3 (nelfinavir use during pregnancy), virologic response was a viral load below 50 copies/mL at the last viral load measurement before delivery. For the latter scenario, we included the time on nelfinavir as an extra independent variable in our logistic regression models.

We used an observed-failure approach in which patients who discontinued their regimen due to virologic failure were considered failures at subsequent time points, whereas patients who discontinued due to other reasons were censored from that moment onwards. Multivariable logistic regression association models were constructed to adjust for potential confounders. All patient-related factors which were used in the analysis for guideline-adherence were tested for confounding.

We used a stepwise selection procedure, by which a parameter was identified as a confounder if its addition to the model resulted in a >10% change of the regression coefficient of TDM on virologic response.

Cox proportional hazards analysis was used to assess the impact of adherence to the TDM guideline on drug discontinuations due to toxicity or patient's choice within the first year of therapy. Patients who discontinued the drug of interest for other reasons were censored from the moment of discontinuation. Confounding was assessed for the same parameters and in the same manner as for the analysis of virologic response.

All data were analyzed with SPSS for MS Windows, version 16.0.1.

Results

Table 1 presents some key characteristics of the Dutch HIV outpatient clinics which were considered potentially relevant for the uptake of the TDM recommendations. Academic outpatient clinics were generally larger than non-academic outpatient clinics; in addition academic outpatient clinics had more frequently a TDM assay locally available.

Scenario 1: Drug interaction lopinavir + efavirenz/nevirapine

Between 2004 and 2008, 304 patients started cART which contained lopinavir/ritonavir plus efavirenz or nevirapine. Within the first three weeks, 47 patients discontinued the use of this combination. Thus, 257 patients were included in the analysis, of which the majority (158, 61.5%) used efavirenz in combination with lopinavir.

A total of 120 of the 257 patients (46.7%) had a lopinavir plasma concentration determined as recommended by the guideline. As shown in Table 2, baseline characteristics for the TDM and non-TDM group were mostly similar.

The use of TDM increased from 32.4% in 2004 (pre-guideline) to 55.3% during introduction of the guideline in 2005-2006. In 2007-2008, the use of TDM remained stable (49.0%). Multivariable logistic regression analysis demonstrated that patients in large non-academic outpatient clinics were significantly less likely to receive TDM compared to patients in academic clinics (see Table 3). Furthermore, treatment-experienced patients were more likely to receive TDM compared to treatment naïve

Table 1 TDM-related characteristics of the 24 Dutch HIV outpatient clinics.

		Academic		Non-academic		P-value
		n	%	n	%	
All		8		16		
Presence of a clinical pharmacist at multidisciplinary HIV treatment-team meetings						
	Never	2	25.0	8	50.0	0.39†
	Regularly	6	75.0	8	50.0	
Presence of a local HIV TDM assay						
	Present	5	62.5	5	31.3	0.20†
	Not present	3	37.5	11	68.8	
		Median	**Range**	**Median**	**Range**	
Number of patients under care at 1 January 2006		354	255-1636	221	69-1490	0.032*

† Fisher's exact test; * Mann-Whitney test

patients, especially those treatment-experienced patients with detectable viral loads at baseline (Table 3).

Forty-eight weeks after baseline, 79.6% of the patients who received TDM had a viral load below 50 copies/mL, compared to 73.9% of the patients who were not monitored by TDM ($p=0.50$). In the univariable logistic regression analysis, baseline CD4 count and baseline viral load were significantly associated with virologic response at week 48. After adjusting for these and other factors that confounded the effect of adherence to the guideline (namely gender, BMI, hepatitis B status, pre-treated status and NRTI backbone), patients who received TDM had an adjusted odds ratio (OR) of 1.77 (95% CI 0.36-8.37, $p=0.48$) for achieving virologic response at week 48. At week 24, adherence to the guideline was also not associated with virologic response (data not shown).
At week 48, 15 out of the 137 patients who did not receive TDM had discontinued lopinavir due to toxicity or patient's choice (TOXP), compared to 3 out of the 120 patients who did receive TDM ($p=0.008$, log-rank test). In bivariable Cox-proportional hazards models, only baseline CD4 count significantly (>10%) modified the effect of adherence to the guideline on the risk of TOXP discontinuations. After

adjusting for this parameter, patients who received TDM still had a significantly lower risk of toxicity-induced lopinavir discontinuations (adjusted HR 0.16, 95% CI 0.036-0.71, p=0.016).

Scenario 2: Routine TDM of efavirenz

A total of 3,057 patients started antiretroviral treatment with efavirenz in combination with two NRTIs between 2004 and 2008. Within the first 36 weeks, 588 patients (19.2%) discontinued efavirenz, which left 2,469 patients for the analysis. Of these patients, 234 (9.5%) had an efavirenz plasma concentration determined at week 4 and 24 as recommended by the guideline. As shown in Table 2, patients who received TDM had generally lower CD4 counts and higher viral loads at baseline compared to patients who did not receive TDM.

The use of TDM of efavirenz decreased significantly from 15.8% in 2004 (pre-guideline) to 11.5% during introduction of the guideline in 2005-2006. In 2007-2008, the adherence to the guideline decreased further to 5.7%. As for scenario 1, patients treated in non-academic outpatient clinics were less likely to receive TDM than patients in academic settings. In addition, large academic clinics had lower adherence to the guideline compared to small academic clinics. The presence of a clinical pharmacist at multidisciplinary team meetings resulted in lower adherence to the 2005 TDM guideline whereas the local availability of a TDM assay resulted in greater adherence to the guideline. Finally, patients with higher baseline viral loads were more likely to receive TDM of efavirenz (see Table 3).

Partial adherence to the guideline, defined as the presence of an efavirenz plasma concentration at week 4, was 30.4%. Partial adherence was stable over time (30.8% in 2004, 30.7% in 2005-2006 and 30.0% in 2007-2008). Baseline viral load, hospital-type, the presence of a local TDM assay, and the presence of a clinical pharmacist at multidisciplinary team meetings predicted partial adherence in the same manner as they did for full adherence to the guideline (data not shown).

Forty-eight weeks after baseline, 89.5% of the patients in the group of patients who received TDM according to the guidelines had a viral load below 50 copies/mL, compared to 93.3% of the patients who did not receive TDM (p=0.054). After adjusting for factors that confounded the effect of adherence to the guideline on virologic response (BMI and baseline CD4 count), patients who received TDM were less likely to achieve virologic response (adjusted OR (95% CI) 0.49 (0.29-0.85), p=0.010).

Table 2 Patient characteristics at the time of starting the regimen of interest.

	Lopinavir + NNRTI				
	TDM		No TDM		p-value
	n	%	n	%	
All	120	46.7	137	53.3	
Gender					
Male	96	80.0	113	82.8	0.61†
Female	24	20.0	24	17.5	
Region of origin					
Western Europe	86	71.7	78	56.9	0.067†
Caribbean / Latin America	13	10.8	16	11.7	
Sub-Saharan Africa	13	10.8	27	19.7	
Other	8	6.7	16	11.7	
Pre-treated status					
Treatment naive	40	33.3	47	34.3	0.22†
Treatment experienced, VL < 50 copies/mL	19	15.8	32	23.4	
Treatment experienced, VL > 50 copies/mL	61	50.8	57	41.6	
	Median	**IQR[1]**	**Median**	**IQR[1]**	
Age (years)	43	37-51	42	36-59	0.37*
Date of starting therapy	06/06	06/05-10/07	04/06	10/04-11/07	0.31*
Body mass index (kg/m^2)	22.5	20-25	22.9	21-25	0.65*
CD4 count ($/mm^3$)	230	120-463	300	140-440	0.63*
Viral load (log_{10} copies/ml)[2]	4.7	3.6-5.8	4.5	3.4-5.5	0.27*

NA = not applicable; † Chi-squared test; * Mann-Whitney test [1] IQR, interquartile range;
[2] includes only data from patients with a detectable viral load at baseline.

Efavirenz					Nelfinavir				
TDM		No TDM		p-value	TDM		No TDM		p-value
n	%	n	%		n	%	n	%	
234	9.5	2235	90.5		79	58.5	56	41.5	
194	82.9	1827	81.7	0.66†	NA	-	NA	-	-
40	17.1	408	18.3		79	58.5	56	41.5	
145	62.0	1413	63.2	0.75†	15	19	4	7.1	0.065†
28	12.0	247	11.1		13	16.5	5	8.9	
41	17.5	349	15.6		45	57.0	44	78.6	
20	8.5	226	10.1		6	7.6	3	5.4	
161	68.8	1516	67.8	0.034†	66	83.5	44	78.6	0.61†
36	15.4	462	20.7		7	8.9	8	14.3	
37	15.8	251	11.2		6	7.6	4	7.1	
Median	**IQR[1]**	**Median**	**IQR[1]**		**Median**	**IQR[1]**	**Median**	**IQR[1]**	
40	35-48	41	35-48	0.38*	29	25-32	29	25-33	0.67*
12/05	10/04-03/07	12/06	07/05-01/08	<0.001*	06/05	09/04-02/06	02/05	08/04-04/06	0.74*
22.7	21-25	23.1	21-25	0.051*	24.7	23-30	26.2	24-28	0.85*
210	100-310	245	160-350	<0.001*	410	283-565	390	281-518	0.51*
5.0	4.6-5.3	4.9	4.4-5.3	0.051	3.9	3.3-4.5	4.0	3.1-4.4	0.96*

Table 3 Factors predictive of adherence to the Dutch TDM guidelines in multivariable logistic regression analyses.

	adjusted OR	95% CI	P
Scenario 1: lopinavir + NNRTI drug interaction			
Treatment period			0.004
Pre-introduction (2004)	0.24	0.10-0.56	0.001
Guideline-introduction (2005-2006)	1.00		
Post-introduction (2007-2008)	0.52	0.23-1.20	0.12
Context of outpatient clinic			0.004
Academic	1.00		
Non-academic-small (<221 patients)	0.49	0.18-1.34	0.16
Non-academic-large (>221 patients)	0.24	0.098-0.57	0.001
Patient's pre-treated status			0.058
Naive	1.00		
Treatment experienced, VL <50 copies/mL	1.16	0.42-3.26	0.082
Treatment experienced, VL >50 copies/mL	2.62	1.08-6.40	0.034
Scenario 2: routine TDM of efavirenz at week 4 and week 24			
Treatment period			<0.001
Pre-introduction (2004)	1.65	1.15-2.37	0.007
Guideline-introduction (2005-2006)	1.00		
Post-introduction (2007-2008)	0.48	0.34-0.67	<0.001

Context of outpatient clinic		
	Academic-small (<354 patients)	1.00
	Academic large (>354 patients)	0.20
	Non-academic-small (<221 patients)	0.076
	Non-academic-large (>221 patients)	0.13
Presence of a local TDM assay for efavirenz		
	Not present	1.00
	Present	2.06
Presence of a clinical pharmacist at HIV MDT[1] meetings		
	Not present	1.00
	Regularly present	0.56
Baseline viral load (copies/mL) (per ^{10}log increase)		1.17
Scenario 3: nelfinavir during pregnancy		
Context of outpatient clinic		
	Academic	1.00
	Non-academic-small (<221 patients)	0.39
	Non-academic-large (>221 patients)	0.18
Presence of a local TDM assay for nelfinavir		
	Not present	1.00
	Present	3.79

[1] MDT, multidisciplinary team.

	<0.001
0.13-0.29	<0.001
0.035-0.17	<0.001
0.089-0.19	<0.001
1.31-3.26	0.002
0.37-0.86	0.009
1.06-1.29	0.003

	0.004
0.12-1.24	0.11
0.066-0.49	0.001
1.45-9.87	0.006

Patients who received TDM of efavirenz at week 4 (i.e., partial adherence to the guideline) were also less likely to achieve virologic response (adjusted OR 0.59 (0.39-0.89), p=0.013).

Because there were only a few patients who discontinued efavirenz because of TOXP after week 32, we examined the association between partial adherence to the guideline (at least a week 4 efavirenz plasma sample available) and TOXP driven discontinuations. Within 48 weeks, 11 out of the 836 patients in the TDM group (2.5%) had discontinued efavirenz because of TOXP versus 41 out of the 1915 (3.5%) of the patients in the non-TDM group (p=0.19, log-rank test). After adjusting for confounding factors in a multivariable Cox proportional hazards analysis (baseline CD4 count and the NRTI backbone), patients who received TDM had an adjusted HR (95% CI) of 0.72 (0.44-1.18) (p=0.19) for TOXP discontinuations of efavirenz.

Scenario 3: TDM of nelfinavir during pregnancy

A total of 161 patients started antiretroviral treatment with nelfinavir during pregnancy or before becoming pregnant. Of these patients, 135 used nelfinavir for more than 8 weeks during pregnancy. The great majority of these patients (n=130, 96.3%) started nelfinavir during pregnancy; 5 patients had already started nelfinavir before they became pregnant.

Table 2 shows that most women (n=79, 58.5%) had at least one plasma concentration of nelfinavir determined during pregnancy. There were no statistically significant differences in baseline characteristics between patients who did or did not receive TDM during pregnancy (see Table 2).

The use of TDM slightly increased from 54.9% in 2004 (pre-guideline) to 61.8% during introduction of the guideline in 2005-2006. After introduction of the guideline, in 2007-2008, the use of TDM decreased (50.0%). In the multivariable logistic regression model, patients treated in large non-academic outpatient clinics were less likely to receive TDM than patients in academic clinics. In addition, the local availability of a TDM assay was associated with more use of TDM during pregnancy (see Table 3).

At the last viral load measurement before delivery, 94.2% in the group of patients who received TDM during pregnancy had a viral load below 50 copies/mL, compared to 94.1% of the patients who did not receive TDM (p=1.00). After adjusting for confounders (baseline CD4, baseline viral load and time on nelfinavir), the adjusted OR (95% CI) to achieve an undetectable load was 1.45 (0.18-11.73), p=0.73 for patients who received TDM. There were 2 TOXP discontinuations of nelfinavir during pregnancy in the TDM group, and 2 in the non-TDM group (p=1.00).

Discussion

The main goal of this study was to evaluate the adherence of clinicians to the Dutch TDM guidelines of 2005. The adherence to the recommendations varied from low for routine TDM of efavirenz (full adherence 9%; partial adherence 30%), to moderate for nelfinavir in pregnancy (59%), and lopinavir concomitantly used with an NNRTI (47%).

We cannot compare these results to those of other countries or databases, because this is, to our knowledge, the first evaluation of HIV physician adherence to TDM guidelines. Part of the explanation for the moderate adherence to the TDM recommendations might be that most recommendations were based on expert opinion. The only exceptions are nelfinavir and indinavir (10;11). In agreement with this, adherence to the TDM recommendations was highest for the nelfinavir scenario. HIV therapy is rapidly changing and improving due to continuous drug development. Between 2004 and 2008, the antiretroviral armamentarium was extended with several potent and relatively well-tolerated drugs. Therefore, physicians have increased opportunities for switching therapy instead of managing drug-related problems with TDM. This may form a second explanation for the moderate adherence to the TDM recommendations.

The adherence to the recommendation to perform routine TDM of efavirenz at week 4 and week 24 was extremely low, and declined from 16% in 2004 to 6% in the period 2007-2008. At present, there is international consensus among clinical pharmacologists that TDM should be applied selectively, and not routinely (1;19-21). It is well possible that during our study period, an increasing number of Dutch HIV physicians, as well as clinical pharmacists (see Table 3), got convinced that TDM is only indicated in selected situations. This may explain the declining rates for routine TDM of efavirenz between 2004 and 2008.

A second important goal of this study was to identify determinants of TDM use. The data from our study indicate that Dutch HIV physicians are more likely to use TDM in patients with a higher baseline viral load (efavirenz scenario) or in treatment-experienced patients who start therapy with a detectable viral load (lopinavir scenario). TDM is thus used in the most vulnerable patients, who have the highest a-priori chance of virologic failure. Other patient-related determinants of TDM use were not identified.

Independent of the context of the outpatient clinic, the local availability of an analytical assay for the measurement of antiretroviral drug concentrations was associated with increased use of TDM. The availability of a local assay will probably ease TDM logistics, thereby shortening the time delay between the moment of blood sampling and the TDM result. As a consequence, HIV physicians might become more prone to apply TDM, which in turn, will lead to increased experience with TDM.
Another health-system related determinant of TDM use was the context of the HIV outpatient clinic. Academic centres were more likely to apply TDM than non-academic centres for all investigated TDM scenarios. It is difficult to explain this result, which is probably caused by multiple factors. One factor might be that academic HIV physicians are more willing to adhere to expert opinion based recommendations. A second possible explanation might be the presence of trainees who specialize in infectious diseases in academic clinics. These trainees were actively educated on the Dutch TDM guidelines and they may have propagated the use of TDM in their outpatient clinics.
Finally, the time period in which therapy was initiated was associated with TDM use for efavirenz (discussed above) and for lopinavir. Only for lopinavir in combination with efavirenz or nevirapine, the results from our study suggest that the introduction of the TDM recommendations led to increased use of TDM.

The ATHENA database offers unique opportunities for TDM research because it comprises detailed information on TDM results as well as clinical information. Although not the primary goal of our analysis, we were also interested whether adherence to the TDM guidelines was associated with virologic response or drug-toxicity (the latter being deduced from toxicity or patient's decision induced drug discontinuations).

For the lopinavir scenario, patients who received TDM appeared less likely to discontinue lopinavir because of toxicity or patient's choice. For efavirenz, patients who received TDM were less likely to achieve an undetectable viral load at week 48. One should cautiously interpret both results, because our study design is observational. Thus, patients were not randomized to a TDM or a non-TDM group; they may have received TDM for a particular reason, which might be associated with the likelihood of achieving the studied outcome. Given the low adherence to the efavirenz routine TDM guideline, it is most probable that efavirenz TDM was applied selectively, rather than routinely. As described above, TDM was mostly applied in those patients who had the highest a-priori chance of virologic failure. In our analysis, we attempted to adjust for such a selection bias, (e.g., by including

baseline viral load in the regression model), but there certainly remains residual confounding (e.g., preferential utilization of TDM in patients suspected of non-adherence). Confounding by indication is in our view the most likely explanation for the worse virologic outcome in patients who received TDM of efavirenz.
The lower discontinuation rate of lopinavir in the TDM group might be caused by dose reduction of lopinavir in patients with lopinavir adverse effects and high lopinavir plasma concentrations. Five patients in the TDM group had their lopinavir dose reduced after initially starting an increased dose, which may have prevented some toxicity induced lopinavir discontinuations in the TDM group. High lopinavir plasma concentrations have been related to hypercholesterolemia (22) and, anecdotally, to gastro-intestinal symptoms, such as nausea and abdominal pain.

Although this study evaluated the adherence to the Dutch TDM guidelines of 2005, drug-drug interactions (lopinavir scenario) and pregnancy (nelfinavir scenario) are still regarded as valid scenarios for TDM by current international HIV treatment guidelines (1;21). Present-day HIV treatment guidelines do not recommend unselected, routine use of TDM anymore. However, efavirenz is one of the few antiretroviral drugs with a high likelihood of concentration-related adverse effects, which is still a valid indication for TDM (3;23).

In conclusion, we have seen moderate to low adherence to the Dutch TDM recommendations of 2005. In addition, we have identified multiple determinants of TDM use. What can we learn from this study for future implementations of TDM guidelines? First, scientific committees that draft TDM guidelines should be cautious in making TDM recommendations based on expert opinion, especially if the impact is large in terms of time and costs (e.g., routine TDM). This might lead to better acceptance of TDM recommendations in clinical practice. Second, the implementation of a TDM guideline might be more successful with a more pro-active implementation by the clinical pharmacologists of the drafting scientific committee. For the Dutch TDM guidelines of 2005, only trainees working in academic clinics were actively and continuously educated. Finally, a denser network of laboratories with TDM assays might lead to better adherence to TDM guidelines.

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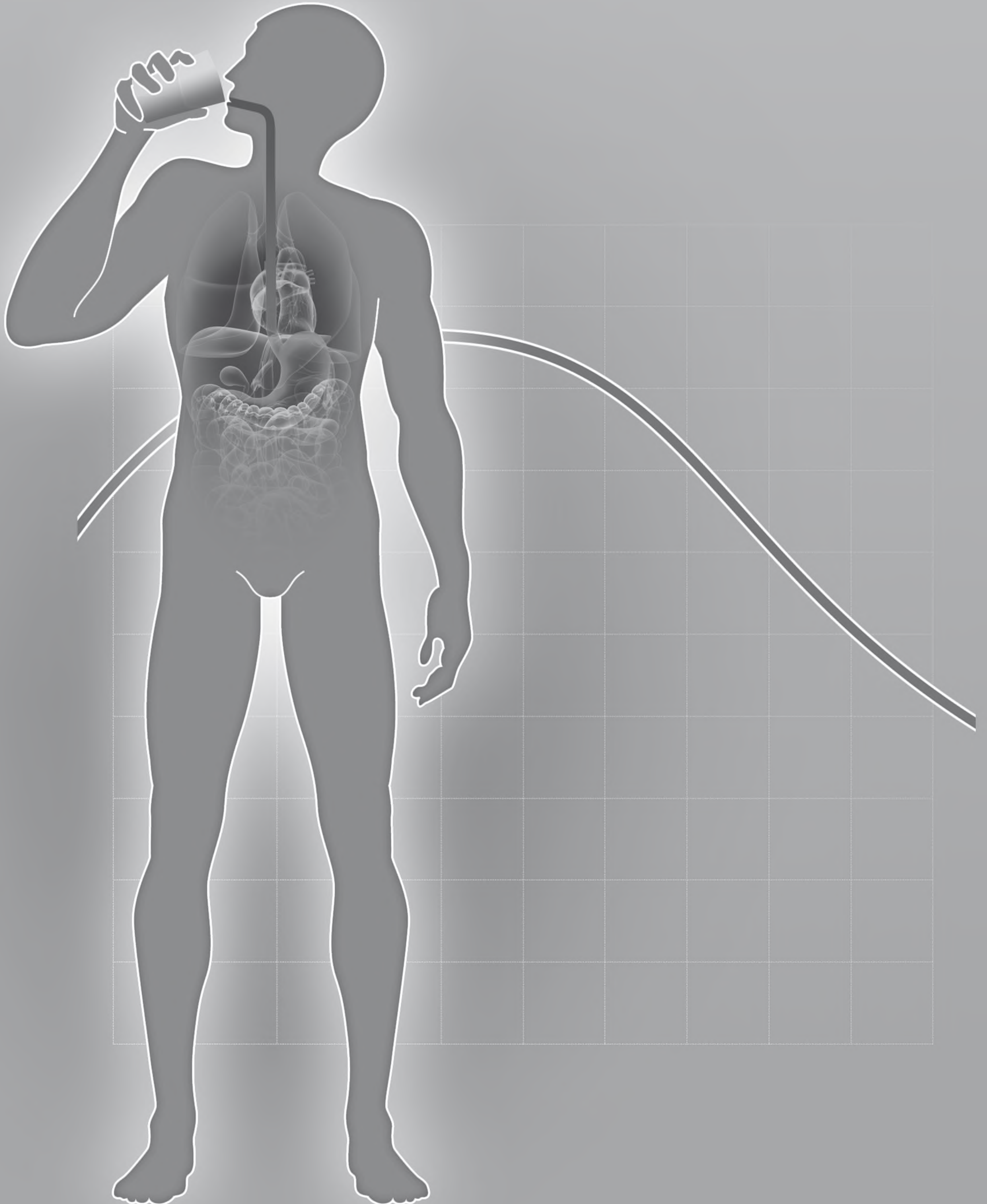

2

Part

Drug-drug interactions of antiretroviral drugs

6

Chapter

Lower atovaquone/proguanil concentrations in patients taking efavirenz, lopinavir/ritonavir or atazanavir/ritonavir

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Abstract

Objective: Atovaquone/proguanil is frequently used as malaria prophylaxis by HIV-infected travellers. The objective of this study was to compare atovaquone/proguanil pharmacokinetics in healthy volunteers to those in HIV-infected patients who were treated with efavirenz, lopinavir/ritonavir or atazanavir/ritonavir.

Methods: This was an open-label, multi-centre, phase-I, single-dose trial. On Day 1, a single dose of atovaquone/proguanil was administered during a strictly fat-standardized breakfast and blood was collected throughout a 168-hour period. Plasma concentrations of atovaquone and proguanil were determined using a validated HPLC method. Pharmacokinetic parameters were calculated using WinNonLin version 5.2.1.

Results: Seventy-six subjects were available for statistical evaluation: 18 healthy volunteers, 20 HIV-infected patients on efavirenz, 19 patients on lopinavir/ritonavir, and 19 patients on atazanavir/ritonavir. The geometric mean (95% confidence interval (95% CI)) atovaquone $AUC_{0\rightarrow t}$ was 103.6 (79-137) h*mg/L in healthy volunteers, compared to 29.6 (23-39), 31.7 (24-42), and 64.3 (49-84) h*mg/L in patients treated with efavirenz, lopinavir/ritonavir, and atazanavir/ritonavir, respectively. The geometric mean (95% CI) atovaquone C_{max} was 1.80 (1.4-2.3) mg/L in healthy volunteers versus 1.06 (0.84-1.3), 1.13 (0.90-1.4), and 1.02 (0.81-1.3) mg/L in patients using efavirenz, lopinavir/ritonavir, and atazanavir/ritonavir, respectively. In addition, the $AUC_{0\rightarrow t}$ of proguanil was 38-43% lower in the three groups of HIV-infected patients versus the healthy controls.

Conclusions: Physicians should be alert for atovaquone/proguanil prophylaxis failures in HIV-infected patients treated with efavirenz, lopinavir/ritonavir or, to a lower extent, atazanavir/ritonavir. In patients treated with efavirenz or lopinavir/ritonavir, an increase of the dose of atovaquone/proguanil should be considered.

Introduction

Atovaquone coformulated with proguanil is used for the treatment and prophylaxis of malaria. Due to its efficacy and favorable safety profile (1), atovaquone/proguanil is frequently used as chemoprophylaxis by HIV-infected patients who travel to malaria endemic destinations.

Nevertheless, there are indications for drug interactions of atovaquone with some frequently prescribed antiretroviral drugs. Despite a lack of data, the summary of product characteristics of lopinavir/ritonavir and ritonavir warn that, theoretically, co-administration may lead to decreased atovaquone plasma concentrations (2;3). The postulated mechanism for this theoretical drug-drug interaction is enhanced glucuronidation of atovaquone (4). Indeed, lopinavir/ritonavir may induce glucuronidation (5). Other ritonavir boosted protease inhibitors (PIs), such as atazanavir/ritonavir, and non-nucleoside reverse transcriptase inhibitors (NNRTIs), such as efavirenz, may induce glucuronidation as well (6-7).

The consequences of these theoretical drug-drug interactions are potentially serious, since diminished exposure to atovaquone may result in suboptimal prophylaxis of malaria. Therefore, we compared atovaquone/proguanil pharmacokinetics in healthy volunteers to those in HIV-infected patients who were on stable antiretroviral treatment with regimens that contained either efavirenz, lopinavir/ritonavir or atazanavir/ritonavir.

Methods

This open-label, multi-centre, phase-I, single-dose trial was conducted from May 2007 until December 2008 at the Erasmus Medical Centre Rotterdam, the Rijnstate Hospital Arnhem, the Radboud University Nijmegen Medical Centre, the Leiden University Medical Centre, and the Elisabeth Hospital Tilburg, all in the Netherlands.

Study design

Participating subjects received one single dose of atovaquone/proguanil 250/100 mg with breakfast in the morning at their clinic. The breakfast in this study was strictly fat-standardized because the absorption of atovaquone is highly dependent on the fat content of the meal taken with the drug (8). The Ethical Review Board of

the Radboud University Nijmegen Medical Centre approved the trial. Local approval by the ethical committees of all other participating centres was obtained as well.

Study population

HIV-infected patients with CD4 positive lymphocyte counts higher than 200 cells/μL, who were for at least one month stable on antiretroviral regimens containing either efavirenz 600 mg once-daily (QD), atazanavir/ritonavir 300/100 mg QD or lopinavir/ritonavir in a dosage of 400/100 mg twice-daily (BD) or 800/200 mg QD, were invited to participate in the trial. The main exclusion criteria were: suspicion of non-adherence to antiretroviral medication and use of medication known to interfere with the pharmacokinetics of atovaquone or proguanil.

The reference group in this study consisted of healthy volunteers, who had to be in a good, age-appropriate health condition as established by physical examination, medical history, biochemical, hematologic, and urinalysis testing within 4 weeks before the single dose. The main exclusion criteria for the healthy volunteers were a positive HIV test result; a positive hepatitis B or C test result and therapy with any drug (for 2 weeks preceding dosing), except for acetaminophen.

All subjects, both the HIV-infected patients and the healthy volunteers, had to be aged 18-65 years on the day of dosing, had to have body mass indexes of 18 to 30 kg/m^2, and had to be willing and able to sign the Informed Consent Form before screening evaluations. The main exclusion criteria for all subjects were: sensitivity/idiosyncrasy to atovaquone/proguanil or chemically related compounds, a relevant medical history or current condition that might interfere with atovaquone/proguanil pharmacokinetics, creatinine clearance less than 60 mL/min (calculated from serum creatinine), and pregnant or breast-feeding females. At screening (within 4 weeks prior to the single dose), eligibility for inclusion was established.

Safety assessments and pharmacokinetic sampling

Blood samples for pharmacokinetics were collected throughout a 168-hour period (0, 0.5, 1, 2, 3, 4, 5, 6, 8, 24, 48, 72, and 168 hours (7 days) after atovaquone/proguanil intake (13 samples) to characterize drug absorption, distribution and elimination. Serum biochemistry, hematology and urinalysis test results were checked on days 1 and 8. Adverse events were assessed during the same visits and on days 2, 3, and 4.

At day 1 and 8 of the study, we determined mid-dose plasma concentrations for efavirenz and trough plasma concentrations for lopinavir and atazanavir. In some cases, the latter was not possible due to practical reasons. In these cases, plasma concentrations were taken at similar (+/- 2 hours) periods after drug-intake on days 1 and 8.

Bioanalysis

Plasma concentrations of atovaquone and proguanil were analyzed at the Department of Clinical Pharmacy of the Rijnstate Hospital (Arnhem, The Netherlands). We used a solid-phase extraction (SPE) - high-performance liquid chromatography (HPLC) method for the simultaneous quantitative analysis of atovaquone and proguanil, which has been described by Lindegårdh et al. (9). Atovaquone and atovaquone internal standard (IS) (compound 59C80) were kindly provided by Glaxo Smith Kline (Hertfordshire, UK); proguanil was kindly provided by Astra Zeneca (Cheshire, UK). 1-(2.5)-dichlorophenylbiguanide (Sigma-Aldrich, Zwijndrecht, The Netherlands) was used as an internal standard for proguanil.

Briefly, to 500 μL plasma, 1,000 μl of atovaquone-IS (2.5 μM) in ice-cold acetonitrile was added. After vortex-mixing, the tubes were centrifuged at 13,400 G for 10 minutes. The supernatant was mixed with 1,000 μL of proguanil-IS 2.5 μM in phosphate buffer (pH 6.8, 0.01M) and then loaded onto an SPE column (Isolute HCX-Q, Biotage, Uppsala, Sweden). The extraction procedure was exactly the same as described by Lindegårdh et al. (9). The eluate was evaporated to dryness under a gentle stream of nitrogen at 60°C. The residue was reconstituted in 100 μL of methanol: water (90:10 v/v). Twenty μL of the resulting solution was run on a Zorbax SB-CN 250 x 4.6 mm 5 μm column (Agilent Technologies, Amstelveen, The Netherlands) with a flow rate of 1.0 mL/min using the mobile phases elution scheme described by Lindegårdh (9). Atovaquone and proguanil were detected by ultraviolet spectroscopy at 245 nm.

The accuracy values for atovaquone were 107, 103 and 99% at 0.275, 0.573 and 4.58 mg/L, respectively. At the same concentrations, the precision values (within day, coefficient of variation) were 7.9, 3.1 and 3.4%, respectively. The calibration curve was linear over a concentration range of 0.183 to 5.50 mg/L. For proguanil, the accuracy values were 101, 98 and 96% at concentrations of 0.029, 0.272 and 0.544 mg/L. The precision values at the same concentrations (within day, coefficient of variation) were 4.8, 2.6 and 2.9%, respectively. The calibration curve for proguanil was linear over a concentration range of 0.019 to 0.580 mg/L.

Efavirenz, lopinavir and atazanavir plasma concentrations were analyzed at the laboratory of the Department of Clinical Pharmacy of the Radboud University Nijmegen Medical Centre by previously described validated HPLC methods (10) (11).

CYP2C19 genotyping

Subjects were tested for the presence of **2* (681G>A, rs4244285), **3* (636G>A, rs4986893), and **17* (806C>T, rs12248560) alleles of *CYP2C19*, which is a key enzyme involved in proguanil metabolism. DNA was isolated from EDTA blood (Total Nucleic Acid isolation kit (Roche Diagnostics, Mannheim, Germany) on a MagnaPure LC (Roche Diagnostics)). Genotyping was performed on 5 ng DNA using Taqman allelic discrimination assays (ABI Prism 7000 Sequence Detection system, Applied Biosystems, Nieuwerkerk a/d IJssel, The Netherlands).
Assay IDs were C_25986767_70 (*CYP2C19*2*) and C_27861809_10), with thermal profiles 50 cycles (15 seconds 92 °C, 90 seconds 60 °C). Genotypes were scored by allele-specific fluorescence using SDS 2.2.2 software (Applied Biosystems). Assays were validated previously via direct sequencing of wild type, heterozygous and homozygous variant samples. Assay performance was monitored by including positive and negative controls. After genotyping, subjects were categorized as follows: homozygous ultra-rapid metabolizers (**17/*17)*, heterozygous ultra-rapid metabolizers (**1/*17*), extensive metabolizers (**1/*1*), intermediate metabolizers (**1/*2, *1/*3,*), and poor metabolizers (**2/*2, *2/*3, *3/*3*). There were 6 subjects with mixed *CYP2C19 *2/*17* genotypes.

Pharmacokinetic analysis

Pharmacokinetic parameters for atovaquone and proguanil were calculated by non-compartmental methods using the WinNonlin® software package (version 5.2; Pharsight, Mountain View, CA, USA) and the log/linear trapezoidal rule. On the basis of the individual plasma concentration-time data, the following pharmacokinetic parameters of atovaquone and proguanil were determined: the area under the plasma concentration-time curve from t=0 (drug intake) to the last quantifiable concentration ($AUC_{0\rightarrow t}$; in hour*milligram/liter), the maximum plasma concentration of the drug (C_{max}; in milligrams per liter), the time to reach C_{max} (T_{max}; in hours), and the apparent elimination half-life ($t_{1/2}$; in hours). The $AUC_{0\rightarrow t}$ was used instead of the $AUC_{0\rightarrow\infty}$ because $AUC_{0\rightarrow\infty}$ contained extrapolated areas greater than 20% in the majority of subjects for both atovaquone and proguanil.

Sample size and statistical analysis

The study was powered to detect a 20% difference in atovaquone AUC between the healthy controls and each of the other three HIV-1 positive groups. The required number of participants was calculated as 20 per study arm.

Patient characteristics at day 1 (baseline) were tabulated for the four groups: the healthy volunteers and the HIV-infected patients treated with efavirenz, lopinavir/ritonavir and atazanavir/ritonavir. Differences between groups were compared using Chi-squared tests or Fisher's exact tests where appropriate for categorical data and the Kruskal-Wallis test for continuous data. All tests were two-sided and a p-value of less than 0.05 was considered statistically significant.

The $AUC_{0\rightarrow t}$, C_{max}, and $t_{1/2}$ were natural log transformed before analysis, and all corresponding confidence intervals (CIs) for means (for the difference of two means) were constructed on the natural log scale. Exponentiation was performed on the means (mean differences and lower and upper limits of these CIs) prior to reporting.

We used multiple variable linear regression models to assess the effect of efavirenz, lopinavir/ritonavir and atazanavir/ritonavir on the $AUC_{0\rightarrow t}$, C_{max}, and $t_{1/2}$ of atovaquone and proguanil. Potential confounding parameters that were assessed were race, age, smoking behaviour, body weight, CYP2C19 genotype, and body height. We used a stepwise selection procedure, by which a parameter was identified as a confounder if its addition to the model resulted in >10% change of the regression coefficient of efavirenz, lopinavir/ritonavir or atazanavir/ritonavir.

To determine the effect of the CD4 count, the HIV-1 RNA viral load and the specific nucleoside analogue reverse transcriptase inhibitor (NRTI) backbone on atovaquone/proguanil plasma exposure, we used linear regression models with the natural logarithm of the $AUC_{0\rightarrow t}$ of atovaquone or proguanil as the dependent variable and the CD4 count (per 100 cells/µL increase), the viral load status (using a viral load <40 copies/mL as reference) and the NRTI backbone as independent variables.

Plasma concentrations of the antiretroviral drugs on days 1 and 8 were compared by paired-samples t-tests after natural log transformation of the data.

Statistical evaluations were carried out using SPSS for Windows, version 16.0.1 (SPSS, Chicago, IL, USA).

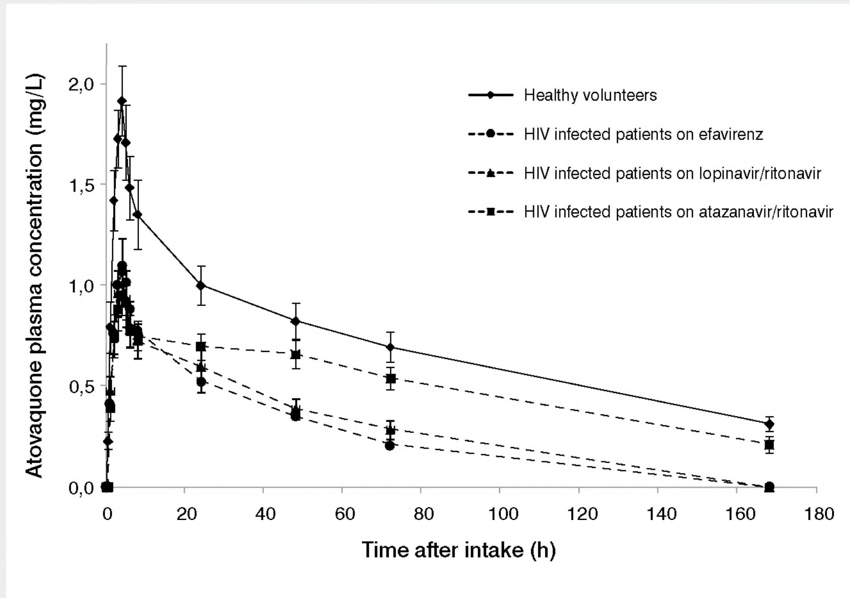
Results

Baseline characteristics

A total of 79 subjects were included: 20 healthy volunteers and 59 HIV-infected patients who were treated with efavirenz (n=20), lopinavir/ritonavir (n=20) or atazanavir/ritonavir (n=19). The blood samples of two healthy volunteers were lost due to an unfortunate accident. In addition, one patient on lopinavir/ritonavir was excluded from statistical evaluations because of unapproved concomitant use of phenytoin, a well-known enzyme inducer. Thus, 76 subjects were evaluable for statistical evaluation.

In Table 1, baseline characteristics are compared between the healthy volunteers and the three groups of HIV-infected patients. Eleven of these patients used lopinavir/ritonavir tablets in a dosage of 400/100 mg BD; eight patients used lopinavir/ritonavir tablets in a dosage of 800/200 mg QD. Compared to the healthy

Figure 1 Atovaquone plasma concentration-time curves after a single dose of atovaquone/proguanil.



Data are presented as arithmetic mean + standard error of the mean.

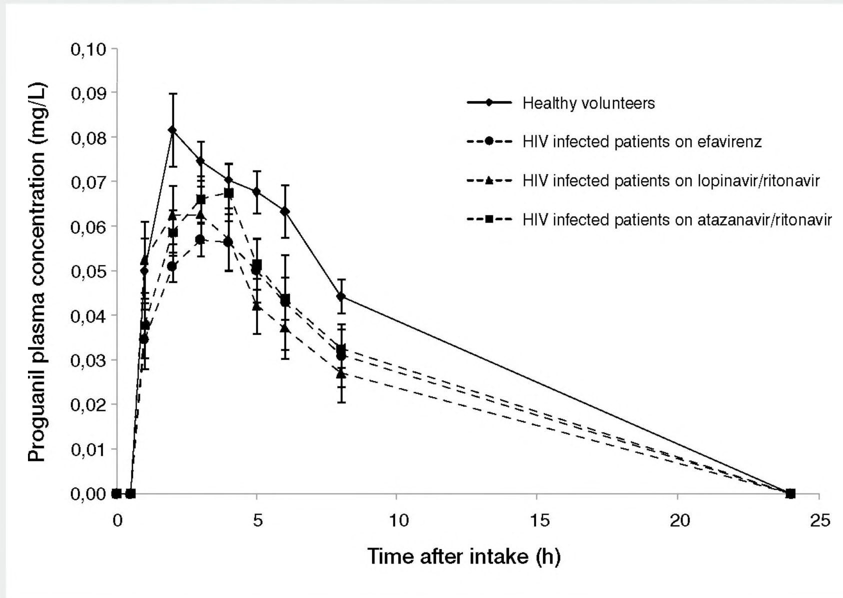
volunteers, there were more males and smokers among all categories of HIV-infected patients. In addition, the healthy volunteers were generally younger than the HIV-infected patients.

Pharmacokinetics of atovaquone / proguanil

Because there were no significant differences in any of the pharmacokinetic parameters of atovaquone or proguanil between patients who took lopinavir/ ritonavir QD or BD, the group of patients who took lopinavir/ritonavir was regarded as one single group in the statistical analyses.

Figure 1 and figure 2 show the atovaquone and proguanil plasma concentration-time curves, respectively, after a single dose of atovaquone/proguanil. The single-dose pharmacokinetic parameters derived from plasma concentration-time data are summarized in Table 2.

Figure 2 Proguanil plasma concentration-time curves after a single dose of atovaquone/proguanil.



Data are presented as arithmetic mean + standard error of the mean.

Table 1 Baseline characteristics.

		Total		Healthy volunteers	
		n	%	n	%
All study participants		76	100.0	18	23.7
Gender					
	Male	61	80.3	10	55.6
	Female	15	19.7	8	44.4
Race					
	Caucasian	59	77.6	15	83.3
	Non-Caucasian	17	22.4	3	16.7
HIV transmission risk group					
	Homosexual	39	67.2	NA	-
	Heterosexual	14	24.1	NA	-
	IDU	2	3.4	NA	-
	Other	3	5.2	NA	-
NRTI backbone					
	TDF + FTC	17	29.3	NA	-
	TDF + 3TC	22	37.9	NA	-
	AZT + 3TC	7	12.1	NA	-
	ABC + 3TC	7	12.1	NA	-
	Other	5	8.6	NA	-
Smoking status					
	Non-smoker	48	63.2	16	88.9
	Smoker	28	36.8	2	11.1

HIV-infected patients on efavirenz		HIV-infected patients on lopinavir/ ritonavir		HIV-infected patients on atazanavir/ ritonavir		
n	%	n	%	n	%	p-value
20	26.3	19	25.0	19	25.0	
						0.025†
19	95.0	16	84.2	16	84.2	
1	5.0	3	15.8	3	15.8	
15	75.0	15	78.8	14	73.7	0.93†
5	25.0	4	21.1	5	26.3	
16	80.0	12	63.2	11	57.9	0.22†
4	20.0	3	15.8	7	36.8	
-	-	2	10.5	-	-	
-	-	2	10.5	1	5.3	
8	40.0	2	10.5	7	36.8	0.032†
10	50.0	6	31.6	6	31.6	
2	10.0	4	21.1	1	5.3	
-	-	3	15.8	4	21.1	
-	-	4	21.1	1	5.3	
						0.028††
9	45.0	10	52.6	13	68.4	
11	55.0	9	47.4	6	31.6	

Viral load at screening											
<40 copies/mL	44	75.9	NA	-	17	85.0	13	68.4	14	73.7	0.47†
>40 copies/mL	14	24.1	NA	-	3	15.0	6	31.6	5	26.3	
CYP2C19 genotype											
Ultra-rapid metabolizers (*17/*17)	6	7.9	1	5.6	3	15.0	1	5.3	1	5.3	0.24†
Heterozygous ultra-rapid metabolizers (*1/*17)	18	23.7	2	11.1	8	40.0	4	21.1	4	21.1	
Extensive metabolizers (*1/*1)	26	34.2	9	50.0	4	20.0	9	47.4	4	21.1	
Mixed genotype (*2/*17)	6	7.9	1	5.6	2	10.0	2	10.5	1	5.3	
Intermediate metabolizers (*1/*2, *1/*3)	17	22.4	5	27.8	2	10.0	3	15.8	7	36.8	
Poor metabolizers (*2/*2)	1	1.3	-	-	1	5.0	-	-	-	-	
Unknown	2	2.6	-	-	-	-	-	-	2	10.5	
	Median	**IQR**	**Median**	**IQR**	**Median**	**IQR**	**Median**	**IQR**	**Median**	**IQR**	
Age (years)	46	32-51	23	20-44	47	38-50	48	45-52	49	35-51	0.001*
CD4 count (/mm^3)	450	338-655	ND	ND	465	318-613	370	260-720	450	400-730	0.57*
Weight (kg)	73	66-80	70	64-77	78	67-83	73	67-78	70	67-79	0.38*
Height (cm)	180	171-184	179	170-181	181	176-186	180	174-185	179	170-181	0.21*

† Fisher's Exact Test; †† Chi-Squared Test; * Kruskal-Wallis Test; IQR, interquartile range; IDU, intravenous drugs use; NA, not applicable; ND, not determined; NRTI, nucleoside analogue reverse transcriptase inhibitor; TDF, tenofovir; FTC, emtricitabine; 3TC, lamivudine; AZT, zidovudine; ABC, abacavir.

Table 3 shows the geometric mean ratios of the pharmacokinetic parameters of atovaquone and proguanil obtained after adjustment for factors that confounded (>10%) the observed effect of efavirenz, lopinavir/ritonavir and atazanavir/ritonavir on the pharmacokinetic parameters of atovaquone and proguanil. Compared to the healthy volunteers, HIV-infected patients who used efavirenz or lopinavir/ritonavir had substantially lower plasma exposure to atovaquone, while patients on atazanavir/ritonavir had modestly lower atovaquone exposure. The $AUC_{0\rightarrow t}$ of proguanil was 38-43% lower in the three groups of HIV-infected patients versus the healthy controls (see Table 3).

Effect of HIV-specific parameters

The CD4 count (β=0.030, p=0.48), nor the viral load status (β= -0.146, p=0.51) nor any of the NRTI backbones were associated with plasma exposure to atovaquone. The same analyses for proguanil yielded similar results.

Plasma concentrations of the antiretroviral drugs

There were no patients with undetectable plasma concentrations of efavirenz, lopinavir or atazanavir. The geometric mean mid-dose efavirenz concentration was 2.1 mg/L at both day 1 and day 8 (p=0.52). The geometric mean plasma concentrations for lopinavir and atazanavir were 4.1 mg/L and 1.1 mg/L on day 1, respectively, compared to 4.3 mg/L (p=0.88) and 1.2 mg/L (p=0.46) on day 8, respectively.

Adverse events and safety assessments

The single dose of atovaquone/proguanil was well tolerated. Six subjects reported a total of 8 non-serious adverse events. The majority of these events (n=7, 88%) were classified as grade I; the remaining event, namely transient diarrhea on the 4th day after the single dose of atovaquone/proguanil, was classified as grade II. Only one adverse event was considered possibly or probably related to the single dose of atovaquone/proguanil: a patient who was treated with lopinavir/ritonavir, zidovudine and lamivudine had a transient headache in the afternoon of study day 1, which did not require additional treatment.

Table 2 Pharmacokinetic parameters of atovaquone and proguanil after a single dose of atovaquone/proguanil (250/100mg).

Pharmacokinetic parameter	Healthy volunteers (n=18)		HIV-infected patients on efavirenz (n=20)		HIV-infected patients on lopinavir/ritonavir (n=19)		HIV-infected patients on atazanavir/ritonavir (n=19)	
Atovaquone	GM	95% CI	GM	95% CI	GM	95% CI	GM	95% CI
$AUC_{0\rightarrow t}$ (h*mg/L)	103.6	(79, 137)	29.6	(23, 39)	31.7	(24, 42)	64.3	(49, 84)
C_{max} (mg/L)	1.80	(1.4, 2.3)	1.06	(0.84, 1.3)	1.13	(0.90, 1.4)	1.02	(0.81, 1.3)
$T_{1/2}$ (h)	93.6	(74, 118)	48.2	(38, 62)	47.7	(37, 62)	90.6	(72,114)
Tmax (h)[a]	4.0	3-5	4.0	3-5	4.0	2-5	4.0	3-5
Proguanil	GM	95% CI	GM	95% CI	GM	95% CI	GM	95% CI
$AUC_{0\rightarrow t}$ (h*mg/L)	0.62	(0.44, 0.87)	0.32	(0.23, 0.44)	0.38	(0.27, 0.53)	0.39	(0.28, 0.55)
C_{max} (mg/L)	0.09	(0.08, 0.11)	0.07	(0.05, 0.08)	0.07	(0.06, 0.07)	0.07	(0.06, 0.09)
$T_{1/2}$ (h)	5.4	(3.9, 7.6)	4.5	(3.4, 6.0)	6.0	(4.3, 8.4)	3.8	(2.7, 5.2)
T_{max} (h)[a]	3.0	2-4	3.0	2-4	2.0	1-3	3.0	2-4

n, number of subjects; GM, geometric mean; CI, confidence interval;
[a] Median and interquartile range reported for T_{max}

Table 3 Unadjusted and adjusted geometric mean ratios (GMRs) of the pharmacokinetic parameters of atovaquone and proguanil in HIV-infected patients treated with efavirenz, lopinavir/ritonavir, or atazanavir/ritonavir versus those obtained in healthy volunteers.

Pharmacokinetic parameters	**HIV-infected patients on efavirenz**		**HIV-infected patients on lopinavir/ ritonavir**		**HIV-infected patients on atazanavir/ritonavir**	
Atovaquone	GMR (95% CI)	Adjusted GMR (95% CI)	GMR (95% CI)	Adjusted GMR (95% CI)	GMR (95% CI)	Adjusted GMR (95% CI)
$AUC_{0\to t}$ (h*mg/L)	0.29 (0.20-0.42)	0.25[a] (0.16-0.38)	0.31 (0.21-0.45)	0.26[a] (0.17-0.41)	0.62 (0.42-0.91)	0.54[a] (0.35-0.83)
C_{max} (mg/L)	0.59 (0.43-0.82)	0.56[b] (0.39-0.80)	0.63 (0.45-0.88)	0.56[b] (0.39-0.82)	0.57 (0.41-0.79)	0.51[b] (0.36-0.73)
$T_{1/2}$ (h)	0.51 (0.37-0.72)	0.58[c] (0.41-0.83)	0.51 (0.36-0.72)	0.53[c] (0.36-0.78)	0.97 (0.70-1.34)	1.05[c] (0.75-1.48)
Proguanil	GMR (95% CI)	Adjusted GMR (95% CI)	GMR (95% CI)	Adjusted GMR (95% CI)	GMR (95% CI)	Adjusted GMR (95% CI)
$AUC_{0\to t}$ (h*mg/L)	0.51 (0.32-0.82)	0.57[d] (0.35-0.93)	0.61 (0.38-0.99)	0.62[d] (0.39-0.99)	0.64 (0.40-1.03)	0.59[d] (0.38-0.93)
C_{max} (mg/L)	0.73 (0.58-0.92)	0.89[e] (0.70-1.14)	0.79 (0.62-1.00)	0.88[e] (0.70-1.10)	0.79 (0.66-1.00)	0.83[e] (0.66-1.05)
$T_{1/2}$ (h)	0.83 (0.54-1.30)	0.90[f] (0.55-1.48)	1.11 (0.69-1.79)	1.12[f] (0.67-1.88)	0.69 (0.43-1.12)	0.60[f] (0.36-0.99)

GMR, geometric mean ratio; [a] adjusted for age; [b] adjusted for age and body weight; [c] adjusted for age, body weight and gender; [d] adjusted for CYP2C19 genotype, body height and smoking status; [e] adjusted for CYP2C19 genotype, body height, body weight and gender; [f] adjusted for CYP2C19 genotype, age, race, and gender.

Discussion

The objective of our study was to compare atovaquone/proguanil pharmacokinetics in healthy volunteers to those in HIV-infected patients who were treated with efavirenz, lopinavir/ritonavir or atazanavir/ritonavir, respectively. The differences in atovaquone exposure appeared to be substantial. HIV-infected patients who used efavirenz or lopinavir/ritonavir had on average 75% lower exposure to atovaquone compared to the healthy controls, while patients on atazanavir/ritonavir had 40-50% lower exposure to atovaquone.

It is not possible to establish from our study the exact mechanism behind the lower atovaquone plasma concentrations in the three groups of HIV-infected patients. The mechanism might be increased atovaquone glucuronidation (4), although there are only indirect data that suggest that atovaquone may be metabolized by glucuronidation (12-14). The lower atovaquone exposure in patients treated with lopinavir/ritonavir compared to those treated with atazanavir/ritonavir is in line with such a mechanism, because lopinavir/ritonavir seems to have stronger inductive effects on glucuronidation enzymes than atazanavir/ritonavir (5;7;15).
Another potential mechanism might be a pharmaceutical interaction in the gastro-intestinal tract, caused by simultaneous intake of the antiretroviral agents and atovaquone/proguanil. However, this is unlikely because atovaquone/proguanil peak plasma concentrations did not differ significantly in patients who took atovaquone/proguanil and antiretroviral medication simultaneously (within a time frame of 3 hours) and those who did not (e.g., patients who took atazanavir/ritonavir in the evening) (data not shown).

Plasma exposure to proguanil was on average 38-43% lower in the three groups of HIV-infected patients. Proguanil, which bolsters atovaquone activity (16), is predominantly metabolized by CYP2C19. Lopinavir/ritonavir and efavirenz may induce CYP2C19 (17;18), which may explain the lower proguanil exposure in these groups. In addition, patients treated with atazanavir/ritonavir had reduced proguanil exposure, which might be due to CYP2C19 induction by ritonavir, atazanavir or both.

Despite the substantially lower atovaquone plasma exposure observed in our study, no clinical reports have been published so far that describe atovaquone/proguanil chemoprophylaxis failure in HIV-infected travellers treated with ritonavir boosted PIs or NNRTIs. In addition, there are no established minimum effective atovaquone plasma concentrations in the setting of malaria prophylaxis, which makes it difficult

to assess the precise clinical relevance of decreased atovaquone plasma concentrations. Nevertheless, the difference in atovaquone plasma exposure with the healthy volunteers was substantial. Therefore, physicians should be alert for atovaquone/proguanil prophylaxis failures in HIV-infected patients treated with efavirenz, lopinavir/ritonavir or atazanavir/ritonavir.

We did not choose for a design in which therapy naïve HIV-infected patients served as a reference group, because the HIV-physicians at our clinics felt it would be very difficult to motivate this group of patients to participate in our trial. Because of the chosen design, it remains unknown whether HIV-infection itself resulted in lower atovaquone plasma concentrations, which is a limitation.
On the other hand: our study mimics the 'real world situation'. HIV-infected patients treated with antiretroviral regimens travel to malaria endemic areas like healthy people do, and phase 3 trials of atovaquone/proguanil for malaria prophylaxis were only conducted in healthy subjects (1).

Another potential limitation of our study is that we used a single dose design for atovaquone/proguanil. However, as shown by Thapar *et al.* (19), the $AUC_{0\rightarrow\infty}$ of atovaquone after a single dose of Malarone® was predictive of the $AUC_{0\rightarrow\tau}$ of atovaquone at steady state (19). Therefore, we judged it would be possible to obtain our main objectives using a single dose design, which appeared to be safe and convenient for the participating subjects.

In summary, our study shows considerably lower plasma exposure to atovaquone in HIV-infected patients treated with efavirenz or lopinavir/ritonavir, and modestly lower plasma exposure to atovaquone in those treated with atazanavir/ritonavir, compared with a group of healthy volunteers. In addition, plasma concentrations of proguanil, which bolsters atovaquone activity, were modestly reduced in patients treated with efavirenz, lopinavir/ritonavir or atazanavir/ritonavir.
Therefore, physicians should be alert for atovaquone/proguanil prophylaxis failures in HIV-infected patients treated with efavirenz, lopinavir/ritonavir or atazanavir/ritonavir. We recommend emphasizing adherence to atovaquone/proguanil in HIV-infected travellers treated with these drugs, i.e., strict daily intake during the main meal. In addition, an increase of the dose of atovaquone/proguanil should be considered in patients treated with efavirenz or lopinavir/ritonavir.

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7

Chapter

The effect of raltegravir on the glucuronidation of lamotrigine

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Abstract

We studied the effect of raltegravir on the pharmacokinetics of the anti-epileptic agent lamotrigine. Twelve healthy volunteers (group A) received 400 mg of raltegravir twice-daily from day 1-5. On day 4, a single dose of 100 mg of lamotrigine was administered. After a wash-out period, subjects received a second single dose of 100 mg of lamotrigine but now without raltegravir (day 32). In group B, 12 subjects received the same treatment as in group A but in reverse order. On days 4 and 32, 48-hour pharmacokinetic curves were drawn. Geometric mean ratios (+90% confidence intervals (CIs) of lamotrigine area under the plasma concentration-time curve ($AUC_{0\rightarrow48}$) and peak plasma concentration (C_{max}) for raltegravir + lamotrigine versus lamotrigine alone were 0.99 (0.96-1.01) and 0.94 (0.89-0.99), respectively. The mean ratio of the $AUC_{0\rightarrow48}$ of lamotrigine-2N-glucuronide to lamotrigine was similar when lamotrigine was taken alone (0.35) or when taken with raltegravir (0.36). Raltegravir does not influence the glucuronidation of lamotrigine.

Introduction

Raltegravir is a newly developed antiretroviral drug that acts by targeting the HIV-1 integrase, thereby preventing the integration of HIV-DNA into the genome of the human host-cell. Raltegravir has demonstrated potent antiretroviral efficacy and is generally well tolerated (1;2). Raltegravir is metabolized in the liver by UDP-glucuronosyltransferase 1A1 (UGT1A1). As a consequence, its pharmacokinetics can be influenced by inhibitors (e.g., atazanavir) or inducers (e.g., etravirine, tipranavir, rifampicin) of UGT1A1 (3-6).

In contrast to protease inhibitors and non-nucleoside transcriptase inhibitors, raltegravir does not inhibit or induce Cytochrome P_{450} (CYP_{450}) enzymes (7). Limited data are available on the influence of raltegravir on substances that share raltegravir's metabolic pathway: glucuronidation. In vitro studies suggest that raltegravir does not potently inhibit (IC_{50} >50 μM) UGT1A1 and UGT2B7 enzymes (8). However, other UGT sub enzymes, such as UGT1A4, have not been evaluated and there are no clinical studies that support the in vitro data.

An example of a UGT-substrate which may be prescribed to HIV-infected patients is the anti epileptic agent lamotrigine, which is hepatically metabolized to lamotrigine-2N-glucuronide (9). Lamotrigine is one of the recommended anti-epileptic agents for the management of seizures in HIV-infected patients (10). Seizures are not rare in HIV-infected patients: retrospective studies indicate that 2-20% of the HIV-infected patients will have seizures at some time during their illness (11). In addition, lamotrigine is one of the few drugs with proven efficacy in the treatment of HIV-associated neuropathic pain (12).

In previous studies, we demonstrated that lopinavir/ritonavir reduces plasma exposure to lamotrigine, by approximately 50% (13). The combination of atazanavir/ritonavir is also able to induce glucuronidation of lamotrigine as demonstrated in a subsequent trial (14). The decrease in lamotrigine exposure (32%), however, was less pronounced as with lopinavir/ritonavir; in addition, this trial showed that atazanavir alone did not influence lamotrigine pharmacokinetics, suggesting that the effect from atazanavir/ritonavir is mainly caused by ritonavir.

These two trials demonstrate that the conversion of lamotrigine to lamotrigine-2N-glucuronide is an appropriate marker to evaluate the effect of concomitant medications on the glucuronidation of lamotrigine. Given the unknown effect of

raltegravir on the glucuronidation of UGT-substrates in vivo, we studied the effect of raltegravir on the pharmacokinetics of lamotrigine.

Methods

Study design

This open-label, randomized, two-period, cross-over, single-centre, phase-I, multiple-dose trial was conducted in March and April 2008 at the Radboud University Nijmegen Medical Centre (Nijmegen, The Netherlands). The study was designed to investigate the effect of raltegravir on the pharmacokinetics of lamotrigine and lamotrigine-2N-glucuronide as determined by intrasubject comparison. Secondary objectives were to assess the effect of lamotrigine on the pharmacokinetics of raltegravir when compared to historical controls and to evaluate the safety of combined use of lamotrigine and raltegravir.

Twenty-four male subjects were randomized to either group A or group B. In group A, 12 subjects received 10 oral doses of 400 mg of raltegravir twice daily (BD) during the first period of 5 days. On day 4 (together with the 7th dose of raltegravir), a single dose of 100 mg of lamotrigine was administered. After a wash-out period of 26 days (study days 6 to 31), all subjects received a second single dose of 100 mg of lamotrigine (day 32). In group B, 12 subjects received the same treatments in reverse order.

The trial was approved by the Review Board of the Radboud University Nijmegen Medical Centre.

Study population

The trial was conducted in healthy young men aged between 18 and 55 years on the day of first dosing. For inclusion in the trial, subjects had to be in a good, age-appropriate health condition as established by physical examination, medical history, electrocardiography and biochemical, hematologic, and urinalysis testing within 4 weeks before the first dose. Subjects had to be able and willing to sign the Informed Consent Form before screening evaluations.

The main exclusion criteria were as follows: a history of sensitivity or idiosyncrasy to medicinal products or excipients; a positive HIV, hepatitis B or C test result and therapy with any drug (for 2 weeks preceding dosing), except for acetaminophen.

Study drug and dosing

The raltegravir dosage that was used in this trial (400 mg twice-daily with or without food) is the recommended dosage for raltegravir (8;15). At the days of pharmacokinetic sampling, both raltegravir and lamotrigine were taken on an empty stomach because at the time of study design, available data on raltegravir pharmacokinetics were obtained in the absence of food (5). Fasting (no food, no fluid) was continued until 2 hours after dosing, followed by a standardized breakfast. Previous work demonstrated that steady-state conditions for raltegravir are present after 2 to 3 days of chronic dosing (16). Therefore, the single dose of lamotrigine was administered on the fourth day of raltegravir administration. We used single doses of lamotrigine to minimize the risk of rash (13;14).

Safety assessments and pharmacokinetic sampling

Blood samples for pharmacokinetics were collected throughout a 48-hour period (0, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 24 and 48 hours) after dosing on day 4 and 32 for lamotrigine and lamotrigine-2N-glucuronide to characterize drug absorption, distribution and elimination. Blood samples for pharmacokinetics of raltegravir were collected during an 8-hour period (0, 0.5, 1, 1.5, 2, 3, 4, 5, 6, and 8 hours) after dosing raltegravir on day 4 (group A) or day 32 (group B). In addition, blood samples were collected to determine trough levels of raltegravir, just before intake of the drug on day 1, 2, and 4 (group A); or day 29, 30 and 32 (group B).

Serum biochemistry and hematology test results were checked on days 1, 2, 4, 6, 29, 30, 32 and 34. Adverse events were assessed during the same visits and on days 5 and 33. Screening for drugs of abuse was performed in urine on days 1 and 29; urinalysis was carried out on days 4 and 32.

Compliance

Study personnel supervised all intake of medication at the clinical trial unit. The exact times of dosing were recorded. Drug intake at home was monitored by use of microelectronic monitoring system (MEMS) caps (Aardex Ltd, Zug, Switzerland), which record the opening of the medication bottle. Furthermore, pill counts and trough level measurements at days 2, 4, 30, and 32 were used to assess adherence. Finally, subjects were asked to write down the exact times of medication intake in a booklet.

Bioanalysis of raltegravir, lamotrigine and lamotrigine-2N-glucuronide in plasma

Plasma concentrations of lamotrigine and lamotrigine-2N-glucuronide were analyzed by use of a validated reversed-phase high-performance liquid chromatography method (13). The accuracy values for lamotrigine were 103%, 103%, and 104% for concentrations of 0.358, 1.79, and 11.94 mg/L, respectively. At these same concentrations, the precision values (within-day, coefficient of variation) were 2.34%, 1.82%, and 1.87%, respectively. For lamotrigine-2N-glucuronide, the accuracy values were 99%, 100%, and 99% at concentrations of 0.218, 1.09, and 7.25 mg/L, respectively. The precision values (within-day, coefficient of variation) were 4.08%, 2.43%, and 1.06%, respectively, for the same concentrations.

Plasma concentrations of raltegravir were analyzed by means of liquid-liquid extraction followed by reversed-phase HPLC with fluorescence detection. In brief, to 500 μL of plasma was added: 500 μL acetate buffer (pH 4.0, 0.2 M); 5 mL hexane:dichloromethane 1:1 (v/v); and 50 μL of internal standard (lormetazepam in methanol: water 1:1 (v/v)). The sample was mixed on a vortex mixer for 5 minutes, followed by centrifugation at 11,000 rpm for 5 minutes. Afterwards, the organic phase was evaporated at 37^{0}C under a gentle stream of nitrogen gas, and reconstituted in 200 μL of eluens (acetonitrile: phosphate buffer (pH 4.80, 20 mM) (35:65 v/v)). Forty μL of the resulting solution was run on a 10-cm Symmetry Shield reversed phase C18 column (flow rate 1.5 mL/min) and raltegravir was detected by use of a fluorescence detector ($\lambda_{excitation}$ 240 nm, $\lambda_{emission}$ 412 nm).
The accuracy values for raltegravir were 99%, 101% and 97% at 0.050, 0.140 and 0.500 mg/L, respectively. At the same concentrations, the precision values (within day, coefficient of variation) were 2.4%, 2.5% and 1.9%, respectively. The calibration curve was linear over a concentration range of 0.014 to 1.40 mg/L.

Pharmacokinetic analysis

Pharmacokinetic parameters for lamotrigine, lamotrigine-2N-glucuronide and raltegravir were calculated by non-compartmental methods using the WinNonlin software package (version 4.1; Pharsight, Mountain View, CA) and the log/linear trapezoidal rule. Based on the individual plasma concentration-time data, the following pharmacokinetic parameters of lamotrigine were determined: the area under the plasma concentration-time curve from 0 to 48 hours after intake ($AUC_{0\rightarrow48}$; in hour*milligram/liter), the maximum plasma concentration of the drug (C_{max}; in milligrams per liter), the time to reach C_{max} (t_{max}; in hours), and the apparent elimination half-life ($t_{1/2}$; in hours).

For raltegravir the same pharmacokinetic parameters were calculated. To be able to compare raltegravir AUC with historical data, we calculated steady state $AUC_{0\rightarrow12}$ by extrapolation to 12 hours.

Sample size and statistical analysis

The study was powered to detect a 20% difference in lamotrigine AUC. In our previous single dose lamotrigine trial, the intersubject coefficient of variation in lamotrigine AUC was 22.2% (14). With a conservative approach, we assumed intrasubject variability to be equal to intersubject variability and we calculated the required number of participants as 20. With an estimated dropout rate of 15%, 24 subjects were included in the trial to ensure complete data from 20 subjects.

For the identification of a clinically relevant drug interaction, we used the bioequivalence approach (17). Geometric mean ratios (GMRs) with 90% confidence intervals (CIs) were calculated for $AUC_{0\rightarrow48}$, C_{max} and $t_{1/2}$ after log transformation of within-subject ratios. GMRs with 90% CIs falling entirely within the range of 0.80 to 1.25 were considered to indicate no significant interaction.

We calculated AUC ratios of lamotrigine-2N-glucuronide vs. lamotrigine obtained by use of lamotrigine alone and by use of lamotrigine in combination with raltegravir to determine the effect of raltegravir on the glucuronidation of lamotrigine. To check whether auto induction of lamotrigine metabolism might have influenced our results, we also compared the AUC ratios of lamotrigine-2N-glucuronide and lamotrigine in subjects who took lamotrigine alone on day 32 (group A) to those obtained in subjects who took lamotrigine alone on day 4 (group B).

Statistical evaluations were carried out using SPSS for Windows, version 16.0.1 (SPSS, Chicago, IL, 1989-2005).

Results

Baseline characteristics

Twenty-four healthy male subjects were included in this trial. The mean (range) age, body weight and body mass index were 34 (20-52) years, 79 (63-94) kg and 24 (20-28) kg/m^2, respectively. There was one black subject and one hispanic subject; the other subjects were Caucasians. There were no dropouts: all subjects completed the trial and were available for statistical analyses.

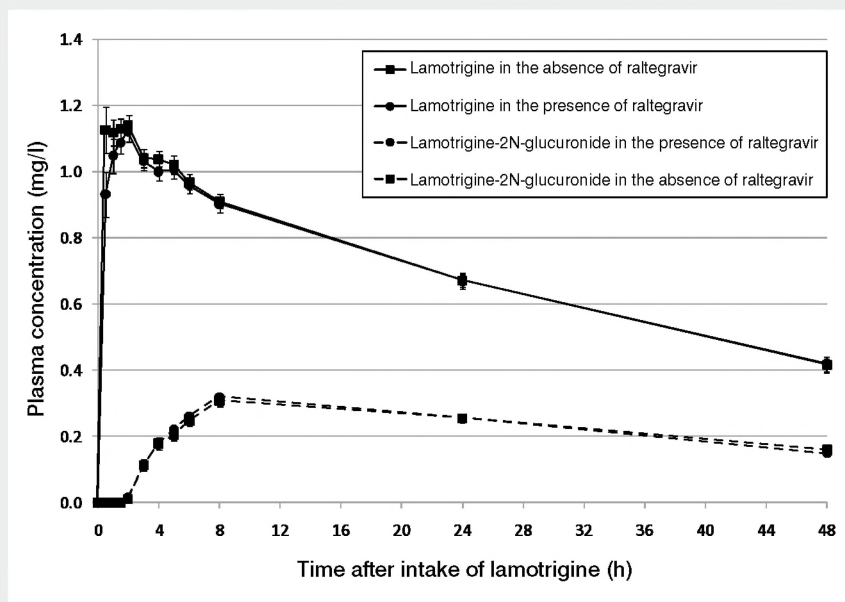
Compliance

The compliance of all 24 subjects was good, as indicated by their statements about the intake of the drug doses, the number of tablets in the returned vials, the raltegravir trough concentrations, the booklets, and the MEMS caps (data not shown).

Pharmacokinetics

All included subjects completed day 34 of the trial and were included for statistical evaluation. Figure 1 shows the lamotrigine and lamotrigine-2N-glucuronide plasma concentration versus time curves obtained in the presence and absence of steady-state raltegravir. The mean pharmacokinetic parameters of lamotrigine are shown in Table 1. Raltegravir did not appear to influence the pharmacokinetics of single-dose lamotrigine. The geometric mean ratios of lamotrigine $AUC_{0\rightarrow48}$, C_{max}

Figure 1 Arithmetic mean plasma concentrations of lamotrigine and lamotrigine-2N-glucuronide following the administration of a single dose of 100 mg of lamotrigine in the presence and absence of raltegravir.



Error bars represent the standard error of the mean.

Table 1 Comparison of lamotrigine pharmacokinetics following administration of a single dose of 100 mg of lamotrigine in the presence or absence of co-administration of multiple doses of 400 mg raltegravir twice daily to healthy male subjects.

Pharmacokinetic parameter	Lamotrigine alone		Lamotrigine + Raltegravir		Lamotrigine + Raltegravir: Lamotrigine alone	
	Geometric mean	95% CI for geometric mean	Geometric mean	95% CI for geometric mean	GMR	90% CI for GMR
$AUC_{0\rightarrow48}$ (h*mg/L)	33.1	(31.3, 35.0)	32.6	(30.7, 34.7)	0.99	(0.96, 1.01)
C_{max} (mg/L)	1.28	(1.20, 1.37)	1.20	(1.13, 1.28)	0.94	(0.89, 0.99)
$t_{1/2}$ (h)	35.0	(31.0, 39.5)	36.0	(31.9, 40.7)	1.03	(0.97, 1.09)

AUC, area under the plasma concentration-time curve; C_{max}, peak plasma concentration; $t_{1/2}$, elimination half-life; GMR, geometric mean ratio; CI, confidence interval.

and $t_{1/2}$ for lamotrigine + raltegravir vs. lamotrigine alone were are all close to 1.0 and the corresponding 90% CIs were within the predefined interval of 0.80 - 1.25, indicating no interaction occurred (see Table 1). In agreement with this observation, the mean (SD) ratio of the $AUC_{0\rightarrow48}$ of lamotrigine-2N-glucuronide / lamotrigine was similar when lamotrigine was taken alone or when taken with raltegravir.
The geometric mean (95% CI) $AUC_{0\rightarrow48}$ of lamotrigine-2N-glucuronide was 11.0 (10.1-12.0) h*mg/L after intake of 100 mg of lamotrigine alone, leading to a mean (SD) AUC ratio of metabolite vs. parent compound of 0.35 (0.08). The mean (SD) ratio was 0.36 (0.10) when lamotrigine was taken in the presence of steady state raltegravir (p=0.35, paired samples t-test).

The mean (SD) AUC ratios of lamotrigine-2N-glucuronide vs. lamotrigine were 0.37 (0.07) and 0.32 (0.08) in subjects who took lamotrigine alone on day 4 and day 32, respectively (p=0.16, independent samples t-test), indicating no period effect occurred. In addition, the elimination half-life of lamotrigine was not significantly different in subjects who took lamotrigine alone on day 4 vs. on day 32: the mean elimination half-lives were 37.0 and 36.0 hours, respectively (p=0.83, independent samples t-test).

Table 2 Pharmacokinetic parameters of raltegravir when compared to historical controls.

Pharmacokinetic parameter	This study (n = 23[c])	Wenning et al.(18) (n=9)	Anderson et al.(6) (n = 19)
$AUC_{0\rightarrow12}$ (h*mg/L)	3.9 (2.7, 5.7)[a]	5.0[b]	3.4 (2.4, 4.7)[b]
C_{max} (mg/L)	1.2 (0.8, 1.8)	1.4 (0.8, 2.3)[b]	0.8 (0.6, 1.2)[b]
T_{max} (h)	1.5 (0-8)	1.5	1.5 (0-12)

AUC, area under the plasma concentration-time curve; C_{max}, peak plasma concentration; T_{max}, time to reach C_{max}.
Data are geometric means + 95% confidence intervals, except for T_{max} (median + range)

[a] The raltegravir $AUC_{0\rightarrow12}$ was obtained by extrapolation from the raltegravir $AUC_{0\rightarrow8}$

[b] Data for AUC and C_{max} from historical controls were converted from h*µM and µM to h*mg/L and mg/L, respectively, using the molecular weight of raltegravir (482.51 g/mol)(8).

[c] One subject was excluded from the pharmacokinetic analyses of raltegravir because raltegravir's half-life and thus $AUC_{0\rightarrow12}$ could not be determined reliably in this individual.

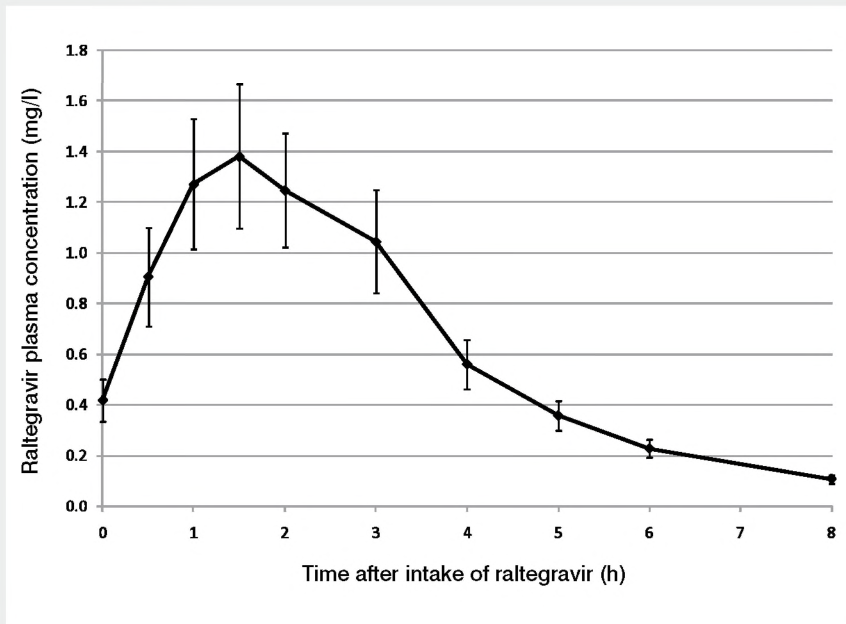
The arithmetic mean plasma raltegravir concentration-time curve following administration of raltegravir with lamotrigine is shown in Figure 2. One subject was excluded from the pharmacokinetic analyses of raltegravir, because raltegravir's half-life and thus $AUC_{0\rightarrow12}$ could not be determined reliably. The geometric mean of the $AUC_{0\rightarrow8}$ of raltegravir was 3.70 h*mg/L. Extrapolation in Winnonlin to the $AUC_{0\rightarrow12}$ resulted in a slightly higher (4.6%) geometric mean: 3.87 h*mg/L. The pharmacokinetic parameters of raltegravir, which are presented in Table 2, were similar to those of historical controls (6;18).
Raltegravir pharmacokinetics displayed large inter-individual variability: the coefficient of variation (CV) for raltegravir $AUC_{0\rightarrow8}$ was 77%. In addition, we observed that steady state raltegravir trough levels in the morning were 3 to 4 times higher than raltegravir levels obtained 8 hours after dosing (see Figure 2).

Adverse events and safety assessments

Study medication was generally well tolerated. Thirteen subjects reported a total of 37 non-serious adverse events. The majority of these events (n=25, 68%) were classified as grade I; the remaining 12 events were classified as grade II (n=11, 30%), or grade III (n=1, 3%).

Three adverse events were considered possibly or probably drug-related. One subject reported transient nausea on the first day of raltegravir administration, which disappeared within 6 hours without need of additional treatment. The other two adverse events were reported in one subject who developed a grade III ASAT elevation (385 U/L) and a grade II ALAT elevation (101 U/L) on day 6, following five days of raltegravir administration. The subject reported no complaints and ALAT and ASAT values returned to normal values within 10 days.

Figure 2 Arithmetic mean plasma raltegravir concentration profile following the administration of multiple doses of 400 mg raltegravir twice daily.



Error bars represent the standard error of the mean.

Discussion

The primary objective of this study was to determine the effect of raltegravir on the pharmacokinetics of single-dose lamotrigine. The lamotrigine pharmacokinetics clearly met the predefined bioequivalence criteria for no clinically relevant interaction. In addition, the mean ratio of the $AUC_{0\rightarrow48}$ of lamotrigine-2N-glucuronide to lamotrigine was similar when lamotrigine was taken alone (0.35) or when taken with raltegravir (0.36), which indicates that raltegravir does not inhibit or induce the glucuronidation of lamotrigine.

In our previous single dose lamotrigine study, the AUC ratio of lamotrigine-2N-glucuronide vs. lamotrigine was slightly higher (0.45) after the same dose of lamotrigine (14). This is probably caused by the longer sampling period in that study (120 hours vs. 48 hours in the current study) (14). Lamotrigine-2N-glucuronide has a longer elimination half-life than lamotrigine (see Figure 1). As a consequence, determining the $AUC_{0\rightarrow\infty}$ for both lamotrigine and lamotrigine-2N-glucuronide instead of the $AUC_{0\rightarrow48}$ which we did in the current study, will increase the absolute value of the AUC of lamotrigine-2N-glucuronide relatively more when compared to the AUC of lamotrigine, which results in a higher ratio of lamotrigine-2N-glucuronide versus lamotrigine.

Because raltegravir and lamotrigine share a common metabolic pathway, we hypothesized that raltegravir might inhibit lamotrigine glucuronidation, although results from in vitro experiments had indicated that raltegravir did not potently inhibit (IC_{50} >50 μM) UGT2B7-mediated glucuronidation. However, as explained by Kiang and colleagues (19), one must be cautious when predicting in vivo effects of UGT enzymes based on data obtained from in vitro experiments.

Moreover, there are several examples of drug-drug interactions between drugs that are both metabolized via glucuronidation, potentially based on hepatic competition for glucuronidation (20;21). Therefore, and because of the importance of drug-drug interactions in the management of seizures in HIV-infected patients (10), we felt it was appropriate to investigate raltegravir's influence on the glucuronidation of the UGT substrate lamotrigine in a clinical study.

In our previous lamotrigine study, we considered lamotrigine a phenotypic probe for UGT1A4 and possibly UGT2B7 substrates (14). Based on this view, we originally designed the current study as a 'phenotypic probe' study to investigate the influence

of raltegravir on UGT1A4 and UGT2B7 enzymes in vivo. Meanwhile, lamotrigine metabolism by UGT1A4 and UGT2B7 has become in part controversial: a recent publication suggests that lamotrigine is metabolized by UGT1A3 and UGT 1A4, but not by UGT2B7 (22). Thus, it is possible that lamotrigine is metabolized by at least three different UGT enzymes, which questions the appropriateness of using lamotrigine as a selective probe for UGT phenotyping. For instance, if raltegravir inhibits UGT1A4 enzymes and not UGT1A3 or UGT2B7, it is possible that lamotrigine is still glucuronidated at a similar velocity by using UGT1A3 and/or UGT2B7 as escape routes.

As depicted in Table 2, pharmacokinetic parameters of raltegravir were comparable to those of healthy volunteers in other studies, indicating no significant effect of a single dose of lamotrigine on raltegravir pharmacokinetics. Because assessing the effect of a single dose of lamotrigine on the pharmacokinetics of raltegravir was a secondary objective of our trial, we did not include a 12-hour pharmacokinetic sample in our study. We extrapolated the raltegravir $AUC_{0\rightarrow8}$ to $AUC_{0\rightarrow12}$ in order to compare our data to historical controls. Because the percentage of the AUC extrapolated was smaller than 5%, we do not expect the extrapolation to confound the comparison to the historical controls.
The pharmacokinetics of raltegravir were characterized by large interindividual variability (CV 77%), which was reported by others as well (23). Proposed explanations for large interindividual variability are differences in co-administration with food and concomitant medications (8). Nonetheless in our study, raltegravir was taken on an empty stomach and concomitant medications, except for lamotrigine, were not allowed. Since omeprazole increases raltegravir exposure by 321% (15), differences in gastric pH might have contributed to interindividual variability. Another contributing factor may be genetic polymorphism of UGT1A1, the enzyme that metabolizes raltegravir. Genetic polymorphism of UGT1A1 is relatively common: 7-19% of the Caucasian population is homozygous for UGT1A1*28, which leads to decreased UGT1A1 expression and reduced elimination of UGT1A1 substrates, such as irinotecan and raltegravir (19).

Raltegravir trough concentrations taken in the morning of study day 4 (i.e., around 8 AM), before drug intake for pharmacokinetic sampling, were on average 3 to 4 times higher than raltegravir concentrations obtained 8 hours after dosing (see Figure 2). Because this might have been caused by too late intake of the raltegravir evening dose before the day of pharmacokinetic sampling, we checked our MEMS data thoroughly. However, according to MEMS, all subjects opened the medication

bottle between 7:42 PM and 8:56 PM in the evening of day 3 or 31 (scheduled intake time 8:00 PM).
Further investigation learned that the same phenomenon can be noted in the work of Anderson et al. (6) In their paper, raltegravir (morning) trough concentrations were about 2-fold higher than C_{8h} or C_{12h} raltegravir concentrations. A possible explanation for these findings could lie in circadian variations in pharmacokinetics (24). For instance, acetaminophen glucuronidation occurs at a higher rate in the daytime compared to the night (25). Glucuronidation rates of raltegravir might vary in a similar way, although this hypothesis requires further investigation. Another factor that may have contributed to this phenomenon is that raltegravir morning C_{trough} levels may have been increased in subjects who took raltegravir with food at the evening before pharmacokinetic sampling days. The food effect on raltegravir is substantial (35% decrease in Cmax and 8.5-fold increase in Cmin), although not considered clinically relevant (26). Unfortunately, we cannot test this hypothesis, because we did not note whether subjects took raltegravir with or without food at home.

Combined use of single-dose lamotrigine and raltegravir was generally well tolerated. However, there was one subject with a reversible grade III ASAT and grade II ALAT elevation after 5 days of raltegravir administration. Lamotrigine as the causal agent seems unlikely in this case, because ASAT had already risen from 16 to 190 U/L between day 1 and day 4, i.e., before intake of lamotrigine. Indeed, hepatitis is among the serious drug-related adverse events of raltegravir, although its frequency is defined as 'uncommon' (≥ 1/1,000 to <1/100) (8;15).

This is the third study in which we studied the influence of antiretroviral drugs on the glucuronidation of lamotrigine. In the first trial we encountered a relatively high incidence (25%) of lamotrigine-related rashes (13), especially among women (42%). Therefore, we decided to include only male subjects in future trials with lamotrigine. In an attempt to further reduce the incidence of rash, we used single doses of lamotrigine instead of chronic dosing. The effect of these measures is satisfactory: rash occurred in only one subject in the second trial (4.8%) and it did not occur in the present trial.
In the first study that used single doses of lamotrigine, we used a sequential design, i.e., all subjects received a single dose of lamotrigine on day 1 (reference) and again on days 13 and 27, after administering atazanavir without and with ritonavir, respectively. In this situation, it could not be excluded that auto-induction of lamotrigine metabolism had confounded our results (9;14). Therefore, we modified

the design of the current study and used a randomized cross-over design with a wash out period of 26 days. In addition, we were now able to check whether our data were confounded by lamotrigine auto-induction, which clearly did not occur. Therefore, we consider the current study design optimal for testing whether drugs influence the glucuronidation of lamotrigine.

In conclusion, our study shows that raltegravir does not affect exposure to the UGT substrate lamotrigine.

Acknowledgements

We thank the healthy volunteers for participating in this trial. The technicians at the Department of Clinical Pharmacy, Radboud University Nijmegen Medical Centre, are kindly acknowledged for processing and analyzing the plasma samples of lamotrigine, lamotrigine-2N-glucuronide, and raltegravir. This study was funded by a research grant from Merck.

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8

Chapter

Drug–drug interactions between raltegravir and pravastatin in healthy volunteers

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Abstract

Background: To evaluate the potential drug-drug interaction between raltegravir and pravastatin.

Methods: This was an open-label, randomized, 3-period, cross-over, single-centre trial in 24 healthy volunteers. Subjects received the following treatments: pravastatin 40 mg q.d. for 4 days, raltegravir 400 mg b.d. for 4 days, and pravastatin 40 mg q.d. + raltegravir 400 mg b.d. for 4 days. The treatments were separated by wash-out periods of 10 days. On day 4 of each treatment period, blood samples for pharmacokinetics were collected throughout a 24-hour period.

Results: Geometric mean ratios (GMRs) (90% confidence interval (90% CI)) for pravastatin + raltegravir versus pravastatin alone were 0.96 (0.83–1.11) for $AUC_{0\rightarrow24}$ and 1.04 (0.85–1.26) for C_{max}. The mean low-density lipoprotein cholesterol decrease after 4 days of pravastatin was 0.42 mmol/L both in the presence and the absence of raltegravir. The GMR (90% CI) $AUC_{0\rightarrow12}$, C_{max}, and C_{12} for raltegravir + pravastatin versus raltegravir alone were 1.13 (0.77–1.65), 1.31 (0.81–2.13), and 0.59 (0.39–0.88), respectively.

Conclusion: Raltegravir did not influence the pharmacokinetics or the short-term lipid-lowering effects of pravastatin, whereas pravastatin increased the C_{max} but decreased the C_{12} of raltegravir. The effects of pravastatin on raltegravir pharmacokinetics are not likely to be clinically relevant.

Introduction

Highly active antiretroviral therapy (HAART) has changed HIV infection from a fatal disease to a manageable chronic medical condition. As a result, the HIV-infected population is becoming older (1). Among this aging population, risk factors for the development of cardiovascular disease are highly prevalent. These include HIV itself and the relatively high number of males and smokers (2;3).

In addition, the use of HIV-protease inhibitors and certain nucleoside analogue reverse-transcriptase inhibitors, i.e. abacavir and didanosine, seems to be associated with an increased risk of myocardial infarction (4;5). The mechanism by which HIV and some antiretroviral drugs increase the risk of cardiovascular events is not entirely understood, although it may be partly explained by HIV- and HAART-induced dyslipidemia (4;6). A common strategy to manage dyslipidemia is the use of lipid-lowering drugs, such as 3-hydroxy-3-methyl-glutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins) and fibrates (7).

A complicating factor in the concomitant use of antiretroviral agents and lipid-lowering drugs is the occurrence of drug-drug interactions. Most statins are metabolized by Cytochrome P450-3A isoenzyme (CYP450-3A), while protease inhibitors are strong inhibitors of this enzyme. Drug–drug interaction studies on the co-administration of statins and protease inhibitors have demonstrated significant increases in plasma concentrations of simvastatin (8), atorvastatin (8), and rosuvastatin (9;10), and cases of rhabdomyolysis in HIV-infected patients have been attributed to these interactions (11;12). Pravastatin is not metabolized by CYP450 and protease inhibitors do not elevate its plasma concentration (8;13). Therefore, and because of pravastatin's well-established benefit in the prevention of cardiovascular events (14;15), pravastatin is considered a first-choice statin for HIV-infected patients (13;16).
The newly developed HIV-1 integrase inhibitor raltegravir is likely to be used by patients that suffer from HIV or HAART-associated dyslipidemia. Hence, co-administration of pravastatin and raltegravir can be expected in the HIV-infected population.

Raltegravir is glucuronidated by uridine diphosphate (UDP)-glucuronyltransferase 1A1 (UGT1A1) (17). Pravastatin metabolism is complex and involves multiple oxidative pathways and glucuronidation (18). Because both agents share glucuronidation as a common metabolic pathway, there is a potential for a pharmacokinetic drug-drug

interaction. In addition, raltegravir use has been associated with myopathy and rhabdomyolysis in heavily pre-treated HIV-infected patients (17;19). Although the causal relationship to raltegravir has not been clearly established, the Summary of Product Characteristics of raltegravir warns that raltegravir must be taken with caution in patients receiving statins (17).

In the context of this warning and the potential for a pharmacokinetic drug interaction between raltegravir and pravastatin, there was a clear need to perform a formal drug–drug interaction study. The primary objective of this study was to determine the effect of raltegravir on pravastatin pharmacokinetics and vice versa. Secondary objectives were to evaluate the safety of the combined use of pravastatin and raltegravir, and to investigate the effect of raltegravir on the short-term lipid-lowering effects of pravastatin.

Methods

Study design

This open-label, randomized, three-period, cross-over, single-center, phase I, multiple-dose trial was conducted from June to September 2008 at the Radboud University Nijmegen Medical Centre (Nijmegen, the Netherlands).

Twenty-four healthy volunteers (12 females, 12 males) were stratified according to gender and the presence of a fasting elevated serum total cholesterol at screening ≥ 6.5 mmol/L. The 24 participants were subsequently divided into 6 groups of 4 participants. In group 1, participants received pravastatin 40 mg q.d. for 4 days. After a wash-out period of 10 days, participants received raltegravir 400 mg b.d. for 4 days. After a second wash-out period of 10 days, participants received both pravastatin 40 mg q.d. and raltegravir 400 mg b.d. for 4 days. The other 5 groups were exposed to exactly the same drug regimens, but each in a different order to prevent bias that might result from period effects.

The trial was approved by the Review Board of the Radboud University Nijmegen Medical Centre.

Study population

The trial was conducted in healthy men and women aged between 18 and 55 years. For inclusion in the trial, participants had to be in a good, healthy condition for their

age, as established by physical examination, medical history, electrocardiography, and biochemical, hematologic, and urinalysis testing conducted within 4 weeks before the first dose. Participants had to be able and willing to sign the Informed Consent Form before screening evaluations were carried out.

The main exclusion criteria were: a history of sensitivity or idiosyncrasy to medicinal products or excipients; a fasting triglyceride level >8.0 mmol/L (see below); a positive HIV test result; a positive hepatitis B or C test result; and therapy with any drug (for 2 weeks preceding dosing), except for acetaminophen.

Study drug and dosing

The raltegravir dosage that was used in this trial (400 mg b.d. with or without food) is the recommended dosage for raltegravir (17;20). On the days of pharmacokinetic sampling, both raltegravir and pravastatin were taken on an empty stomach because pravastatin's systemic bioavailability is reduced by food (21), and because, at the time of study design, available data on raltegravir pharmacokinetics were obtained in the absence of food (22). Fasting (no solids or fluids) was continued for 2 hours after dosing, followed by a standardized breakfast.

Safety assessments and pharmacokinetic sampling

Blood samples for pharmacokinetics were collected just before dosing and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, and 24 hours after dosing on day 4 of each treatment period to characterize drug absorption and elimination. After administration of raltegravir alone, no sample was taken at 24 hours after dosing. In addition, blood samples were collected to determine trough levels of either raltegravir or pravastatin or both, just before intake of the drug on days 1 and 2 of each treatment period.

Serum biochemistry, hematology, and urinalysis test results were checked at screening and on days 1, 2, 4, and 5 of each treatment period. Adverse events were assessed during the same visits. Special attention was paid to myopathy. Screening for drugs of abuse was performed at screening and on day 1 of each of the three treatment periods.

Compliance

At visits to the clinical trial unit, study personnel supervised the intake of medication and recorded the exact times of dosing. Drug intake at home was monitored by the use of microelectronic monitoring system (MEMS) caps (Aardex Ltd, Zug, Switzerland), which record the opening of the medication bottle. Furthermore,

pill counts and plasma trough level measurements on days 2 and 4 were used to assess adherence. Finally, participants were asked to write down the exact times of medication intake in a booklet.

Bioanalysis of pravastatin and raltegravir in plasma

Plasma concentrations of raltegravir were analyzed at the Department of Clinical Pharmacy at the Radboud University Nijmegen Medical Centre by means of a validated reversed-phase high-performance liquid chromatography (HPLC) method, which has been described previously (23).

Plasma concentrations of pravastatin were measured at the Analytical Biochemical Laboratories (Assen, the Netherlands). In the assay procedure, pravastatin, along with its internal standard, d3-pravastatin, were extracted from the plasma samples by protein precipitation followed by on-line solid-phase extraction using C18 cartridges on a Symbiosis Pharma system (Spark, Emmen, the Netherlands). Separation of pravastatin and d3-pravastatin was performed on the same system, equipped with a C8 HPLC column (75 × 4.6 mm, 3.5 µm). Detection and quantification of pravastatin and d3-pravastatin was carried out using an API 4000 tandem mass spectrometry (MS/MS) detector (MDS Sciex, Concord, ON, Canada). The accuracy values for pravastatin were 106%, 105%, and 103% at 1.50, 15.0, and 400 µg/L, respectively. At the same concentrations, the precision values (within day, coefficient of variation) were 3.7%, 1.6%, and 3.2%, respectively. The calibration curve was linear over a concentration range of 0.500 to 500 µg/L.

Pharmacokinetic analysis

Pharmacokinetic parameters for pravastatin and raltegravir were calculated by non-compartmental analysis of the plasma-concentration data using WinNonlin software version 5.2.1 (Pharsight Corporation, Mountain View, CA, USA). On the basis of the individual plasma concentration–time data, the following pharmacokinetic parameters of pravastatin were determined: the area under the plasma concentration–time curve from 0 to 24 hours after intake ($AUC_{0\rightarrow24}$; in µg·hour/L); the maximum plasma concentration of the drug (C_{max}; in µg/L), the time to reach C_{max} (T_{max}; in hours); the apparent oral clearance (CL/F) (in L/hour); the volume of distribution (V/F) (in liters); and the apparent elimination half-life ($t_{1/2}$; in hours). For raltegravir, the same pharmacokinetic parameters were calculated, except that the trough concentration in plasma 12 hours after intake (C_{12}; in mg/L) was also calculated and the AUC was calculated over the dosing interval from 0 to 12 hours.

Lipid-lowering effects: low-density lipoprotein cholesterol

On days 1 and 5 of each treatment period, serum total cholesterol, triglycerides, and high-density lipoprotein cholesterol concentrations were obtained under fasting conditions (≥ 12 hours from last food ingestion). The Friedewald equation was used to calculate LDL cholesterol (24). Because this equation cannot be applied in subjects with triglyceride levels above 8.0 mmol/L, we excluded those who had higher fasting triglyceride levels. We used paired t-tests to compare LDL changes during the 4 days of treatment with pravastatin, raltegravir, and pravastatin + raltegravir, respectively.

Sample size and statistical analysis

The required number of participants was calculated to be 20 (to detect 20% differences in the AUCs of pravastatin and raltegravir). To account for a dropout rate of 15%, 24 participants had to be included.

For the identification of a clinically relevant drug interaction, we used the bioequivalence approach (25). Geometric means were calculated for the AUC, C_{max}, C_{12}, CL/F, V/F, and $t_{1/2}$. GMRs with 90% CIs were calculated after log transformation of within-patient ratios. GMRs with 90% CIs falling entirely within the range of 0.80 to 1.25 were considered to indicate no significant interaction.

Statistical evaluations were carried out using SPSS for Windows, version 16.0.1 (SPSS, Chicago, IL, 1989–2005).

Results

Baseline characteristics

Twenty-four healthy volunteers (12 females and 12 males) were included in the trial. The mean (range) age, body weight, and body mass index were 34 (20–53) years, 70 (49–103) kg and 23 (18–29) kg/m^2, respectively. There were two Hispanic participants; the other 22 participants were Caucasian. There were no dropouts: all participants completed the trial and all were available for statistical analyses.

Compliance

The compliance of all 24 participants was good, as indicated by their statements about the intake of the drug doses, the MEMS caps, the number of tablets in the returned vials, the plasma trough concentrations of raltegravir, and the booklets (filled in by the participants) recording intake of medication.

Figure 1 Geometric mean plasma pravastatin concentration profile in the presence and absence of raltegravir (note the semilog scale on the inset).

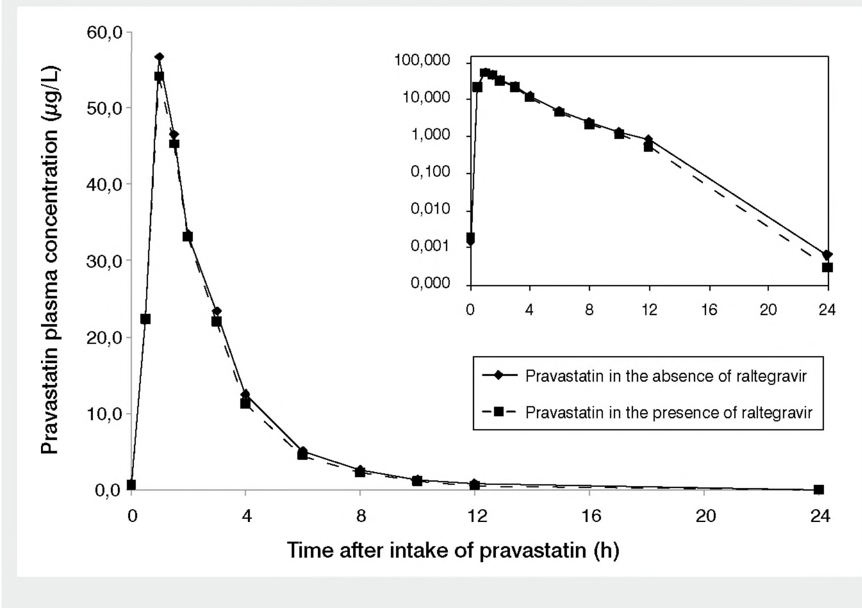

Table 1 Comparison of steady-state pravastatin pharmacokinetics with or without co-administration of multiple doses of 400 mg of raltegravir twice daily to healthy volunteers.

Pharmacokinetic parameter	Pravastatin alone		Pravastatin + Raltegravir		Pravastatin + Raltegravir: Pravastatin alone	
	Geometric mean	95% CI for geometric mean	Geometric mean	95% CI for geometric mean	GMR	90% CI for GMR
$AUC_{0\rightarrow24}$ (h·µg/L)	157	(127, 194)	150	(121, 187)	0.96	(0.83, 1.11)
C_{max} (µg/L)	58.4	(45.1, 75.6)	60.5	(47.6, 77.0)	1.04	(0.85, 1.26)
t_{max} (h)*	1.00	(1.00, 1.00)	1.00	(1.00, 1.00)		
CL/F (L/h)	255	(206, 315)	266	(214, 331)	1.04	(0.90, 1.21)
V/F (L)	1048	(788, 1394)	1026	(672, 1566)	0.98	(0.67, 1.42)
$t_{1/2}$ (h)	2.85	(2.14, 3.80)	2.68	(1.93, 3.70)	0.94	(0.68, 1.30)

CI, confidence interval; GMR, geometric mean ratio; AUC, area under the plasma concentration–time curve; C_{max}, peak plasma concentration; T_{max}, time to reach C_{max}, CL/F, apparent oral clearance; V/F, volume of distribution; $t_{1/2}$, elimination half-life. *For T_{max}, median + interquartile range is reported.

Pharmacokinetics

Figure 1 shows the geometric mean pravastatin plasma concentration versus time curves obtained in the presence and absence of raltegravir. The mean pharmacokinetic parameters of pravastatin are described in Table 1. Raltegravir did not influence the pharmacokinetics of pravastatin. For pravastatin co-administered with raltegravir relative to pravastatin alone, the GMR (90% CI) was 0.96 (0.83–1.11) for $AUC_{0\rightarrow24}$, 1.04 (0.85–1.26) for C_{max} and 0.94 (0.68–1.30) for $t_{1/2}$.

The geometric mean plasma raltegravir concentration–time curves following administration of raltegravir with and without pravastatin are shown in Figure 2. The mean pharmacokinetic parameters of raltegravir are presented in Table 2. Pravastatin increased the C_{max} but decreased the C_{12} of raltegravir. For raltegravir co-administered with pravastatin relative to raltegravir alone, the GMR (90% CI) was 1.13 (0.77–1.65) for $AUC_{0\rightarrow12}$, 1.31 (0.81–2.13) for C_{max}, 0.59 (0.39–0.88) for C_{12}, and 0.99 (0.88–1.22) for $t_{1/2}$. At days 2 and 4, the GMR (90% CI) C_{trough} for raltegravir + pravastatin versus raltegravir alone were 0.70 (0.48-1.02) and 0.90

(0.60-1.33), respectively. Of note, steady-state plasma trough concentrations of raltegravir obtained in the morning were approximately 10 times higher than plasma concentrations obtained 12 hours after dosing in the evening (see Figure 2).

Lipid-lowering effects: low-density lipoprotein cholesterol

Table 3 summarizes the changes in low-density lipoprotein (LDL) cholesterol observed in the three treatment regimens. After short-term administration (8 doses) of raltegravir alone, serum LDL cholesterol levels were not significantly altered. Short-term administration (4 doses) of pravastatin alone resulted in a statistically significant decrease of the mean serum LDL cholesterol concentration. The mean LDL decrease after 4 days of pravastatin administered with raltegravir was similar to the effect of pravastatin alone, indicating no influence of raltegravir on the short-term lipid-lowering effects of pravastatin.

Figure 2 Geometric mean plasma raltegravir concentration profile in the presence and absence of pravastatin (note the semilog scale on the inset).

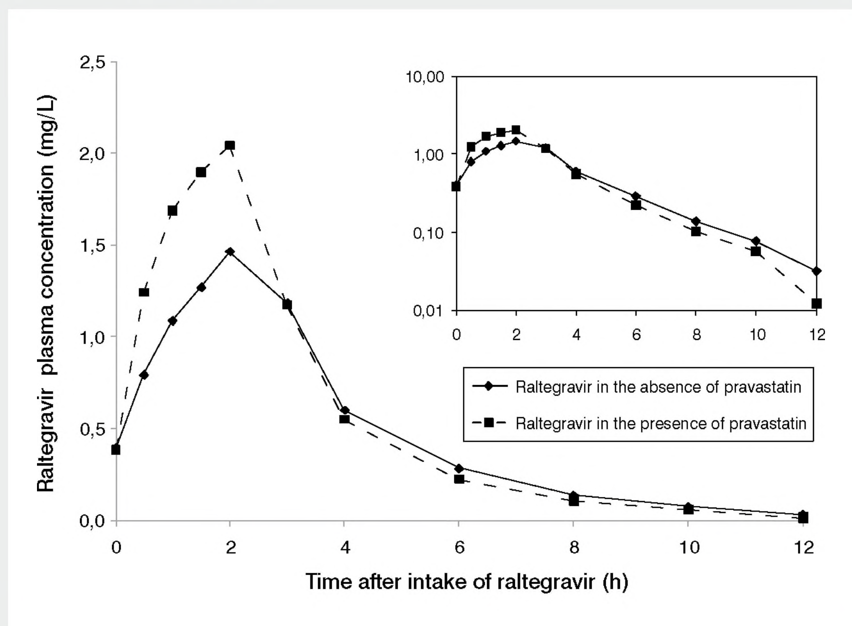

Table 2 Comparison of steady-state raltegravir pharmacokinetics with or without co-administration of multiple doses of 40 mg of pravastatin once-daily to healthy volunteers.

Pharmacokinetic parameter	Raltegravir alone		Raltegravir + Pravastatin		Raltegravir + Pravastatin: Raltegravir alone	
	Geometric mean	95% CI for geometric mean	Geometric mean	95% CI for geometric mean	GMR	90% CI for GMR
$AUC_{0\rightarrow12}$ (h·mg/L)	6.93	(5.24, 9.16)	7.83	(5.39, 11.4)	1.13	(0.77, 1.65)
C_{max} (mg/L)	2.05	(1.43, 2.94)	2.69	(1.71, 4.23)	1.31	(0.81, 2.13)
t_{max} (h)*	1.52	(1.00, 2.44)	1.50	(0.89, 2.00)		
C_{12} (mg/L)	0.061	(0.038, 0.098)	0.036	(0.021, 0.060)	0.59	(0.39, 0.88)
CL/F (L/h)	57.7	(43.7, 76.4)	51.1	(35.2, 74.2)	0.88	(0.60, 1.29)
V/F (L)	178	(124, 256)	156	(93.7, 261)	0.88	(0.52, 1.48)
$t_{1/2}$ (h)	1.99	(1.72, 2.30)	1.96	(1.53, 2.52)	0.99	(0.80, 1.22)

CI, confidence interval; GMR, geometric mean ratio; AUC, area under the plasma concentration–time curve; C_{max}, peak plasma concentration; T_{max}, time to reach C_{max}, C_{12}, plasma concentration 12 hours after intake of raltegravir; CL/F, apparent oral clearance; V/F, volume of distribution; $t_{1/2}$, elimination half-life. *For T_{max}, median + interquartile range is reported.

Adverse events and safety assessments

There were no discontinuations due to adverse events, and no serious adverse events were reported. The 24 study participants reported a total of 91 adverse events. The most common adverse event was headache, which was reported by 5 different participants (4 reports on raltegravir, 2 on pravastatin, and 2 on pravastatin + raltegravir). Twenty adverse events (22%) were considered to be possibly drug-related. The great majority of these events (*n*=19, 95%) were classified as grade 1; one adverse event (i.e. transient headache on raltegravir) was classified as grade 2. There were no creatinine kinase (CK) elevations or cases of myopathy that were considered to be possibly related to the study drugs.

Table 3 Changes in fasting serum LDL cholesterol (mmol/L) after 4 doses of 40 mg of pravastatin.

	Mean	95% CI	P
Change in LDL after 4 doses of 40 mg of pravastatin taken alone (day 5 – day 1)	−0.42	(−0.61; −0.24)[†]	<0.001[††]
Change in LDL after 4 doses of pravastatin taken with raltegravir (day 5 – day 1)	−0.42	(−0.59; −0.25)[†]	<0.001[††]
Change in LDL after 8 doses of raltegravir alone (day 5 – day 1)	+0.12	(−0.02; +0.26)[†]	0.10[††]
Differences in pravastatin – induced LDL change: change without raltegravir (day 5 – day 1) minus change with raltegravir (day 5 – day 1)	−0.002	(−0.24; +0.23)[†]	0.98[††]

LDL, low-density lipoprotein; CI, confidence interval.
[†] 95% confidence interval around the mean.
[††] paired-samples t-test.

Discussion

In this study, raltegravir did not increase pravastatin plasma concentrations and no drug-related myopathy or CK elevations occurred during the short-term co-administration of pravastatin with raltegravir. In addition, raltegravir had no influence on the short-term lipid-lowering efficacy of pravastatin.

On the other hand, concomitant use of pravastatin and raltegravir resulted in a mean 30% increase of the raltegravir C_{max}, and a slightly increased raltegravir mean $AUC_{0\rightarrow12}$ (13%). The elimination half-life of raltegravir was unaffected, because both raltegravir clearance and volume of distribution decreased to a similar degree after the addition of pravastatin (see Table 2). Therefore, the higher raltegravir C_{max} in the presence of pravastatin is probably not caused by inhibition of raltegravir metabolism, but rather by a change in the bioavailability of raltegravir.

It is tempting to speculate about the potential mechanism for the increased raltegravir peak plasma concentration in the presence of pravastatin. Raltegravir is a P-glycoprotein (P-gp) substrate; P-gp inhibition might thus have increased the bioavailability of raltegravir (26). However, this is not a likely mechanism because pravastatin is not an inhibitor or a substrate of P-gp (27). Pravastatin is a substrate of the organic anion-transporting polypeptide (OATP) 1B1, formerly known as OATP2 or OATP-C (28-30). OATP1B1 is a transporter which is located at the basolateral membrane of hepatocytes (31). If OATP1B1 also mediates raltegravir uptake into hepatocytes, which is currently unknown, competition for OATP1B1 may have resulted in decreased raltegravir hepatic uptake and increased raltegravir peak plasma concentrations.

The increase in raltegravir plasma concentrations with pravastatin is smaller than observed with tenofovir (raltegravir AUC +49%, C_{max} +64%) (32) or atazanavir (raltegravir AUC +41%, C_{max} +24%) (33). Clinical data from phase II and phase III trials showed that the combined use of raltegravir with these drugs did not raise any safety issues (34;35). In addition, no dose- or pharmacokinetic parameter-related toxicities have been identified for raltegravir so far. A phase I trial, in particular, showed that a raltegravir dosage as high as 800 mg b.d. was as well tolerated as the standard dosage of 400 mg b.d. (36). Therefore, the modest increase in raltegravir peak plasma concentrations in the presence of pravastatin is not likely to cause any safety concerns.

Raltegravir C_{12} was approximately 40% decreased in the presence of pravastatin. Because we encountered considerable variability in raltegravir C_{trough} and C_{12} values (coefficient of variation: 50%), this finding may be caused by chance. Another explanation would be induction of raltegravir metabolism by pravastatin. Nonetheless, this is not a likely scenario. First, pravastatin had no effect on the elimination half-life of raltegravir (see Table 2). Second, enzyme induction is known to increase with time, which is in contrast with our data (the GMR C_{trough} for raltegravir + pravastatin versus raltegravir alone were 0.70 and 0.90 on day 2 and day 4, respectively). Finally, there are no indications of pravastatin being an enzyme inducer (37).

When interpreting the results of our study, it is important to realize which pharmacokinetic parameter of raltegravir is associated with antiviral efficacy. Intensive investigations of pharmacokinetic-pharmacodynamic data obtained in phase II and phase III trials did not find any relationship between raltegravir C_{12} and raltegravir efficacy (38;39).

These relationships were established for raltegravir plasma concentrations which were randomly taken during the dosing interval, which indicates that raltegravir AUC is the pharmacokinetic parameter related to efficacy (39). In agreement with this, in vitro data provided evidence that the AUC/EC_{50} ratio of raltegravir is the pharmacokinetic variable that explains viral inhibition by raltegravir (40). Because the AUC of raltegravir was 13% increased in our study, we do not expect diminished raltegravir efficacy when combined with pravastatin.

Plasma trough concentrations of raltegravir obtained on the morning of study day 4, before drug intake for pharmacokinetic sampling, were on average 10 times higher than raltegravir C_{12} concentrations (see Figure 2). This finding is consistent with previously published results (23;41). Possible explanations are circadian variations in glucuronidation (42;43) and the effect of food on raltegravir pharmacokinetics. The food effect on raltegravir is considerable (e.g. 8.5-fold increase in C_{min} (44)). Therefore, raltegravir morning C_{trough} levels may have been increased in participants who took raltegravir with food on the evening before the pharmacokinetic sampling days. In this study, participants were free to take raltegravir at home with or without food, and the intake of food was not recorded.

In conclusion, short-term co-administration of raltegravir and pravastatin was well tolerated in healthy volunteers and did not result in altered pravastatin plasma exposure. In accordance with this, raltegravir did not influence the short-term lipid-lowering effects of pravastatin. The effects of pravastatin on raltegravir pharmacokinetics are not likely to be of clinical importance. The data from our study support the co-administration of raltegravir and pravastatin without dose-adjustments.

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9

Chapter

Effect of posaconazole on the pharmacokinetics of fosamprenavir and vice versa in healthy volunteers

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Abstract

Objective: To manage the interaction between fosamprenavir/ritonavir and posaconazole, we hypothesized that ritonavir could be replaced by posaconazole as an alternative booster of fosamprenavir with no significant influence on posaconazole pharmacokinetics.

Methods: This was an open-label, randomized, 3-period, cross-over, single-centre trial in 24 healthy volunteers. All subjects received the following three treatments for 10 days, separated by washout periods of 17 days: posaconazole 400 mg twice daily; fosamprenavir/ritonavir 700/100 mg twice daily; fosamprenavir 700 mg twice daily with posaconazole 400 mg twice daily.

Results: Twenty subjects completed the trial. Geometric mean ratios (GMR; +90% CI) of posaconazole AUC and C_{max} when taken with fosamprenavir versus posaconazole alone were 0.77 (0.68-0.87) and 0.79 (0.71-0.89), respectively. The GMRs of amprenavir AUC and C_{max} when taken as fosamprenavir and posaconazole versus fosamprenavir/ritonavir were 0.35 (0.32-0.39) and 0.64 (0.55-0.76), respectively. No serious adverse events were reported during the trial.

Conclusion: Unboosted fosamprenavir should not be used concomitantly with posaconazole.

Introduction

Infections with fungi and yeasts frequently occur in patients infected with HIV. Since the introduction of combination antiretroviral therapy (cART), the incidence and prevalence of most opportunistic infections has decreased (1;2) but they can still pose a problem in, for instance, resource limited settings or in non-compliant patients.

Azole antifungal drugs are first line therapy in the prophylaxis and treatment of invasive fungal infections. Posaconazole is a second generation triazole with a broad antifungal spectrum against yeasts and moulds. It has proven to be effective in the prevention and treatment of invasive fungal infections in high-risk patients, including those who are immunosuppressed (3-5). Once absorbed, 76.9% of the administered dose of posaconazole is excreted with feces. 14% of the administered dose is retrieved in the urine as a glucuronide metabolite (6). UDP-glucuronyltransferase 1A4 (UGT1A4) has been identified as the key enzyme responsible for posaconazole glucuronidation (7). Posaconazole is a potent inhibitor of the cytochrome P450 isoform 3A4 (CYP3A4) (8).

Fosamprenavir is a protease inhibitor that is used to treat HIV-infection (9). Once hydrolysed to amprenavir, this substance is both a substrate and an inhibitor of CYP3A4. Fosamprenavir is given concomitantly with ritonavir which serves as a booster of the pharmacokinetics of amprenavir (10). Although ritonavir is capable of potent CYP3A4 inhibition, ritonavir induces other metabolic pathways including glucuronidation and CYP2C19 (11-14).
The combination of antiretroviral drugs with azole antifungal drugs is not without risk. First, combining fosamprenavir/ritonavir with posaconazole may lead to subtherapeutic posaconazole exposure due to induction of UGT by ritonavir. Second, inhibition of CYP3A4 by posaconazole may (further) increase exposure to amprenavir with an increased risk of fosamprenavir toxicity.

To manage the interaction between fosamprenavir/ritonavir and posaconazole, we hypothesized that ritonavir could be replaced by posaconazole as an alternative booster of the pharmacokinetics of fosamprenavir, with the additional advantage of eliminating the potential negative effect of ritonavir on posaconazole. Based on these theoretical considerations, we performed a trial to determine the effect of unboosted fosamprenavir on posaconazole and vice versa.

Methods

Study design

This open-label, 3-period, cross-over, single-centre, phase-I, multiple-dose trial was conducted from March to May 2009 in the Clinical Research Centre of the Radboud University Nijmegen Medical Centre, The Netherlands. The study was designed to examine the effect of fosamprenavir on the pharmacokinetics of posaconazole and vice versa by intrasubject comparison. The secondary objective of this trial was to evaluate the safety of combined administration of posaconazole and fosamprenavir. The trial (ClinicalTrials.gov Identifier NCT00817765) was approved by the review board of the Radboud University Nijmegen Medical Centre and conducted according to the declaration of Helsinki.

Study population

This trial was conducted in healthy male and female volunteers, aged 18-55 years with a body mass index of 18 to 30 kg/m^2. Subjects who were included had to be able and willing to sign the Informed Consent Form prior to screening evaluations. Subjects had to be in a good age-appropriate health condition as established by medical history, physical examination, electrocardiography, biochemistry, hematology and urinalysis testing within 4 weeks prior to the first day of dosing. The main exclusion criteria were a history of sensitivity / idiosyncrasy to any of the study drugs, a HIV positive test, a positive hepatitis B / C test or therapy with any drug (two weeks preceding dosing), except for acetaminophen. Other exclusion criteria were: participation in a drug trial or donation of blood within 60 days prior to the first dose. Pregnant females were also excluded.

Study drug and dosing

In this cross-over design three treatment arms were investigated. Subjects were randomized to start with different treatment arms (6 different treatment sequences in total) (Table 1). Each period consisted of 10 days of treatment with either one of the three regimens. After each treatment period, there was a wash-out period of 17 days. The interaction arm contained fosamprenavir 700 mg twice daily together with posaconazole. Posaconazole was dose escalated from 200 mg once daily on day 1, 200 mg twice daily on day 2 and 400 mg twice daily from day three onward. In the first comparator arm, posaconazole was given in a similar fashion as in the interaction arm. In the second comparator arm, subjects used fosamprenavir 700 mg twice daily with ritonavir 100 mg twice daily. The chosen dose escalation for posaconazole, which is not listed in the label, was chosen to minimize toxicity since this regimen had never been tested.

Table 1 Study Design.

Arm	Period 1 (Days 1 – 10)	Washout (Days 11-28)	Period 2 (Days 29-38)	Washout (Days 39-56)	Period 3 (Days 57-66)
1	POS*		FPV / RTV		POS + FPV
2	POS		POS + FPV		FPV / RTV
3	FPV / RTV#		POS		POS + FPV
4	FPV / RTV		POS + FPV		POS
5	POS + FPV$		POS		FPV / RTV
6	POS + FPV		FPV / RTV		POS

* POS = POS 200 mg QD on day one, 200 mg BID on day two, 400 mg BID from days 3-10.
# FPV / RTV = FPV 700 mg BID + RTV 100 mg BID from days 1-10.
$ POS + FPV = POS 200 mg QD on day one, 200 mg BID on day two, 400 mg BID from days 3-10 + FPV 700 mg BID from days 1-10.

Fosamprenavir, ritonavir and posaconazole were taken with food at approximately 8 AM and 8 PM. Subjects visited the trial centre approximately every other day during the treatment periods for supervised dosing and blood sampling. On every visit day of each treatment period, subjects received a standardized breakfast at around 8 AM followed by the supervised intake of an oral dose of posaconazole, fosamprenavir/ritonavir or fosamprenavir/posaconazole (depending on the treatment arm). Fosamprenavir tablets and ritonavir capsules were swallowed whole with 200 mL of water. The posaconazole suspension was swallowed with 200 mL water. The breakfast consisted of 2 slices of wheat bread (one slice with 48+ cheese and one with luncheon meat or cervelat) and one glass of full milk or full chocolate milk. This breakfast contained, depending on the choice of topping and milk, 488 - 553 kcal and 26-28 gram fat (43-50% fat).

Pharmacokinetic sampling and safety assessments

Blood samples for pharmacokinetics were collected throughout a 12-hour period at 11 pre-defined time points (0, 1, 2, 3, 4, 5, 6, 7, 8, 10 and 12 hours after dosing) at day 10 of every treatment period to characterize drug absorption and elimination. Trough concentrations, just before intake of the drugs, were collected on study days 1, 3, 5, and 8 of each treatment period.

Serum biochemistry and hematology were checked at screening and on days 1,3,5,8 and 10 of each treatment period. Adverse events assessment, blood glucose and urinalysis were performed at screening and on days 1 and 5 of each treatment

period. A pregnancy test for women was conducted at screening and a screening for drugs of abuse was conducted before dosing on day 1 of each treatment period. Electrocardiogram and blood pressure / pulse rate (supine) were checked at screening and on days 1 and 5 of each treatment period.

Compliance

Study personnel supervised all medication intakes at the clinical trial unit on visit days. The times of dosing were recorded. Drug intake of subjects at home was monitored by the use of MEMS caps (Aardex Ltd., Zug, Switzerland), which record the opening of the medication bottle. Furthermore, subjects were asked to write down exact times of medication intake in a diary.

Pharmacokinetic analysis

Pharmacokinetic parameters for posaconazole, amprenavir and ritonavir were calculated by non-compartmental methods using the WinNonLin software package (version 5.2.1; Pharsight, Mountain View, CA) and the log/linear trapezoidal rule.
On the basis of the individual plasma concentration-time data, the following pharmacokinetic parameters were determined: the area under the plasma concentration-time curve from 0 to 12 hours after intake ($AUC_{0\rightarrow12}$; in mg*hour per liter), the maximum plasma concentration of the drug (C_{max}; in mg per liter), the time to reach C_{max} (T_{max}; in hours), the apparent clearance after oral administration (CL/F) (in liters per hour), the apparent volume of distribution (V/F) (in liters), the trough concentration in plasma (C_{12} [12 hours after intake]) and the apparent elimination half-life ($t_{1/2}$; in hours).

Analytical procedure

All plasma samples were analyzed at the Department of Clinical Pharmacy, Radboud University Nijmegen Medical Centre.
Amprenavir and ritonavir were determined by a validated High Performance Liquid Chromatography method with UV-detection (15). Samples were pre-treated using a liquid-liquid extraction from plasma. The dynamic range of the amprenavir assay was 0.10 - 30 mg/L and for ritonavir 0.045 - 30 mg/L. The assay had an accuracy range (five replicates of three concentrations of QC samples), dependent on the concentration, from 102-105% for amprenavir and 101-104% for ritonavir, respectively. Intraday precision (n=15) for amprenavir varied between 2.55 - 4.05% and 0.89 - 3.22% for ritonavir. The interday precision (n=3) for amprenavir was 1.18 - 5.04% and 1.10 - 3.64% for ritonavir, respectively.

Posaconazole samples (total and free fraction) were measured by a validated High Performance Liquid Chromatography method with fluorescence detection. Samples were pretreated using a protein precipitation procedure. The dynamic range of the assay was 0.05 - 10 mg/L. The assay had an accuracy range (five replicates of three concentrations of QC samples), dependent on the concentration, from 97.9 - 104.1%. Intraday precision varied between 1.56 - 3.03% and interday precision was between 1.37 - 4.11%.
To determine the free fraction of posaconazole, we used plasma samples drawn at or around T_{max}. This plasma was then transferred into Centrifree Centrifuge tubes (30 kDa). Samples were centrifuged during 10 minutes at 2000 RCF (rpm 3310) (Rotante 46 R, radius 164 mm, angle 45 degrees, temperature 25 C). The analysis was modified to be able to determine very low concentrations of unbound posaconazole and had a lower limit of quantification of 0.01 mg/L without loss of accuracy and precision. Both assays are externally validated by an international proficiency testing program (16-18).

Sample size calculation and Statistical Analysis

To determine bioequivalence with sufficient power, the sample size calculation was performed on posaconazole since this drug has the highest estimated degree of intrasubject variation (19-21). The study was powered (power of 80%) to detect a 20% difference in posaconazole AUC. The required number of participants was 16 and compensating for drop-outs, 24 subjects were included.
For the identification of a clinically relevant drug interaction, we used the bioequivalence approach "Guidance for Industry: Statistical Approaches to Establishing Bioequivalence" (22). Geometric mean ratios (GMRs) with 90% confidence intervals (CIs) were calculated for $AUC_{0\rightarrow 12}$ and C_{max} after log transformation of within-subject ratios. GMRs with 90% CIs falling entirely within the range of 0.80 to 1.25 were considered to indicate no significant interaction.

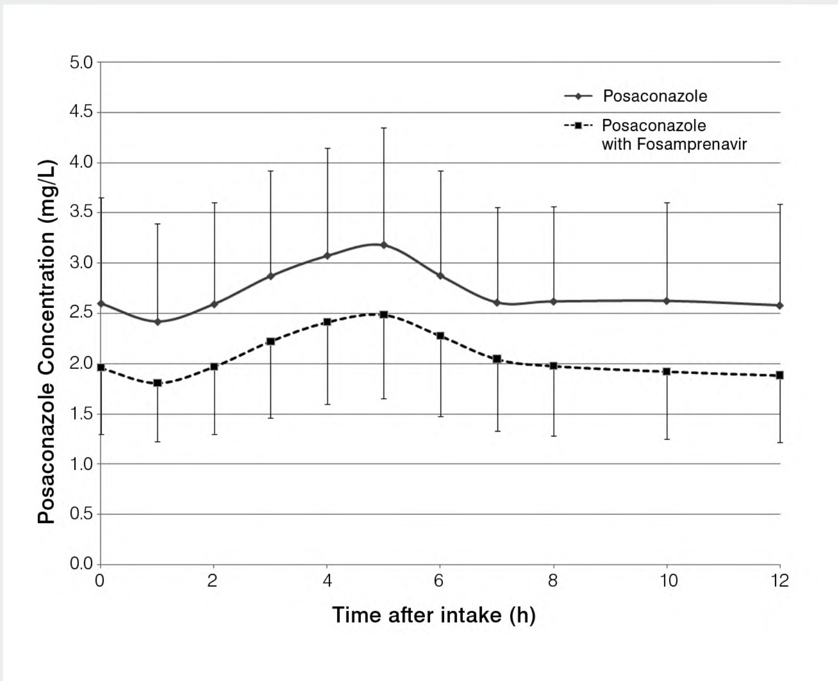
To assess the carry-over and period effect of concomitant administration of posaconazole on the pharmacokinetics of fosamprenavir, linear mixed model analyses were performed on the log(AUC_{0-12}) of amprenavir. Similar analysis were performed on fosamprenavir. In this approach, treatment, period, and a carry-over variable were treated as fixed factors and patients effects as random.
Statistical evaluations were carried out using SPSS for Windows, version 16.0.1 (SPSS, Chicago, IL, 1989-2005).

Results

Baseline characteristics

Twenty-four healthy volunteers (10 females and 14 males) were included. The mean (range) age, body weight and body mass index were 36 (18-54) years, 73 (44-104) kg and 23 (18-29) kg/m^2, respectively. Twenty-one participants were Caucasian, three participants were from Hispanic ethnicity. Three subjects prematurely withdrew from the study due to adverse events and a fourth subject withdrew at own request, not related to treatment. Twenty participants completed the trial (9 female and 11 male) and were available for pharmacokinetic analyses.

Figure 1 Arithmetic mean plasma posaconazole concentration profile following the administration of multiple doses of 400 mg posaconazole twice daily alone versus posaconazole 400 mg twice daily with fosamprenavir 700 mg twice daily.



Error bars represent the standard error of the mean.

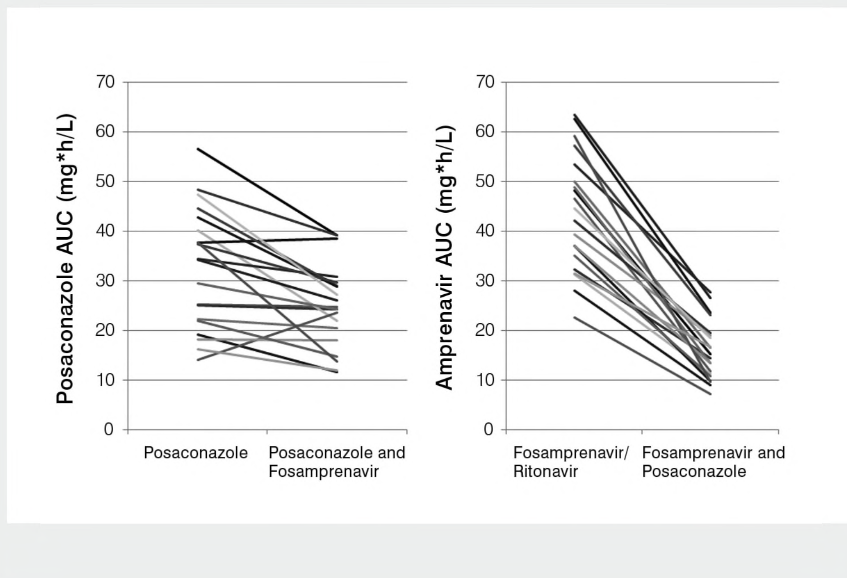
Compliance

The compliance of all participants was good, as indicated by their statements about the intake of the drug, the number of tablets in the returned vials, the trough drug concentrations, and the MEMS caps (data not shown).

Pharmacokinetics

Figure 1 shows the posaconazole plasma concentration versus time curves obtained in the absence and the presence of fosamprenavir. The mean pharmacokinetic parameters of posaconazole are described in Table 2. Fosamprenavir reduced the exposure to posaconazole. For posaconazole co-administered with fosamprenavir relative to posaconazole alone, the GMR (90% CI) was 0.77 (0.68-0.87) for $AUC_{0\rightarrow12}$, and 0.79 (0.71-0.89) for C_{max} (Table 2; Figure 2).

Figure 2 Individual changes in area under the concentration time curve of posaconazole alone versus posaconazole combined with fosamprenavir and of fosamprenavir / ritonavir versus fosamprenavir combined with posaconazole

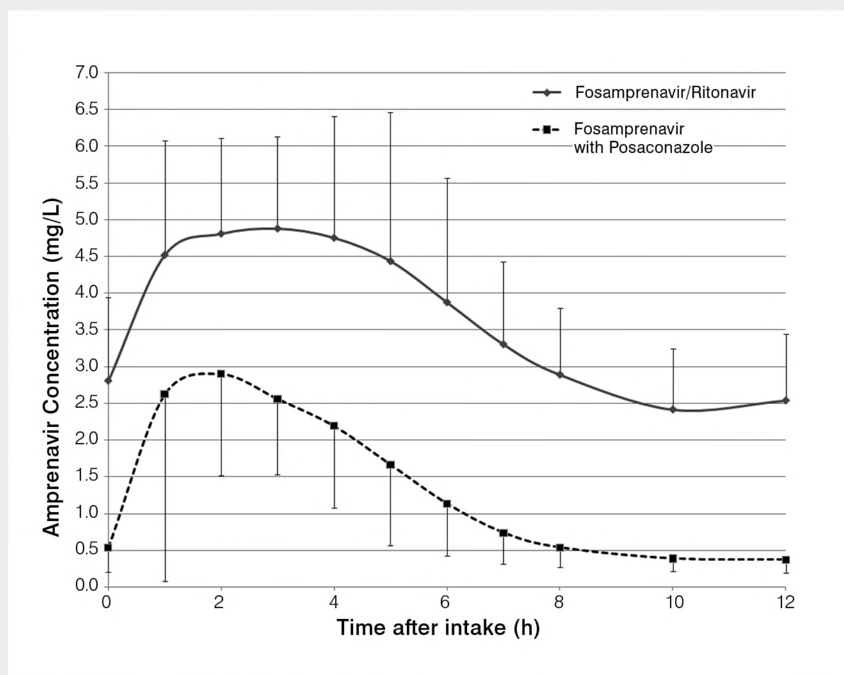


Geometric mean free posaconazole concentration in the posaconazole arm was 0.029 mg/L (95% CI: 0.023-0.036) and 0.029 mg/L (95% CI: 0.022-0.038) in the combination arm. There was no statistically significant difference in geometric

mean (range) free fractions of posaconazole (n=20) in the posaconazole alone arm versus in the combination arm: 0.988% (0.63 - 1.64%) versus 1.10% (0.63 - 1.74%) (p=0.18, paired samples T-test).

The amprenavir plasma concentration-time curves following administration of fosamprenavir with ritonavir versus fosamprenavir with posaconazole are shown in Figure 3. The mean pharmacokinetic parameters of amprenavir are presented in Table 2. Posaconazole did not increase the AUC and C_{max} of amprenavir to the same extent as ritonavir did. For fosamprenavir with posaconazole relative to fosamprenavir with ritonavir, the GMR (90% CI) was 0.35 (0.32–0.39) for $AUC_{0\rightarrow12}$, and 0.64 (0.55–0.76) for C_{max} (Table 2, Figure 2,3).

Figure 3 Arithmetic mean plasma amprenavir concentration profile following the administration of multiple dose of 700 mg of fosamprenavir twice daily with 100 mg ritonavir twice daily versus fosamprenavir 700 mg twice daily with posaconazole 400 mg twice daily.



Error bars represent the standard error of the mean.

Table 2 Steady state pharmacokinetic parameters as determined on day 10 of treatment; geometric mean ratios of area under the concentration time curve and maximum concentration of posaconazole and amprenavir.

	Steady-state plasma pharmacokinetic parameter estimates, geometric mean (95% CI)				**Treatment comparisons, geometric mean ratio (90% CI)**	
	Posaconazole		Amprenavir			
Pharmacokinetic Parameter	POS	POS + FPV	FPV / RTV	FPV + POS	POS + FPV vs POS alone	FPV + POS vs FPV / RTV
$AUC_{0\rightarrow12}$ (mg*h/L)	30.4 (25.2-36.7)	23.5 (19.7-27.9)	42.0 (36.7-47.9)	14.8 (12.4-17.7)	0.77 (0.68 - 0.87)	0.35 (0.32 – 0.39)
C_{max} (mg/L)	3.0 (2.5-3.6)	2.4 (2.0-2.8)	5.8 (5.0-6.6)	3.7 (3.0-4.5)	0.79 (0.71 - 0.89)	0.64 (0.55 – 0.76)
T_{max} (h)	5 (4-5)	5 (4-5)	3 (2-4)	2 (1-3)	-	-
C_{min} (mg/L)	2.2 (1.8-2.7)	1.6 (1.4-2.0)	2.1 (1.8-2.5)	0.3 (0.2-0.4)	-	-
CL/F (L/h)	13.2 (10.9-15.9)	17.1 (14.4-20.3)	16.7 (14.6-19.1)	47.2 (39.5-56.4)	-	-
V/F (L)	609 (489-759)	471 (385-576)	173 (137-218)	241 (171-341)	-	-
$t_{1/2}$ (h)	32.1 (26.0-39.7)	19.1 (16.3-22.5)	7.2 (6.1-8.5)	3.5 (2.7-4.7)	-	-

$AUC_{0\rightarrow12}$, area under the plasma concentration-time curve over the 12 hour dosing interval; C_{max} , peak plasma concentration; T_{max}, time to reach C_{max};
C_{min}, plasma concentration 12 hours after intake of study drug; CL/F, apparent oral clearance; V/F, volume of distribution; $t_{1/2}$, elimination half-life; CI, confidence interval
* For T_{max} median + interquartile range is reported

There was no carry-over effect of fosamprenavir on posaconazole as assessed by the contribution of the variable defined to be 1 respectively 0 in case a treatment with fosamprenavir preceded or did not precede the treatment with posaconazole ($p = 0.9$). Likewise, there was no carry-over effect of posaconazole on fosamprenavir ($p = 0.6$). For both outcome variables there was no indication of a period effect to be present ($p=0.5$ for posaconazole and $p=0.9$ for fosamprenavir, respectively).
A regression analysis was performed to determine if there was a concentration dependent inhibition of amprenavir metabolism by posaconazole. No significant correlation was found between posaconazole exposure and amprenavir exposure ($p=0.099$; $r^2=0.144$).

Adverse events and safety assessments

A total of 141 adverse events were reported by a total of 23 subjects. The severity of 28 adverse events was grade 2; two adverse events were grade 3 and two grade 4 (three occasions of increased CK in one subject and one grade 4 occasion of increased CK in a second subject, all were judged not to be related to the study medication); all other (109) adverse events were grade 1. No serious adverse events were reported. There was no notable difference in adverse events in the different treatment arms.
The relation to the study drug was judged to be definite in eight occasions reported by three subjects: a grade II rash occurred in two subjects (one on fosamprenavir / ritonavir and one on posaconazole / fosamprenavir) and one subject experienced a grade III rash on posaconazole / fosamprenavir treatment. All three subjects discontinued treatment after which they recovered from the rash. Other side effects definitely related to the study drug were reported by a single subject: loose stool (2 occasions), flatulence (2 occasions) and pruritus. Seventeen adverse events were judged to be probably related and 36 possibly related.

Discussion

This trial showed a significant, bidirectional effect on the pharmacokinetics of posaconazole and fosamprenavir. Exposure to both posaconazole (-23%) and fosamprenavir (-65%) were significantly lower than in the comparator arms.

We could think of four possible explanations for the decrease in exposure of posaconazole by fosamprenavir: 1) induction of UGT1A4; 2) induction of P-glycoprotein; 3) decreased absorption of posaconazole 4) protein displacement of posaconazole.

It is generally thought that ritonavir is responsible for induction of glucuronidation (12;13) although an effect of the boosted protease inhibitor cannot be ruled out (12;13;23). Fosamprenavir has been shown to significantly reduce plasma raltegravir exposure, likely through UGT1A1 induction (24), however the effect on UGT1A4 remains unknown (10;25). Based on the average 23% decrease in posaconazole exposure, fosamprenavir may be a less potent UGT1A4 inducer than efavirenz and phenytoin, which have shown a reduction of 50% in exposure to posaconazole (26;27).

Posaconazole is a substrate for P-glycoprotein. Fosamprenavir has been shown to induce intestinal expression of P-glycoprotein in rats (28). This mechanism could be an additional explanation for an increase in intestinal efflux of posaconazole with subsequent lowered exposure.

Posaconazole absorption is significantly influenced by gastric pH, prandial state and timing of intake relative to the meal (29). In the literature there are no reports of an effect of fosamprenavir on the absorption of other drugs by, for instance, alterations in gastric pH. The T_{max} of posaconazole was not changed after addition of fosamprenavir, indicating that at least the rate of absorption was not influenced.

In general, an increased free fraction due to protein displacement, will result in a lower total plasma concentration (30). Posaconazole is >98% bound to serum albumin (27) while amprenavir is 90% bound to alpha-1-acid glycoprotein and albumin (31); hence an interaction based on protein displacement is possible. In our study posaconazole free drug fraction was unaltered. Therefore, protein displacement can be ruled out as an explanation.

In a recent study the AUC and C_{max} of atazanavir combined with posaconazole were comparable to the AUC and C_{max} of atazanavir boosted with ritonavir (33.4 mg*h/L and 3.57 mg/L versus 35.4 mg*h/L and 3.93 mg/L, respectively) (26). This suggests that posaconazole may be an equipotent inhibitor of CYP3A4 when compared to ritonavir.
In our study, the AUC and C_{max} of amprenavir after intake of fosamprenavir with posaconazole were 2.9 and 1.6 fold lower compared with administration of fosamprenavir with ritonavir. Yet, when compared with a historical control group, an effect on the pharmacokinetics of fosamprenavir by posaconazole can be noted compared with unboosted fosamprenavir 700 mg twice daily: $AUC_{0\rightarrow12}$ 14.82 mg*h/L (95% CI 12.41-17.70; this study) versus $AUC_{0\rightarrow12}$ 9.51 mg*h/L (95% CI 7.81-11.6) (32).

The extent of boosting of fosamprenavir by posaconazole is an indication that there might be a moderate effect of posaconazole on fosamprenavir pharmacokinetics, but clearly not to an extent similar to ritonavir. In fact, the exposure of amprenavir given as fosamprenavir 700 mg twice daily with posaconazole approximates that of unboosted fosamprenavir 1400 mg twice daily, which is a FDA-licensed dose for the treatment of therapy naïve patients (10;33). However, unboosted fosamprenavir dosed 1400 mg twice daily dose is considered a non-favorable regimen according to the Panel on Antiretroviral Guidelines for Adults and Adolescents (9).

No serious adverse events were reported during this trial and none of the included subjects experienced irreversible damage due to the use of trial medication. Three subjects dropped out because of a rash, but the other adverse events during this trial were mild or moderate. We expected rash to be an adverse event of fosamprenavir, as it is described as “common” in the Summary of Product Characteristics (SPC) of fosamprenavir (10).

Once again our study demonstrates the complexity of combined use of antiretroviral and antifungal drugs. From the results of our study we conclude that combined use of fosamprenavir with posaconazole results in subtherapeutic amprenavir concentrations compared to ritonavir boosted fosamprenavir and therefore this combination should not be used in this way. Future studies must reveal whether ritonavir boosted fosamprenavir can be safely combined with posaconazole. With regard to posaconazole, concentrations must be monitored by means of therapeutic drug monitoring to assure adequate exposure in order to warrant efficacy.

Acknowledgements

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General Discussion

The first section of the general discussion will discuss the history and the present role of TDM and TDM research. The second section will focus on drug-drug interaction studies which are performed during phase 4 drug development, thus after introduction into clinical care. Which studies should be performed? How should these studies be performed? These questions are addressed in the second section of the general discussion. Finally, the general discussion will conclude with future research agendas for TDM and drug-drug interaction studies.

1. Therapeutic Drug Monitoring

1.1 Therapeutic drug monitoring: from 1996 to 2010

The drugs that were used in the early era of combination antiretroviral therapy (cART) had unfavorable pharmacokinetic properties. This resulted in numerous problems, such as frequent daily intake of pills with strict food requirements, high interindividual variability in plasma concentrations (1;2) and high rates of virologic failure (3). These problems led to strong interest for TDM, which had the potential of ameliorating HIV treatment by providing the optimal dose for the individual patient.

Before TDM can be applied, drugs need to fulfil a number of criteria, such as the existence of a relationship between the plasma concentration of the drug and its effect and/or adverse effects (4). Intensive research learned that protease inhibitors and non-nucleoside reverse transcriptase inhibitors (NNRTIs), but not nucleoside analogue reverse transcriptase inhibitors (NRTIs), met these criteria (5-9).

The next question in the development of TDM was to find out which patients would most likely benefit from it. Two prospective randomized controlled trials investigated whether routine and unselected use of TDM would benefit patient outcome. In 2002 and 2003, the results of these trials were published, demonstrating less virologic failures and less toxicity-induced drug discontinuations in patients in whom therapy was monitored by TDM (1;10). These results led to increased enthusiasm for TDM. Typical for the enthusiasm those days, the Dutch HIV treatment guideline of 2005 recommended TDM of all available protease inhibitors and NNRTIs in all patients who started a new antiretroviral regimen (see **chapter 5**).

It is important to realize however, that HIV therapy is rapidly changing and improving due to continuous drug development. The randomized controlled trials that demonstrated improved therapeutic outcome with TDM were performed with indinavir- and nelfinavir-based cART in treatment naïve patients, a scenario which has almost completely vanished from current clinical practice.

Currently preferred antiretroviral drugs, such as efavirenz, possess much better pharmacokinetic profiles. As mentioned in **chapter 3**, standard dosing of efavirenz leads to therapeutic plasma concentrations in at least 80% of the HIV infected individuals (6;11), compared to just 40% for nelfinavir (1). As a consequence, it would require large randomized controlled trials (with more than 1,000 patients (12)) to have sufficient statistical power to investigate the value of unselected routine use

of TDM for efavirenz. For most clinical research groups, the organization of such trials is not feasible, also because it is difficult to obtain the financial resources for the conductance of such trials.

To give a rough idea of the use of routine TDM of efavirenz, it is interesting to estimate the number needed to treat with TDM in order to prevent virologic failure or discontinuation of efavirenz because of CNS toxicity.
A close look at the landmark study of efavirenz TDM, conducted by Marzolini and colleagues, learns that 7.7% of the patients had efavirenz plasma concentrations below 1.0 mg/L. Half of these patients developed virologic failure. The majority of patients, 79.2%, had efavirenz plasma concentrations in the therapeutic range. Virologic failure occurred in 22.3% of these patients. Finally, 13% of the patients had efavirenz plasma concentrations above 4.0 mg/L, associated with an increased risk of central nervous system (CNS) toxicities. The risk of virologic failure in this group was 17.6% (6).
Without TDM, the risk of virologic failure in a randomly selected patient would therefore be: (0.077*0.500)+(0.792*0.223)+(0.13*0.176)= 0.238; 23.8%. Assuming that the application of TDM would result in therapeutic concentrations for all patients, the risk of virologic failure would be reduced to 22.3%. Therefore, the number of patients 'needed to TDM' in order to prevent one patient from virologic failure would be 1/(0.238-0.223) = 67.

In addition, routine TDM of efavirenz may prevent toxicity-induced efavirenz discontinuations. The data presented in **chapter 3** show that patients with efavirenz plasma concentrations above 4.0 mg/L who continued the standard efavirenz dose had a risk of 11.5% of toxicity-induced efavirenz discontinuations. Patients who had their dose reduced had a 2.3% risk of efavirenz discontinuation. According to Marzolini, patients who had a plasma concentration below 1.0 mg/L had 0% risk of persistent CNS toxicity. Assuming that patients with a therapeutic plasma concentration would also have a risk of 2.3% for efavirenz discontinuations because of toxicity, like patients with high plasma concentrations who had their dose reduced, the risk for a randomly selected patient for efavirenz discontinuations would be: (0.077*0)+(0.792*0.023)+(0.13*0.115) = 0.033; 3.3%. With TDM, all patients would ideally get the risk of patients with a therapeutic plasma concentration, namely 2.3%. Therefore, the number 'needed to TDM' to prevent a randomly selected patient from virologic failure or from discontinuing efavirenz because of toxicity can be estimated to be 1/((0.238-0.223)+(0.033-0.023)) = 40.

It should be realized that this is merely an estimation with data from two observational studies. In the absence of evidence from randomized controlled trials that demonstrate benefits of routine TDM of efavirenz, it seems rational to restrict TDM to those patients who are suspected of non-compliance, who take medication which may interact with efavirenz, who experience persistent CNS toxicities and to treatment-experienced patients with limited treatment-options (9;13).

1.2 Therapeutic drug monitoring research

Current international HIV treatment guidelines recommend TDM for selected scenarios, such as in patients with drug-drug interactions (9;13). The adherence of clinicians to these recommendations is unknown. In order to improve the implementation of future guidelines and to adapt TDM guidelines, it is important to understand which factors are associated with adherence to TDM guidelines. Therefore, we evaluated the clinicians' adherence to the Dutch TDM recommendations of 2005 **(chapter 5).**

As described in **chapter 5**, the adherence to the TDM guidelines in The Netherlands appeared to be low to moderate, depending on the indication for TDM. Part of the explanation for the moderate implementation of the TDM guidelines might be that most indications for TDM were based on expert opinion. In order to improve the level of evidence for TDM recommendations, TDM research should focus on demonstrating its use in specific clinical situations. Studies should, for instance, investigate the use of TDM in pregnant women or in patients with renal dysfunction. In this thesis, we performed one such study, in which we investigated the use of TDM to manage efavirenz related CNS disturbances in patients with high plasma concentrations **(chapter 3)**.

In principle, the best design for investigating the use of TDM in a specific clinical situation would be a prospectively randomized controlled design, in which one group would receive TDM and the other group would not. Unfortunately, there are some problems that hinder the performance of such trials. First, most accepted clinical scenarios for TDM are relatively seldom. Therefore, it would require large multi-centre trials to recruit a sufficiently high number of patients, which complicates the organization of these trials. Second, TDM has entered into the hearts and minds of numerous European HIV-physicians. Despite the fact that the TDM indications in international guidelines are predominantly based on expert opinion, it is questionable whether HIV physicians would, for instance, be willing to withhold TDM from randomly selected pregnant women. This illustrates how treatment guidelines

based on expert opinion may unintentionally hamper the conductance of prospective randomized controlled trials. Third, it is doubtful whether funding can be obtained for the multi-centre trials described above. After all, there is no commercial interest for performing these kind of studies. In addition, it is questionable whether governmental organizations would give this type of TDM studies a high enough priority rating for funding them.

In conclusion, it seems not feasible to perform prospective studies of TDM. An alternative would be to perform retrospective studies at TDM services with data from the own laboratory. Our research group, for instance, used data from our own TDM service to study determinants of plasma exposure to lopinavir (14). Nonetheless, if the dependent variable of an analysis is a relatively seldom categorical variable, such as discontinuation of efavirenz because of toxicity, large datasets are needed in order to have sufficient statistical power.

Fortunately, we have the availability of such datasets in the Netherlands. In 1996, the Dutch Minister of Health, Els Borst, made several new antiretroviral drugs accelerated available through a subsidized early-access program. Two years later, a large investigation was started to measure the effects of cART in all patients who had commenced antiretroviral therapy from 1996. This study, which was conducted between 1998 and 2001, became known as the ATHENA (AIDS Therapy Evaluation in the Netherlands) cohort study. Once ATHENA was finished, it was decided that the research should be continued. This led to the establishment of the HIV Monitoring Foundation in 2001. Although officially incorrect, the HIV monitoring Foundation still refers to the 'ATHENA cohort study' in its scientific publications (15;16). Almost all Dutch HIV-infected patients are included in ATHENA. Currently, the central database, maintained by the HIV Monitoring Foundation, contains data from 16,715 patients (16). As can be noted from Table 1, the ATHENA database contains much of the information that is needed to evaluate the use of TDM in specific clinical situations. ATHENA has one limitation. The reason for performing TDM is not collected in ATHENA. It would, for instance, be relevant to know whether a TDM sample is taken because a patient is suspected of non-adherence. This thesis comprises two studies with data from ATHENA (**chapters 3 and 5**).

In addition, this thesis contains one retrospective study with data from another database, called EuroSIDA. Like ATHENA, EuroSIDA is a prospective observational cohort study. The EuroSIDA database contains over 16,000 patients followed in 103 hospitals in 32 European countries plus Israel and Argentina (17). Table 1 compares

Table 1 Comparison of the EuroSIDA and the ATHENA database.

	EuroSIDA	ATHENA
General features		
Number of patients in database (2009)	16,505	16,715
Number of participating hospitals (2009)	103	25
Number of participating countries (2009)	34	1
Important features for TDM studies		
Database contains viral load data	Yes	Yes
Database collects reasons for discontinuation of antiretroviral drugs	Yes	Yes
Database contains information on dosages of antiretroviral drugs	No	Yes
Database contains TDM results	No[1]	Yes[2]
Database contains information on co-medication	Partially[3]	Complete

[1] At 6 monthly intervals, a blood sample is taken and stored for each patient, which has led to the accumulation of a large sample repository which can be used to determine plasma concentrations of antiretroviral drugs. However, the time between the last intake of the patient's antiretroviral drugs and time of sampling is not noted.
[2] The time between sampling and intake of the antiretroviral drug is included in the database.
[3] EuroSIDA collects data on drugs which are used for the treatment and prophylaxis of opportunistic infections, and drugs being used for cardiovascular disease. EuroSIDA does not collect data on all drugs that patients may be taking, such as anti-depressants or anti-convulsants.

EuroSIDA and ATHENA. Because of the manner that EuroSIDA collects its data (see Table 1), this database is not suitable to evaluate TDM-driven interventions. Nonetheless, the half-yearly blood samples that are taken in EuroSIDA can be used to explore relationships between plasma concentrations of antiretroviral drugs and pharmacodynamic endpoints. Such studies are only appropriate for antiretroviral drugs with long elimination half lives. Because EuroSIDA does not collect the time between sampling and the latest intake of the antiretroviral drug, the influence of this factor on the drug plasma concentration should be negligible. This is only the case for drugs with long elimination half lives (6).
There is another problem though, when performing retrospective studies in EuroSIDA. Since plasma samples are only taken on a half-yearly basis, it will be hard to detect correlations between plasma concentrations and clinical endpoints

that have the highest incidence just after starting therapy. All patients who discontinue the drug before their half-yearly plasma sample has been taken are not included in such analyses. This problem was encountered in **chapter 2**.
In conclusion, the EuroSIDA database can be used to explore relationships between plasma concentrations of antiretroviral drugs and pharmacodynamic endpoints. Studies should only be performed on clinical endpoints that occur with a stable incidence in time. In addition, only drugs with long-elimination half lives can be appropriately studied.

2. Drug-Drug Interactions

In a general sense, the effects of a drug are related to its concentration at the sites of action, which is a function of the administered dose and the drug's absorption, distribution and elimination. The latter three processes can be influenced by food and other drugs, potentially resulting in diminished drug-efficacy or increased drug-toxicity.

For antiretroviral drugs, diminished drug efficacy may have serious consequences, such as drug (-class) resistance and virologic failure. Validly, the evaluation of potential drug-drug interactions has become an important part of the drug approval process (18).

Notwithstanding this, drug–drug interactions involving antiretroviral drugs are frequently not unraveled until after introduction into clinical care (19). There are several explanations for this phenomenon. First, new antiretroviral drugs often receive accelerated drug-approval, which obviously benefits HIV-infected patients with limited treatment-options, but decreases the available information on potentially hazardous drug-drug interactions. A recent example is the drug-drug interaction of raltegravir and rifampicin. After accelerated FDA approval of raltegravir in October 2007 (20), it lasted until January 2009 before the summary of product characteristics recommended to double the raltegravir dose when combined with rifampicin (21).
A second explanation for the discovery of relevant drug-drug interactions during the post-marketing phase is that polypharmacy is common among HIV-infected patients. For instance, HIV-infected subjects are known to have a high incidence of psychotropic agent use (22;23). With the increasing age of the HIV-infected population (24), the use of concomitant drugs will probably increase even more, for instance to treat cardiovascular disease or cancer (25).

Because of this polypharmacy, it is not realistic to expect all potentially relevant drug-drug interactions to be elucidated before marketing approval. Two recent examples of clinically relevant drug-drug interactions that were discovered long after introduction into clinical care are the effect of ginkgo biloba on efavirenz (26) and the effect of lopinavir/ritonavir on the anticancer drug irinotecan (27).

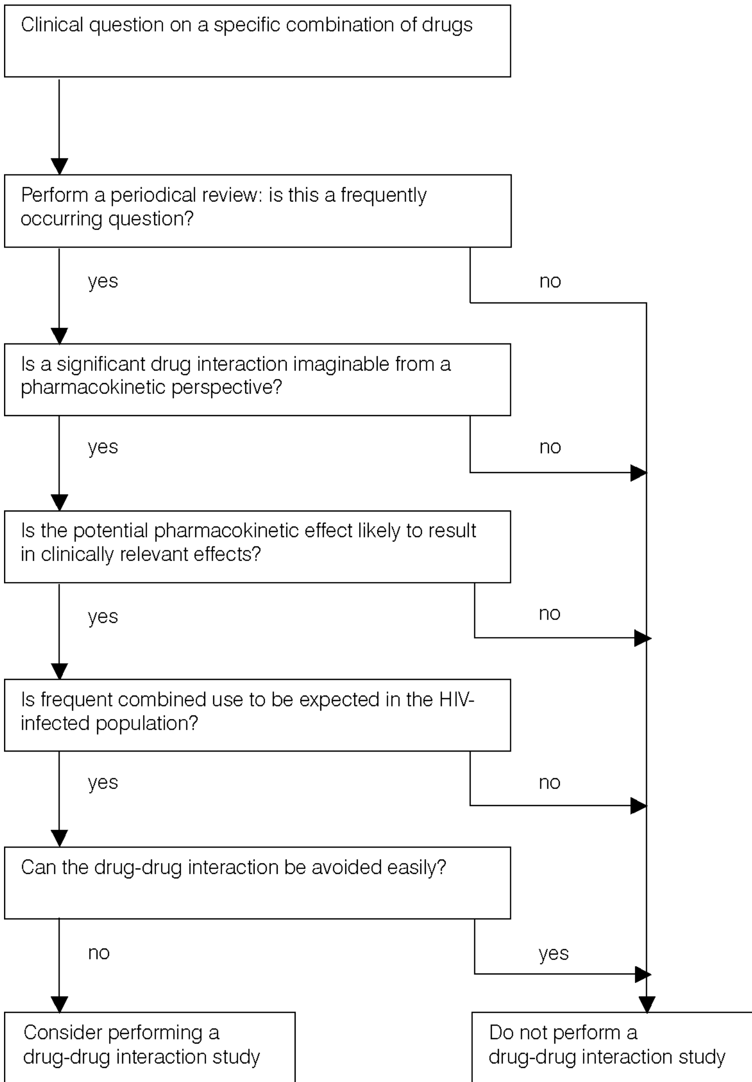
For academic researchers who want to perform pharmacokinetic drug-drug interaction studies in the post-marketing phase, the challenge is to identify those combinations that are the most useful to be studied. To be able to do this, close collaboration with HIV-physicians is essential. Drug-drug interaction questions which arise in clinical HIV practice can be used to identify relevant research questions. To identify interesting topics, it may be helpful to register pharmacological questions and answers in a database. By regularly evaluating the registered questions, the pharmacological researcher may identify drug-drug interactions that need to be studied. Naturally, the clinical pharmacologist should use his or her pharmacological knowledge to predict the probability of the occurrence of a hypothetical drug-drug interaction. Furthermore, he or she should assess, in consultation with the clinician, the potential clinical relevance of a drug-drug interaction. Finally, if an interaction can be easily bypassed for instance by replacing a drug for a suitable alternative drug, it is unnecessary to perform a drug-drug interaction study. The former reasoning can be expressed in the algorithm depicted in Figure 1.

The study described in **chapter 6,** which aimed to describe the effect of two boosted protease inhibitors and efavirenz on the pharmacokinetics of atovaquone/proguanil, is an example of a study that was undertaken after proceeding the steps depicted in the algorithm.

Another way to come to a relevant research question is to think ahead of clinical practice. By attending scientific clinical pharmacology meetings, ideas for interesting drug-drug interaction studies may come up. This is especially true for new antiretroviral drugs, or new drugs that are expected to be used frequently by HIV-infected patients. In this scenario, one can enter the algorithm in step 3 (*is a significant drug interaction imaginable…*). The studies described in **chapters 8 and 9** fit into this scenario.

The drug-drug interaction study described in **chapter 7** does not entirely fit into the algorithm, since frequent combined use of raltegravir and lamotrigine is not expected in the HIV-infected population. The original goal of this study was broader

Figure 1 Decision tree to decide whether it is useful to perform a drug-drug interaction study.



than investigating the influence or raltegravir on lamotrigine pharmacokinetics. Based on previous studies (28), we had designed this study as a 'phenotypic probe' study (see below and see **chapter 7**).

2.1 Study designs of drug-drug interaction studies

2.1.1 Cross-over design or parallel design?

Most drug-drug interaction studies employ a crossover design. This allows for a within-subject comparison between treatments because each subject serves as his or her own control, eliminating the bias introduced by intersubject variability (29). The pitfall of this approach is carry-over effects. For instance, a long wash-out period may be required to prevent bias resulting from enzyme induction. The study described in **chapter 7** (raltegravir/lamotrigine trial) illustrates this. In this study, we used a wash-out period of 26 days to prevent bias which could have resulted from auto-induction of lamotrigine metabolism.

A parallel study design can be considered when drugs cannot be stopped or repeatedly administered. This is the case if there is a risk for the development of resistance in HIV-infected patients after drug withdrawal. Thus, it would be undesirable to utilize a crossover design with mono therapy of efavirenz or nevirapine in HIV-infected patients. Because the intersubject pharmacokinetic variability of most drugs is considerably higher than the intraindividual variability, the sample size of studies with parallel designs must be higher, which is a clear disadvantage of parallel designed drug-drug interaction studies.

2.1.2 Study population: HIV-infected patients or healthy volunteers?

Most drug interaction studies of antiretroviral drugs are performed in healthy volunteers, because it allows to control for as many factors as possible. A major disadvantage of conducting drug interaction studies in HIV-infected patients is the use of co-medication, which may bias the assessment of the interaction between two drugs. Another disadvantage of conducting these trials in HIV-infected patients is the risk of lowered plasma concentrations of antiretroviral drugs, which might lead to the development of resistant virus.

An obvious disadvantage of conducting drug interaction studies in healthy volunteers is that healthy people are exposed to (antiretroviral) drugs, and potential concomitant toxicities. Before designing a drug interaction trial, one should therefore carefully balance the importance of the information that a trial may provide against the risk for the participating subjects.

If the risk of adverse events is disproportionally high, one should seek for a design with HIV-infected patients who have a true indication for the drug combination of interest. Such a scenario should for instance be preferred for drug-drug interaction studies between antiretroviral drugs and anticancer drugs. In addition, studies of drug-drug interactions between rifampicin or rifabutin and protease inhibitors should preferably have HIV-infected patients as study population. For both drugs, toxicity appears to be more prevalent among healthy volunteers than in HIV-infected patients.

Rifampicin is a strong inducer of Cytochrome P450 (CYP) 3A mediated metabolism of protease inhibitors. Three recent drug-drug interaction studies which aimed to study combined use of rifampicin with the protease inhibitors atazanavir, saquinavir, and lopinavir in healthy volunteers were prematurely terminated because of high incidences of gastro intestinal intolerance and hepatotoxicity (30-32). Hepatotoxicity of this magnitude has not been reported in HIV-infected populations who used protease inhibitors concomitantly with rifampicin (33-36).

Rifabutin can be used as an alternative for rifampicin in the treatment of HIV-associated tuberculosis, because CYP3A enzyme induction is lower than with rifampicin. However, combined use with protease inhibitors is complex, since protease inhibitors inhibit CYP3A-mediated metabolism of rifabutin. This may lead to higher rifabutin plasma concentrations and an increased risk of rifabutin associated neutropenia and uveitis.

Clinical trials that included both HIV-infected and non-HIV-infected individuals reported a significantly higher frequency of rifabutin-associated neutropenia among HIV-negative study participants (37;38). In addition, recent drug-drug interaction studies of rifabutin with lopinavir and atazanavir in healthy volunteers reported high incidences (57-72%) of neutropenia and other rifabutin associated adverse reactions (39;40). Meanwhile, a drug interaction study of rifabutin and lopinavir in HIV-infected subjects, which used the same dosage of rifabutin, found a 40% lower rifabutin C_{max} and better tolerance as compared to the healthy volunteer trial. Based on the latter data, the authors recommended a higher dose of rifabutin for HIV-infected patients than the originally recommended rifabutin dose that was based on healthy volunteer trials (41). From above, it becomes clear that further drug-drug interactions studies of rifabutin or rifampicin in healthy volunteers are undesirable.

It is unclear why some adverse effects seem to occur at a higher rate in HIV-negative subjects. An obvious explanation would be an immune mediated mechanism. However, there are no clear data to support this hypothesis. In addition, little is known about the potential differences in pharmacokinetics between HIV negative and HIV positive subjects (42).

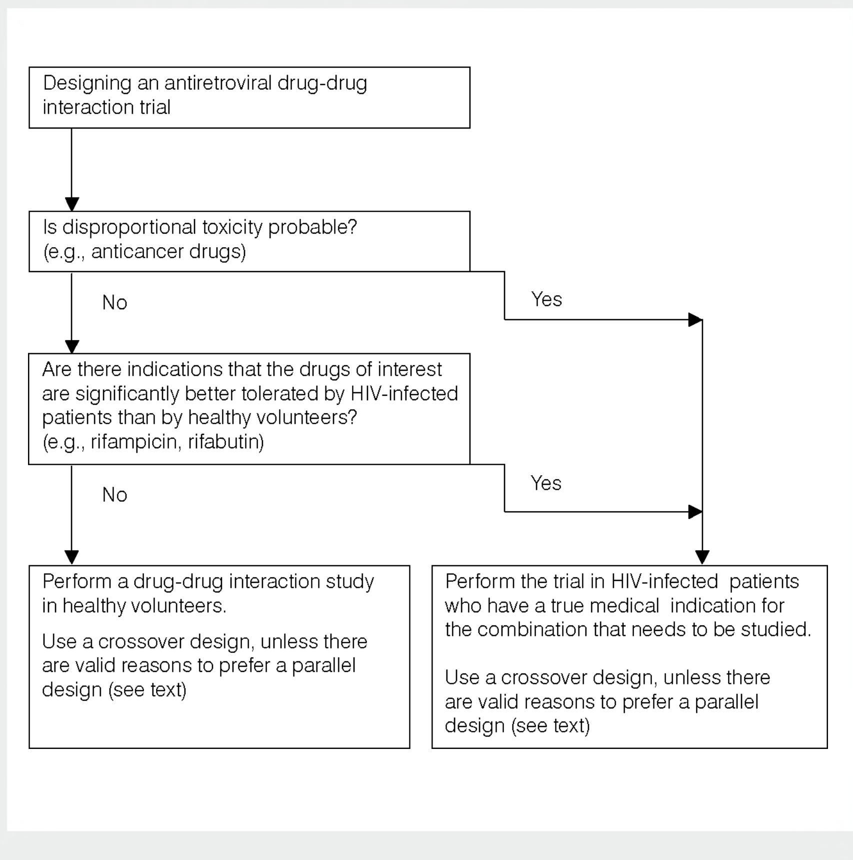
What is, apart from rifabutin, rifampicin, and other highly toxic drugs, the optimal design for a drug interaction study of antiretroviral drugs? In my opinion, a drug-drug interaction study with a crossover design in healthy volunteers is still best capable of assessing whether two drugs influence each other's pharmacokinetics. However, the pharmacokinetic behaviors and in some cases toxicities of drugs, might differ as a result of HIV-disease (42). Therefore, one should be cautious when making firm dose recommendations based on healthy volunteer data alone. If data in HIV-infected patients are lacking, TDM should be used to support the management of drug-drug interactions.

Figure 2 displays an algorithm for the choice of the study population of drug-drug interaction trials involving antiretroviral agents. The drug-drug interaction studies performed described in **chapters 7 to 9** fit into this algorithm, whereas the study described in **chapter 6**, does not. The latter study aimed to determine the effect of lopinavir/ritonavir, atazanavir/ritonavir and efavirenz on the pharmacokinetics of a single dose of atovaquone/proguanil. The chosen design was a comparison of single dose atovaquone/proguanil pharmacokinetics between healthy volunteers and HIV-infected patients who were using lopinavir/ritonavir, atazanavir/ritonavir or efavirenz.

The reason to choose for this design was that we wanted to prevent healthy volunteers from taking antiretroviral drugs for 10 days to obtain enzyme induction. According to the algorithm in Figure 2, it would have been appropriate to conduct this trial in healthy volunteers using a cross over design. After all, there was a highly relevant research question and the administration of a boosted protease inhibitor alone is not expected to result in disproportional toxicity.

In fact, the atovaquone study is not a pure drug-drug interaction study, but a parallel comparison of single dose atovaquone/proguanil pharmacokinetics in healthy volunteers and HIV-infected patients who were taking antiretroviral drugs that may have influenced the metabolism of the two anti malaria drugs. However, the pharmacokinetics of the anti malaria drugs may have been altered by HIV-infection as well.

Figure 2 Decision tree for selecting the most appropriate study population for a drug-drug interaction trial.



This does not make the data useless: the study mimics the 'real world situation'. The manufacturer of atovaquone/proguanil does not recommend to adjust the atovaquone/proguanil dose for people with HIV-infection, while phase 3 trials for malaria prophylaxis were conducted in healthy subjects (43).

2.2 Probe Studies

Based on in vitro data, an antiretroviral drug can be suspected to affect the metabolism of other drugs. In that case, a sensitive and specific probe can be used to investigate the drug-drug interaction potential of that antiretroviral drug in vivo (44). For instance, a method to assess CYP2C19 activity is by calculating the 5-OH omeprazole / omeprazole ratio in plasma after administration of omeprazole (45).

There are several requirements for phenotypic probes. First, it is essential that individual enzyme or transporter activity is stable. Second, it must be validated that the phenotypic metric that is used reflects enzyme/transporter activity under various circumstances. Thus the metric should change when patients are treated with inhibitors or inducers of the enzyme/transporter, there should be proven in vitro specificity of the metabolic step/transport, there should be a low coefficient of variation for repeated tests and the metric should not depend on other factors not related to enzyme activity (44).

Instead of using one probe at a time, a combination of probes may also be used, provided that no drug interactions occur between the probes. There are several examples of these so called phenotypic cocktails, such as the Leiden cocktail, the Pittsburgh cocktail, the Cologne cocktail and the Cooperstown (5+1) cocktail (44). The latter cocktail includes validated metrics and has been used extensively (46). An excellent example of the use of this cocktail was given by Yeh and colleagues, who demonstrated that the administration of 10 days of lopinavir/ritonavir resulted in significant enzyme induction of CYP1A2, CYP2C9 and CYP2C219 (45).

There are no widely validated phenotypic methods in use for most phase II metabolic enzymes, such as glucuronyltransferases, sulfotransferases and glutathione transferases (44).
In the past, our research group had evaluated the effect of lopinavir/ritonavir and atazanavir on the glucuronidation of lamotrigine. In this thesis, we studied the effect of raltegravir on the glucuronidation of lamotrigine (**chapter 6**).
Although these studies provide important information of the inductive potential of ritonavir on the glucuronidation of the UDP-glucuronyltransferase (UGT) substrate lamotrigine, and the lack of such an effect of unboosted atazanavir and raltegravir, these data cannot be straightaway extrapolated to other UGT substrates. Lamotrigine has not been validated as a phenotypic probe for specific UGT enzymes in vitro or in vivo. In fact, it has become unclear whether lamotrigine is metabolized solely by UGT1A4, or by a UGT1A4 and UGT2B7, or by UGT1A4 and UGT1A3 (28;47;48). Therefore, lamotrigine cannot be considered a selective phenotypic UGT probe.

2.3 Funding of drug-drug interaction studies after marketing approval

The algorithm depicted in Figure 1 ignores the funding which is required for performing drug-drug interaction studies after marketing approval. In our experience, it is difficult to require funding from non-commercial organizations for clinically oriented pharmacology research. Consequently, investigator-driven clinical

pharmacology research is often funded by the pharmaceutical industry. This does not mean that the quality of such research is at stake, but this may have implications for the choice of the drug-drug combinations that are studied.

Obviously, funding of clinical pharmacology HIV research by an unbiased expert committee would be preferable. For the topic of drug-drug interactions, such committees should collect data and questions arising from the clinical field and congresses, in order to decide which drug-drug combinations would need to be studied after marketing approval. In addition, academic researchers who want to perform investigator initiated drug interaction studies should be able to submit study proposals to such expert committees.

The United States have the Aids Clinical Trials Group (ACTG). The ACTG is funded by the US Department of Health and Human Services, the National Institute of Allergy and Infectious Diseases and by the National Institutes of Health, division of AIDS (http://www.aactg.org). The ACTG has published many high-impact papers, such as the recent comparison of the efficacy between the NRTI backbones lamivudine/abacavir and tenofovir/emtricitabine (49). In addition, several key drug-drug interaction studies of antiretroviral drugs with statins were funded by the ACTG (50-52). Since 2007, Europe has the European Aids Treatment Network (NEAT). The future must learn whether NEAT will provide funding for drug-drug interaction studies in the way ACTG does.

3. Future Perspectives

3.1 Therapeutic Drug Monitoring

As explained in section 1 of the general discussion, TDM research should now focus on demonstrating its use in specific clinical scenarios. As can be noted from Table 1, the ATHENA database contains much of the information that is needed to evaluate the use of TDM in those specific situations.

A scenario that could be investigated in ATHENA would be the drug-drug interaction between efavirenz and rifampicin (see **chapter 4).** It would be of interest to investigate whether clinicians use TDM in this situation. In addition, the study could describe the number of subtherapeutic efavirenz plasma concentrations and the number of successful TDM-driven dose increases of efavirenz. Another example of a study that could be conducted in ATHENA would be to investigate the use of TDM

in patients who receive lopinavir in a once-daily dosage. A third potential study would be to describe the use of TDM of all protease inhibitors and NNRTIs during pregnancy, including the number of subtherapeutic drug concentrations in the third trimester and the number of successful TDM-driven interventions in this situation.

A complete other part of future TDM research should focus on new antiretroviral drugs, such as raltegravir, darunavir and etravirine. For none of these drugs, a lower threshold concentration for efficacy has been established and the role of TDM seems therefore limited at this moment. However, a minimum trough concentration for efficacy has been suggested for maraviroc (9) and raltegravir efficacy seems to be related to the raltegravir AUC (53;54).

As noted above, EuroSIDA cannot be used to evaluate the use of TDM. Nonetheless, some interesting research questions on potential correlations between plasma concentrations and clinical endpoints could be investigated in this database. Given the short elimination half-lives of most antiretroviral drugs, only tenofovir, nevirapine and efavirenz would be suitable agents for future pharmacokinetic research in EuroSIDA.

Future EuroSIDA studies could for instance investigate the potential relationship between tenofovir plasma concentrations and nephrotoxicity, which can be expressed in a continuous variable (e.g., serum creatinine) and which develops gradually in time (55). A second idea would be to explore the potential correlations between plasma concentrations and age, which may provide important information in the context of the aging HIV-infected population (24). A third idea, which we are currently working out, is to measure plasma concentrations of protease inhibitors in patients who had virologic failure on protease inhibitor-based cART in the absence of documented resistance. The goal of this study is to assess the role of non-adherence in this specific situation.

3.2 Drug-Drug Interactions

Looking at the future, several aspects need to be addressed. First, there is an ageing population of HIV-infected patients. Therefore, increased use of anticancer drugs and cardiovascular drugs can be expected in the HIV-infected population. Especially for anticancer drugs, studies aimed at investigating the potential drug-drug interactions with antiretroviral drugs are lacking (56). Alternative study designs, using HIV-infected patients who need treatment for cancer, are required to study these interactions. An elegant example of a study with an alternative design

is given by Corona and colleagues. They studied HIV-infected patients with Kaposi's sarcoma who were treated with irinotecan. The researchers managed to perform a crossover design to investigate the pharmacokinetics of irinotecan and its active metabolite SN-38 in the presence and the absence of lopinavir/ritonavir (27).
A second development that needs our attention is the great number of new drugs for the treatment of the hepatitis C virus (HCV) (57). Treating HIV-HCV co-infected patients is challenging, and there will be a clinical need for information on drug-drug interactions between antiretroviral drugs and the new anti-HCV drugs.
Third, there is very little information available on the potential differences between healthy volunteers and HIV-infected patients with regards to the pharmacokinetics of (antiretroviral) drugs. This subject deserves more attention.
Fourth, the use of phenotypic probe studies has the promise of decreasing the number of required subjects for drug interaction trials. However, phenotypic measures need to be validated thoroughly. This is another subject that deserves more attention.
Finally, it would be valuable to evaluate the adoption of dose recommendations coming from drug-drug interaction trials with data from ATHENA or other databases.

4. Conclusions

Clinical pharmacology continues to be highly relevant for HIV treatment. Data from drug-drug interaction trials, pharmacological knowledge, and TDM are essential input factors for the decision-making process in the management of drug-drug interactions. Although the role of TDM has become smaller (i.e., selected use rather than routine use), **chapters 3 and 4** once more demonstrate the important role that TDM can have in individual patient management.

TDM of protease inhibitors and NNRTIs should be employed in vulnerable patient populations (children, pregnant women) and in complex situations, such as with drug-drug interactions. Future TDM research should provide evidence for TDM in these scenarios. In the context of the HIV-infected population, studies aimed at investigating the potential drug-drug interactions between antineoplastic agents and antiretroviral drugs are highly required. Other aspects that need to be explored are the use of phenotypic probes and the adherence to dose recommendations coming from drug-drug interaction studies.

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Summary

Introduction

This thesis has presented a variety of clinical pharmacology studies of HIV therapy. **Part I** of the thesis focuses predominantly on therapeutic drug monitoring (TDM) in HIV-infected adults. **Part II** contains four drug-drug interaction studies of antiretroviral drugs with drugs that are used for the prevention and treatment of co-morbidities in HIV-infected patients.

I. Therapeutic Drug Monitoring

Chapter 1 contains a brief review which discusses the role of TDM in present-day antiretroviral therapy. The review discusses the main arguments in favor and against the use of TDM. The most important arguments in favor of TDM are the correlation of plasma concentrations with virologic response and the high interindividual variability in plasma concentrations among patients taking the same dose. An important argument against the implementation of routine TDM is the lack of randomized controlled trials demonstrating that TDM improves therapeutic outcomes. Therefore, it is concluded that TDM should be used for specific indications, such as in patients with manifestations of concentration-dependent toxicities, patients suspected of non-adherence, pregnant women and children.

The central theme of **chapters 2 to 4** is TDM of efavirenz. Efavirenz is a non-nucleoside reverse-transcriptase inhibitor (NNRTI) that is used as a first line agent in the treatment of HIV infection. A well-known disadvantage of efavirenz are its central nervous system (CNS) side effects, like insomnia, dizziness and headache. More than 50% of the patients treated with efavirenz experience CNS adverse effects after starting efavirenz. These adverse effects are usually transient. Nonetheless, some patients experience persistent CNS toxicity.

A number of studies have described a relationship between persistent CNS toxicity and high efavirenz plasma concentrations (i.e., >4.0 mg/L). However, there is controversy on this subject, because several other studies did not establish an increased risk of CNS toxicity in patients with high efavirenz plasma concentrations.

Given these conflicting data, we performed a large retrospective study with data from the EuroSIDA study (**chapter 2**). The goal of this study was to determine whether patients with high efavirenz plasma concentrations had an increased likelihood of toxicity-driven efavirenz discontinuations. A total of 843 patients were

included in the study. Of these patients, 138 patients (16.4%) discontinued efavirenz due to toxicity or patient's/physician's choice (TOXPC) and 705 (83.6%) patients continued EFV for ≥ 2 years. A total of 20 (14.5%) patients in the TOXPC group had high EFV plasma concentrations (>4.0 mg/L) compared to 99 (14.0%) of the patients in the no toxicity group, p=0.89. A positive hepatitis C status (p=0.026), but not the EFV plasma concentration, was an independent predictor of toxicity-driven EFV discontinuations. Part of the explanation for this result might have been limited power, since most patients who discontinued efavirenz because of CNS toxicity had no plasma sample available. Nonetheless, the results of this study suggest that there is a large number of patients with high efavirenz plasma concentrations that do not experience CNS toxicity to such an extent that they have to stop taking the drug.

Despite the conflicting data described above, pharmacists at our TDM practice advise dose reduction of efavirenz in patients with plasma concentrations above 4.0 mg/L who suffer from persistent CNS toxicity. Anecdotally, this has been reported to be an effective intervention, but the outcome of this intervention had never been formally evaluated.

The retrospective analysis with data from the ATHENA cohort study, described in **chapter 3,** evaluates the effect of this intervention. The analysis included 180 patients with high plasma efavirenz levels. Of these patients, 49 (27.2%) had their efavirenz dose reduced. After one year, the Kaplan-Meier estimated cumulative incidence of toxicity-induced efavirenz discontinuations was 11.5% in patients who continued the standard dose versus 2.3% in patients who had a dose reduction (p=0.07). As expected, dose reduction was not associated with loss of virologic suppression. Therefore, we concluded that dose reduction of efavirenz in patients with high plasma concentrations is safe with regards to maintenance of virologic efficacy. In addition, the results of this study suggest that dose reduction may prevent toxicity-induced discontinuations of efavirenz.

International guidelines suggest that HIV-tuberculosis (TB) co-infected patients are treated with efavirenz -based antiretroviral regimens, because rifampicin decreases efavirenz plasma concentrations only modestly (22-35%). It is recommended to use the standard efavirenz dose (600 mg QD) in patients weighing <50 kg and to consider an increase of the dose (800 mg QD) in patients weighing >50 kg. **Chapter 4** describes a 50 kg weighing rifampicin-treated HIV-TB co-infected patient who had high efavirenz plasma concentrations (10.6 mg/L), and concomitant CNS

toxicities on the standard dosage of efavirenz. Despite concomitant use of rifampicin, the efavirenz dose was decreased to 200 mg once-daily under the guidance of TDM, which resulted in an alleviation of CNS symptoms.

In an attempt to understand this, we performed pharmacogenetic testing of the Cytochrome P450 2B6 (CYP2B6) enzyme, which plays a major role in efavirenz metabolism. The patient turned out to be homozygote for *CYP2B6*15,* which results in very little CYP2B6 enzyme activity. Although rifampicin induces CYP2B6 activity by increasing CYP2B6 gene transcription, increased formation of CYP2B6 mRNA in this patient merely led to enhanced production of poor-functioning CYP2B6 enzyme. Apparently, this does not reverse a poor metabolizer phenotype into a normal metabolizer phenotype. This chapter illustrates the value that TDM and pharmacogenetic testing can have in individualized patient management.

In 2005, the Dutch Association of AIDS Physicians (NVAB) issued guidelines for the treatment and management of HIV-infected patients, including recommendations for TDM. **Chapter 5** provides an evaluation of the uptake of these recommendations in the Netherlands. We selected three scenarios for which the guideline recommended TDM from the ATHENA observational cohort study: i) start with a combination of lopinavir/ritonavir + efavirenz or nevirapine (drug-drug interaction); ii) start with efavirenz (routine TDM), iii) use of nelfinavir during pregnancy. The adherence to the TDM guideline was low for routine TDM in patients who started efavirenz (10 to 30%, depending on the definition), and moderate (45-60%) for the two other scenarios. Patients treated in clinics with the local availability of a TDM assay, and patients treated in academic clinics were more likely to receive TDM. A higher baseline HIV viral load was also predictive for the use of TDM. A potential explanation for the moderate adherence to the guidelines is that most recommendations were based on expert opinion.

II. Drug-drug Interactions

The second part of this thesis contains four drug-drug interaction studies between antiretroviral drugs and drugs being used for the prevention and treatment of co-existing medical conditions.

Atovaquone co-formulated with proguanil is frequently used by western HIV-infected patients who travel to malaria-endemic destinations. Atovaquone is considered a substrate for glucuronidation. Chronic use of protease inhibitors and NNRTIs may induce glucuronidation. Therefore, HIV-infected patients who use protease inhibitors

or NNRTIs might have diminished atovaquone plasma concentrations when taking atovaquone/proguanil, which might in turn lead to suboptimal malaria prophylaxis. The study described in **chapter 6** compares single dose atovaquone/proguanil pharmacokinetics between 18 healthy volunteers and 58 HIV-infected patients, who were using efavirenz (n=20), lopinavir/ritonavir (n=19), or atazanavir/ritonavir (n=19) as part of their antiretroviral regimens. After a single dose of atovaquone/proguanil, the geometric mean (95% confidence interval (95% CI)) atovaquone $AUC_{0\to t}$ was 104 (79-137) h*mg/L in healthy volunteers, compared to 30 (23-39), 32 (24-42), and 64 (49-84) h*mg/L in patients treated with efavirenz, lopinavir/ritonavir, and atazanavir/ritonavir, respectively. The $AUC_{0\to t}$ of proguanil was 38-43% lower in the three groups of HIV-infected patients versus the healthy controls. The study concludes that physicians should be alert for atovaquone/proguanil prophylaxis failures in HIV-infected patients who are treated with efavirenz, lopinavir/ritonavir or, to a lower extent, atazanavir/ritonavir.

Chapter 7 and **chapter 8** contain two drug-drug interaction studies with the recently approved HIV-1 integrase inhibitor raltegravir. Raltegravir is metabolized by UDP glucuronyltransferase (UGT) 1A1 and hence its pharmacokinetics can be influenced by inhibitors (e.g., atazanavir) or inducers (e.g., etravirine, tipranavir, rifampicin) of UGT1A1. The influence of raltegravir itself on UGT substrates had not been evaluated in clinical studies. Therefore, we undertook the study described in **chapter 7** to investigate the influence of raltegravir on the UGT substrate lamotrigine. The results of this study, which was conducted in 24 healthy male volunteers, show no effect of raltegravir on the glucuronidation of lamotrigine.

Dyslipidemia is a common complication during chronic HIV infection. Pravastatin is considered a preferred lipid-lowering drug for HIV-infected patients. As a consequence, frequent combined use of raltegravir and pravastatin can be expected in the ageing HIV-infected population. Therefore, and because both drugs share a common metabolic pathway, we studied the effect of raltegravir on pravastatin pharmacokinetics and vice-versa in 24 healthy male and female volunteers (**chapter 8)**. The results of this study show no effect of raltegravir on the pharmacokinetics or the short-term lipid-lowering effects of pravastatin. On the other hand, pravastatin increased the C_{max} (+31%) and the AUC of raltegravir (+13%), whereas the raltegravir C_{12} was 41% decreased in the presence of raltegravir. Because the AUC of raltegravir appears to be related to virologic efficacy, and not the raltegravir C_{min}, the effects of pravastatin on raltegravir pharmacokinetics are not likely to be clinically relevant.

Finally, **chapter 9** contains a drug-drug interaction study of the combined use of the protease inhibitor fosamprenavir and the antifungal agent posaconazole. Fosamprenavir is normally used with ritonavir, which serves as a booster of the pharmacokinetics of amprenavir through inhibition of CYP3A. Combining fosamprenavir/ritonavir with posaconazole may lead to a bidirectional drug-drug interaction. First, inhibition of CYP3A4 by posaconazole (above that already caused by ritonavir) may further increase exposure to amprenavir with an increased risk of fosamprenavir toxicity. Second, ritonavir might induce posaconazole glucuronidation. Thus, combined use of fosamprenavir/ritonavir with posaconazole might lead to subtherapeutic posaconazole exposure.

To manage the interaction between fosamprenavir/ritonavir and posaconazole, we hypothesized that ritonavir could be replaced by posaconazole as an alternative booster of the pharmacokinetics of fosamprenavir, with the additional advantage of eliminating the potential negative effect of ritonavir on posaconazole. Therefore, we performed a clinical trial in 24 healthy volunteers to determine the effect of unboosted fosamprenavir on posaconazole and vice versa. The geometric mean ratios (GMR; 90% CI) $AUC_{0\rightarrow12}$ and C_{max} for posaconazole + fosamprenavir versus posaconazole alone were 0.77 (0.68-0.87) and 0.79 (0.71-0.89), respectively. In addition, posaconazole was not capable of boosting the amprenavir pharmacokinetics in a similar way as ritonavir: the GMR (90% CI) of the amprenavir $AUC_{0\rightarrow12}$ and C_{12} for fosamprenavir + posaconazole versus fosamprenavir + ritonavir were 0.35 (0.32-0.39) and 0.64 (0.55-0.76), respectively. Therefore, the conclusion of this study was that the combination of posaconazole and unboosted fosamprenavir should not be used in HIV-infected patients.

Discussion

The discussion starts with a short description of the history of TDM in HIV therapy. Whereas there is evidence that routine TDM benefits patient outcome for indinavir and nelfinavir, the evidence is lacking for current first-line antiretroviral drugs. Therefore, it is concluded that TDM should not be used routinely, but for specific indications, such as with drug-drug interactions or in patients with manifestations of concentration-dependent toxicities. Because there is also little evidence for TDM in these situations, TDM research should be aimed at investigating the potential benefits of TDM for these specific clinical scenarios. It seems most feasible to perform these studies retrospectively, for instance by using data from the ATHENA cohort study.

The second part of the discussion focuses on drug-drug interaction studies during phase 4, thus after marketing approval. The general discussion presents an algorithm for phase 4 drug-drug interaction studies, which may help to decide whether a potential drug-drug interaction should be studied.

The study design of drug-drug interaction studies is also discussed. In principle, the optimal design would be a cross-over trial in healthy volunteers. However, there are several situations in which another design, for instance in HIV-infected patients, seems preferable. Other subjects in the second part of the general discussion are the use of phenotypic probe studies and the funding of drug-drug interaction studies. Finally, the general discussion concludes with recommendations for future research. For instance, given the ageing HIV-infected population, more knowledge will be required on drug-drug interactions between antineoplastic agents and antiretroviral drugs.

Samenvatting

Inleiding

Dit proefschrift bevat negen hoofdstukken. Alle hoofdstukken zijn gewijd aan de behandeling van HIV, waarbij steeds voor een klinisch farmacologische invalshoek is gekozen. **Deel 1** van het proefschrift gaat voornamelijk in op de rol van 'therapeutic drug monitoring' (TDM) als onderdeel van de HIV behandeling. **Deel 2** van het proefschrift bevat 4 onderzoeken naar interacties tussen anti- HIV geneesmiddelen en geneesmiddelen die door HIV geïnfecteerde patiënten gebruikt kunnen worden voor de preventie of behandeling van bijkomende ziektes.

I. Therapeutic Drug Monitoring

Hoofdstuk 1 geeft een overzicht van de verschillende argumenten voor én tegen het gebruik van TDM bij de behandeling van HIV. Het belangrijkste argument vóór het gebruik van TDM is het grote verschil in plasma concentraties tussen personen die dezelfde dosering gebruiken, en het feit dat deze verschillen gevolgen hebben voor het al dan niet slagen van de HIV behandeling. Er zijn namelijk relaties aangetoond tussen de hoogte van de plasmaconcentraties van anti- HIV middelen en de virologische respons. Er zijn ook argumenten tegen de inzet van TDM. Er is maar voor 2 anti- HIV middelen, te weten indinavir en nelfinavir, middels een gerandomiseerd gecontroleerd onderzoek aangetoond dat TDM de behandel uitkomst verbetert. Voor alle andere anti- HIV middelen zijn deze gegevens niet aanwezig, of alleen uit onderzoeksopzetten met een minder sterke mate van bewijskracht. **Hoofdstuk 1** eindigt daarom met de conclusie dat TDM niet zonder meer moet worden toegepast in alle patiënten die anti- HIV middelen gebruiken, maar dat TDM moet worden ingezet in díe situaties waarin er de grootste kans bestaat op afwijkende plasma concentraties. Voorbeelden zijn patiënten met concentratie gerelateerde bijwerkingen, patiënten die worden verdacht van therapie ontrouw, zwangere vrouwen en kinderen.

In de **hoofdstukken 2 tot en met 4** staat het anti- HIV middel efavirenz centraal. Efavirenz is een middel uit de klasse van de non-nucleoside reverse trancriptase remmers (NNRTRs), dat op dit moment veel wordt voorgeschreven aan HIV patiënten. Een nadeel van efavirenz is dat meer dan 50% van de patiënten na aanvang van therapie tijdelijk last krijgt van centraal zenuwstelsel bijwerkingen, zoals hoofdpijn, een slechte nachtrust of duizeligheid. Bij de meeste patiënten verdwijnen deze bijwerkingen binnen enkele weken. Echter, bij sommige patiënten persisteert de bijwerking.

Een aantal onderzoeken heeft een relatie gevonden tussen de hierboven genoemde bijwerkingen en de hoogte van de efavirenz concentratie in plasma. Het bestaan van deze relatie is echter controversieel, omdat andere onderzoeken deze relatie niet hebben kunnen vinden.

Hoofdstuk 2 beschrijft een groot retrospectief onderzoek naar deze relatie, dat werd uitgevoerd met gegevens van de EuroSIDA studie. Het doel van dit onderzoek was om te bepalen of er een relatie was tussen de hoogte van de efavirenz plasma concentratie en het risico op het stoppen met efavirenz vanwege bijwerkingen. Er werden 843 patiënten geïncludeerd. Hiervan stopten er 138 (16.4%) binnen 2 jaar vanwege toxiciteit of op instigatie van arts of patiënt. In de groep patiënten die voortijdig stopte had 14.5% een efavirenz plasma concentratie boven de 4.0 mg/L. In de groep patiënten die ten minste twee jaar efavirenz bleef gebruiken had 14.0% een efavirenz plasma concentratie boven de 4.0 mg/L ($p=0.89$). Uit de multivariabele logistische regressie analyse bleek een positieve hepatitis C status ($p=0.026$), maar niet de efavirenz plasma concentratie, een onafhankelijke voorspeller voor het stoppen van efavirenz vanwege toxiciteit.
Een mogelijke verklaring voor deze resultaten is dat veel patiënten die met efavirenz moesten stoppen vanwege bijwerkingen (en dus misschien een hoge spiegel hadden), al waren gestopt voordat bij hen een bloedmonster was afgenomen. Daarnaast suggereert het onderzoek dat een groot aantal patiënten met een hoge efavirenz spiegel niet in zo'n mate last heeft van bijwerkingen dat dit leidt tot stoppen van efavirenz.

Ondanks de hierboven genoemde tegenstrijdige gegevens, adviseren ziekenhuis-apothekers om bij patiënten met bijwerkingen en een hoge spiegel (>4.0 mg/L) de efavirenz dosering te verlagen. Formeel is niet bekend is of deze interventie veilig en zinvol is. **Hoofdstuk 3** beschrijft een retrospectief onderzoek naar het effect van deze interventie.
Hiertoe werden gegevens gebruikt van de ATHENA cohort studie. Er werden in totaal 180 patiënten met hoge efavirenz spiegels (>4.0 mg/L) geïncludeerd in dit onderzoek. De minderheid van deze patiënten ($n=49$, 27.2%) kreeg een dosisreductie; de andere 131 patiënten continueerden de standaard efavirenz dosering. Na 1 jaar was de met Kaplan-Meier geschatte cumulatieve incidentie van het stoppen van efavirenz vanwege bijwerkingen 11.5% in de groep patiënten die doorgingen met de standaard dosering versus 2.3% in patiënten die een dosisreductie kregen ($p=0.07$). Dosisreductie was niet geassocieerd met een verminderde onderdrukking van de virusreplicatie.

De conclusie van dit onderzoek is dat het veilig is om bij patiënten met efavirenz spiegels boven de 4.0 mg/L de dosering te verlagen op geleide van TDM. Daarnaast lijkt het erop dat deze interventie het stoppen van efavirenz vanwege bijwerkingen kan helpen te voorkomen.

Internationale richtlijnen bevelen aan om HIV patiënten die tevens geïnfecteerd zijn met tuberculose, te behandelen met efavirenz bevattende antiretrovirale regimes. De richtlijnen adviseren om bij patiënten met een lichaamsgewicht kleiner dan 50 kg te starten met de standaard dosering efavirenz (1 x daags 600 mg) en om een hogere dosering (1x daags 800 mg) te overwegen bij patiënten die zwaarder zijn dan 50-60 kg.

Hoofdstuk 4 beschrijft een 50 kg wegende, met rifampicine behandelde HIV-tuberculose geïnfecteerde patiënt die met de standaard dosering efavirenz zeer hoge efavirenz spiegels (tot 10.6 mg/L) had. Daarbij had hij veel last van centrale bijwerkingen. Op basis van de gemeten efavirenz plasma concentraties werd de efavirenz dosering, ondanks gebruik van rifampicine, uiteindelijk verlaagd tot 1x daags 200 mg, waarbij de centrale bijwerkingen aanzienlijk afnamen.

Genetisch onderzoek van het CYP2B6 enzym, dat verantwoordelijk is voor het metabolisme van efavirenz, leerde dat deze patiënt homozygoot was voor *CYP2B6*15.* Dit genotype zorgde ervoor dat deze patiënt een nauwelijks functionerend CYP2B6 enzym had. Toediening van rifampicine zorgde bij deze patiënt dan ook slechts voor een toegenomen productie van niet functionerend CYP2B6 enzym. Hierdoor was bij deze patiënt een zeer lage dosering efavirenz nodig, ondanks gebruik van rifampicine. Deze casus onderstreept de toegevoegde waarde die TDM en farmacogenetisch onderzoek kunnen hebben bij de HIV behandeling.

Hoofdstuk 5 bevat een evaluatie van de implementatie van de Nederlandse HIV TDM richtlijnen uit 2005. Hierbij werd gekeken naar het gebruik van TDM bij een interactie (lopinavir/ritonavir + een NNRTR), het gebruik van nelfinavir TDM in de zwangerschap en het routinematig gebruik van TDM bij patiënten die efavirenz therapie startten. Met name voor dit laatste scenario werd de richtlijn zeer matig nageleefd (10-30%, afhankelijk van de gehanteerde definitie); voor de eerste twee scenario's kreeg 45-60% van de patiënten TDM volgens de richtlijn. Uit het onderzoek bleek dat de TDM richtlijn beter werd nageleefd in die HIV behandelcentra waar lokaal een analytische bepalingsmethode aanwezig was voor de anti- HIV middelen. Verder bleken academische HIV behandelcentra de richtlijn meer te hebben gevolgd. Ten slotte bleek de virale load bij start van therapie voorspellend

voor de inzet van TDM. Een mogelijke verklaring voor de matige navolging van de TDM richtlijn is dat de bewijskracht voor de meeste aanbevelingen niet was gebaseerd op hard bewijs, maar op de mening van experts.

Deel II. Interacties

Deel II van dit proefschrift bevat 4 onderzoeken naar mogelijke interacties tussen anti- HIV middelen en geneesmiddelen die door HIV geïnfecteerde patiënten gebruikt kunnen worden voor de preventie of behandeling van bijkomende ziektes.

Atovaquone/proguanil wordt veelvuldig voorgeschreven aan reizigers die naar de tropen gaan om malaria te voorkomen, en dus ook aan HIV geïnfecteerde reizigers die chronisch anti- HIV medicatie gebruiken. Van atovaquone wordt verondersteld dat het wordt gemetaboliseerd door middel van glucuronidering. Langdurig gebruik van HIV proteaseremmers of NNRTRs kan glucuronidering induceren (versnellen). Daardoor is het mogelijk dat HIV patiënten die proteaseremmers of NNRTRs gebruiken verlaagde atovaquone plasma concentraties hebben wanneer zij atovaquone/proguanil gebruiken. Dit zou verminderde werkzaamheid van atovaquone/proguanil als malaria profylaxe tot gevolg kunnen hebben.
Het onderzoek beschreven in **hoofdstuk 6** vergelijkt de farmacokinetiek van atovaquone en proguanil na een éénmalige dosis atovaquone/proguanil tussen 18 gezonde vrijwilligers enerzijds en 58 HIV geïnfecteerde patiënten anderzijds, die de proteaseremmers lopinavir/ritonavir (n=19), atazanavir/ritonavir (n=19) of de NNRTR efavirenz (n=20) gebruikten. Na een éénmalige dosis atovaquone/proguanil was het geometrisch gemiddelde (95% betrouwbaarheidsinterval, BI) van de $AUC_{0\rightarrow t}$ van atovaquone 104 (79-137) h*mg/L in de gezonde vrijwilligers versus 30 (23-39), 32 (24-42) en 64 (49-84) h*mg/L in HIV patiënten die respectievelijk efavirenz, lopinavir/ritonavir of atazanavir/ritonavir gebruikten. Ook de blootstelling aan proguanil bleek beïnvloed: de $AUC_{0\rightarrow t}$ van proguanil was 38-43% lager in de 3 groepen HIV patiënten in vergelijking met de gezonde vrijwilligers. Er dient dus rekening te worden gehouden met verminderde werkzaamheid van atovaquone/ proguanil in HIV patiënten die efavirenz, lopinavir/ritonavir of atazanavir/ritonavir gebruiken.

Hoofdstuk 7 en **hoofdstuk 8** bevatten interactie onderzoeken met een nieuw anti-HIV middel, raltegravir. Raltegravir heeft een ander werkingsmechanisme dan de middelen die tot nu toe werden gebruikt bij de HIV behandeling. Het voorkomt de incorporatie van HIV DNA in humaan DNA. Raltegravir wordt gemetaboliseerd door

UDP-glucuronyltransferase (UGT) 1A1. Dientengevolge kan de farmacokinetiek van raltegravir worden beïnvloed door remmers van UGT1A1 (zoals atazanavir) of door inducers (zoals etravirine, tipranavir, rifampicine) van UGT1A1.
De invloed van raltegravir zelf op andere stoffen die door middel van glucuronidering worden afgebroken was nog niet onderzocht in een klinische studie. Om deze reden onderzochten wij de invloed van raltegravir op de glucuronidering van het anti epilepticum lamotrigine **(hoofdstuk 7)**. Uit dit onderzoek, dat werd uitgevoerd in 24 gezonde mannelijke vrijwilligers, bleek uiteindelijk geen invloed van raltegravir op de glucuronidering van lamotrigine.

Uit de literatuur en de klinische praktijk is bekend dat HIV patienten relatief vaak een verstoorde lipide huishouding hebben. Statines zijn effectieve geneesmiddelen in de behandeling van dyslipidemie; pravastatine is een statine dat veel door HIV patiënten wordt gebruikt. Veelvuldig gecombineerd gebruik van raltegravir en pravastatine is dus te verwachten in HIV patiënten. Omdat pravastatine en raltegravir beide door middel van glucuronidering worden afgebroken, bestond er de kans op een interactie tussen deze middelen. Om dit te onderzoeken, voerden wij de studie uit welke beschreven is in **hoofdstuk 8.** Uit dit onderzoek, dat werd uitgevoerd in 24 gezonde vrijwilligers, bleek dat raltegravir geen invloed had op de plasma concentraties van pravastatine, noch op de cholesterol verlagende werking van pravastatine.
Pravastatine beïnvloedde wel de raltegravir concentraties in enige mate. Zo werden de gemiddelde C_{max} en AUC van raltegravir met respectievelijk 31 en 13% verhoogd. De C_{min} van raltegravir was echter juist 41% lager met pravastatine. Omdat de AUC van raltegravir en niet de C_{min} van belang is voor het virologisch effect van dit middel, zijn deze effecten naar verwachting niet klinisch relevant.

Hoofdstuk 9 beschrijft ten slotte een onderzoek naar de interactie tussen het anti schimmel middel posaconazol en de HIV- protease remmer fosamprenavir. Fosamprenavir wordt normaal gesproken gebruikt in combinatie met ritonavir, wat door remming van CYP3A zorgt voor voldoende blootstelling aan amprenavir. Posaconazol remt echter ook CYP3A. Hierdoor zou toevoeging van posaconazol aan fosamprenavir/ritonavir tot sterk verhoogde amprenavir spiegels kunnen leiden. Aan de andere kant zou de combinatie van fosamprenavir/ritonavir en posaconazol tot lagere posaconazol spiegels kunnen leiden. Posaconazol wordt namelijk afgebroken door middel van glucuronidering, en het is bekend dat ritonavir glucuronidering kan induceren. In **hoofdstuk 9** werd onderzocht of de hierboven beschreven interactie kon worden ondervangen door ritonavir weg te laten bij

gecombineerd gebruik van fosamprenavir en posaconazol. Hierdoor zou het negatieve effect van ritonavir op posaconazol spiegels verdwijnen. Daarnaast moest worden onderzocht of posaconazol de amprenavir spiegels in dezelfde mate zou verhogen als ritonavir. Dit laatste bleek niet het geval: de ratio van het geometrisch gemiddelde (90% BI) die werd verkregen met fosamprenavir + posaconazol versus fosamprenavir + ritonavir was 0.35 (0.32-0.39) voor de $AUC_{0\rightarrow12}$ van amprenavir en 0.64 (0.55-0.76) voor de C_{max} van amprenavir. Ook bleek de blootstelling aan posaconazol met 23% verlaagd in aanwezigheid van fosamprenavir, ondanks weglaten van ritonavir. De conclusie van dit onderzoek is dan ook dat de combinatie fosamprenavir - posaconazol zonder ritonavir niet aan HIV patiënten moet worden voorgeschreven.

Discussie

Het proefschrift sluit af met een discussie, welke bestaat uit 2 delen. Het eerste deel van de discussie is gewijd aan TDM. Er wordt een korte beschrijving gegeven van de historie van TDM bij de behandeling van HIV. Verder wordt geconcludeerd dat TDM anno 2010 niet routinematig bij iedere patiënt dient te worden ingezet, maar wel bij specifieke indicaties, zoals bij interacties, gedurende de zwangerschap of bij verdenking van therapie ontrouw. Daarnaast wordt er gesteld dat het van belang is om bewijs te vinden voor het (verwachte) nut van TDM bij deze indicaties. Er wordt uitgebreid ingegaan op de vraag hoe dit bewijs te verkrijgen, waarbij een pleidooi wordt gehouden voor retrospectief TDM onderzoek met behulp van de EuroSIDA en ATHENA databases.

Het tweede deel van de discussie gaat nader in op interactie onderzoeken met anti- HIV middelen nadat zij zijn toegelaten op de markt (gedurende fase 4). Er wordt een algoritme gepresenteerd dat gehanteerd kan worden bij de beslissing om al dan niet een mogelijke interactie te onderzoeken. Daarnaast wordt ingegaan op de designs van interactie onderzoeken. Er wordt gesteld dat een cross-over design in gezonde vrijwilligers in principe het beste design is, maar dat er ook situaties zijn waarin een dergelijk design juist niet te verkiezen is. Ook voor dit onderwerp wordt een algoritme gepresenteerd. Andere onderwerpen die in dit deel van de discussie aan bod komen zijn probe studies voor fenotypering en de financiering van interactie onderzoeken.

Tot slot worden aanbevelingen gedaan voor toekomstig onderzoek. Zo wordt er aanbevolen om in de toekomst meer aandacht te besteden aan interacties tussen cytostatica en anti- HIV middelen.

Dankwoord

Dankwoord

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Curriculum Vitae

Matthijs van Luin werd geboren op 20 maart 1978 te Leeuwarden. Hij groeide op in de Friese hoofdstad en behaalde in 1997 zijn VWO diploma aan het Stedelijk Gymnasium. Aansluitend werd begonnen met de studie Farmacie in Groningen, alwaar hij in maart 2003 het doctoraal examen behaalde. Het apothekers-examen werd vervolgens in augustus 2004 behaald. Van september 2004 tot februari 2005 werkte Matthijs als projectapotheker binnen de Alysis Zorggroep (ziekenhuis Rijnstate) te Arnhem. In maart 2005 begon Matthijs met de opleiding tot ziekenhuisapotheker in datzelfde ziekenhuis. Van 1 januari 2007 tot 1 maart 2010 combineerde Matthijs deze opleiding met promotieonderzoek binnen de afdeling Apotheek/Klinische Farmacie van het UMC St Radboud. Sinds 1 maart 2010 werkt Matthijs als ziekenhuisapotheker binnen de Alysis Zorggroep, met als voornaamste aandachtsgebieden de farmaceutische analyse, therapeutic drug monitoring en toxicologie. Matthijs woont samen met zijn vriendin Ellen van Vegten.