Application of Pharmacokinetics to Improve Antiretroviral Treatment

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Application of Pharmacokinetics to Improve Antiretroviral Treatment

Toepassing van farmacokinetiek
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(met een samenvatting in het Nederlands)

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Contents

Chapter 1.

Introduction

HIV infection and AIDS

In 1981 the first cases of the acquired immunodeficiency syndrome (AIDS) were described in previously healthy homosexual males [1,2]. Two years afterwards a retrovirus was isolated and identified as the causative agent of AIDS [3,4], and this virus was eventually classified as human immunodeficiency virus 1 (HIV-1). HIV infection can be acquired through unprotected sexual intercourse, injectable drug use with contaminated needles, receipt of contaminated blood products, and mother-to-child transmission. The virus infects cells of the immune system that bear the CD4+ receptor, specifically the CD4 lymphocyte [5]. The host cells are used by the virus for its replication and are eventually destroyed. This and other complex immune alterations cause profound cellular immunodeficiency.

The clinical implications of infection with HIV-1 can be characterized as an acute (primary) infection, a prolonged asymptomatic phase, and a symptomatic disease, ultimately leading to death from opportunistic infections or malignancies [6]. Acute HIV infection is associated with high levels of plasma viraemia and a transient depletion of CD4 cells, whereas patients may complain of nonspecific flu-like symptoms. The asymptomatic phase is characterized by ongoing viral replication and a gradual decrease in CD4 cell counts. AIDS is diagnosed upon the occurrence of specific opportunistic infections or malignancies. The majority of untreated patients develop AIDS within 10 years after infection with HIV-1, and most untreated patients die within two years after diagnosis of AIDS.
The course of infection with HIV (and the effects of antiretroviral therapy) can be monitored through two surrogate markers, the viral load and the CD4 cell count [7]. The viral load represents the number of circulating HIV RNA particles in the plasma and predicts the rate of decrease in CD4 cell count and progression to AIDS and death. The CD4 cell count indicates the extent of HIV-induced immune damage already suffered.

Infection with HIV-1 is one of the greatest challenges facing the world today. It is estimated that more than 40 million people have been infected with HIV-1 worldwide, of whom 20 million have already died. Another retrovirus, HIV-2, is a prevalent cause

of AIDS in West-Africa, but this virus appears to be less pathogenic than HIV-1 [8]. Fortunately, improved understanding of the pathogenesis of HIV infection has led to rational drug development. The introduction of new antiretroviral drugs since 1995 allowed for chronic treatment of HIV-infected patients with combinations of antiretroviral drugs, which are referred to as "highly active antiretroviral therapy" (HAART). Treatment with HAART enabled a reduction of the viral load to undetectable levels and an increase in CD4 cell count in many patients who have access to these regimens. These effects have translated into a major decline in HIV-related morbidity and mortality, at least in the developed world [9]. Researchers have even speculated about eradicating HIV with HAART, but this is not feasible yet [10].
Despite much progress, it has become increasingly clear that the benefits of antiretroviral therapy are not durable in many patients, due to a number of interrelated factors. As a result, new strategies for the optimization of response to antiretroviral drugs are being proposed and evaluated continuously.

Antiretroviral drugs

Each of subsequent steps in the HIV-1 life cycle, from infection of the host cell to the release of new viruses, represents a potential drug target. Currently available drugs (table 1) act by inhibiting the viral enzymes reverse transcriptase or protease. Reverse transcriptase catalyses the process of reverse transcription of the single stranded viral RNA into double stranded proviral DNA. Protease is involved in the cleavage of polyproteins, an essential step in the maturation of newly formed virions.

Table 1. Currently licensed nucleoside reverse transcriptase inhibitors (NRTIs), nucleotide reverse transcriptase inhibitors (NtRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), and protease inhibitors (PIs)

NRTIs	NtRTI	NNRTIs	PIs
abacavir (ABC)	tenofovir	delavirdine	amprenavir
didanosine (ddI)		efavirenz	indinavir
lamivudine (3TC)		nevirapine	lopinavir (coformulated with ritonavir)
stavudine (d4T)			nelfinavir
zalcitabine (ddC)			ritonavir
zidovudine (ZDV, AZT)			saquinavir

The *nucleoside reverse transcriptase inhibitors (NRTIs)* are phosphorylated intracellularly into their active triphosphate moieties [11]. These triphosphate anabolites are substrates for and inhibitors of reverse transcriptase. Importantly, plasma concentrations of the parent NRTIs do not appear to correlate with intracellular triphosphate concentrations, or the efficacy of these drugs.
Tenofovir is the first approved *nucleotide reverse transcriptase inhibitor (NtRTI)*. This drug acts in a similar way to nucleoside analogues by inhibiting reverse transcriptase, but has an abbreviated intracellular activation pathway [11,12]. Tenofovir is currently licensed for use in patients who have failed antiretroviral therapy.
Non-nucleoside reverse transcriptase inhibitors (NNRTIs) are a class of chemically distinct compounds that block reverse transcriptase activity by binding adjacent to the enzyme's active site, inducing conformational changes in this site [13]. Unlike NRTIs, NNRTIs do not need to be phosphorylated to become active. The compounds in this class are metabolized by the cytochrome (CYP) P450 system and thus are prone to drug interactions. In addition, efavirenz and nevirapine are inducers of CYP3A4 themselves, which may cause interactions with other drugs that are metabolized by this iso-enzyme. The elimination half-lives of efavirenz and nevirapine are long (40-55 and 25-30 h respectively). Consequently once-daily administration of these drugs is approved (efavirenz) or being evaluated (nevirapine). NNRTIs have no activity against HIV-2.
Protease inhibitors (PIs) do not need to be metabolized intracellularly to become active [14,15]. Individual PIs differ with respect to the necessity to ingest them with or without food. All PIs undergo oxidative metabolism by CYP3A4, and additional CYP isoforms metabolize individual PIs. In view of their metabolic pattern, PIs are susceptible to drug interactions involving P450 inhibitors or inducers. In addition, they illicit variable effects on other drugs, acting as inducers or inhibitors of CYP P450 iso-enzymes. Especially ritonavir is a strong inhibitor of CYP3A4. PIs are also substrates and, in some cases, inhibitors of p-glycoprotein.The short half-life of the PIs demands twice or thrice daily dosing regimens. Nelfinavir forms a metabolite that circulates at appreciable levels in plasma [16].

Current guidelines for the use of antiretroviral drugs recommend the combination of at least three antiretroviral drugs in a HAART regimen. More specifically, two NRTIs should be combined with either one PI, a PI combined with a low-dose of ritonavir (to increase plasma levels of the former PI), two PIs, an NNRTI, or a third NRTI.

Reasons for inadequate response to HAART

Inadequate response to HAART is denoted as "treatment failure" or "virological failure". Treatment failure refers to discontinuation or modification of therapy (switching to other drugs) for any reason. In one large cohort study, 36% of patients had discontinued their first HAART regimen after one year of follow-up [17]. Virological failure relates to patients who do not achieve adequate suppression of plasma HIV RNA, i.e. an undetectable viral load. The percentage of treatment-naive patients with an undetectable viral load varies from 10 to 50% after one year of therapy [18-21].
The most prominent causes for the high rates of treatment failure and virological failure in HIV-infection are inadequate adherence, pharmacokinetic variability caused by drug interactions and other factors, emergence of viral resistance, and the occurrence of adverse reactions to HAART. These factors are all related to eachother, with the plasma concentration of antiretroviral drugs serving as an intermediary liaison.

Inadequate adherence

In terms of taking drugs, nonadherence may mean not taking medication at all, omission of doses, not taking doses at prescribed frequencies or intervals, or not matching medication to food requirements. Treatment of HIV infection appears to be very unforgiving for inadequate adherence. One study showed that more than 95% adherence to PIs (i.e percent of doses taken) is required to achieve an undetectable viral load in more than 80% of treated patients [22]. The close association between adherence and virological response is likely to be mediated by plasma drug concentrations, that is only high adherence rates lead to continuously adequate plasma drug levels and satisfactory response.
Although reasons for nonadherence are generally multifaced in nature, the complexity of HAART regimens is assumed to have a weighty, negative impact on adherence rates in the treatment of HIV infection [23]. Consequently, efforts are being made to develop less complex HAART regimens, especially regimens that can be dosed once-daily, while incorporating the necessary potency [24].

Pharmacokinetic interactions and other determinants of pharmacokinetic variability

Drug interactions seriously complicate the treatment of HIV infection [25,26]. Apart from three or more antiretrovirals, patients may be taking drugs for opportunistic infections, concurrent diseases and treatment of adverse events. PIs and NNRTIs are especially prone to be involved in pharmacokinetic drug-drug interactions. Drug-food interactions further accentuate the interaction potential of PIs. All such interactions may cause undesirably low or high plasma concentrations of PIs and NNRTIs in a subgroup of patients, and this may cause inadequate suppression of viral replication and emergence of viral resistance (low plasma concentrations) or concentration-related toxicity (high plasma concentrations). Fortunately, this source of variability can be corrected after evaluation of potential interactions in pharmacokinetic studies. Based on such studies, dose recommendations for specific drug combinations can be given.

Although drug interactions remain a hazardous complication of antiretroviral therapy, they can also be exploited as a means to optimize the pharmacokinetics and pharmacodynamics of antiretroviral drugs. Nowadays, PIs are often combined with a low ("baby") dose of ritonavir, a strong inhibitor of CYP3A, to increase ("boost") the PI plasma levels [27]. This approach permits a reduction in the frequency of dosing and a reduction in pill burden, thereby potentially facilitating adherence. In addition, co-administration of low-dose ritonavir compensates for certain undesirable drug interactions, and higher plasma levels may suppress (or overcome) resistant viral strains.

Apart from pharmacokinetic interactions, there are other determinants of pharmacokinetic variability that affect plasma concentrations of PIs and NNRTIs, e.g. genetics, age and gender. Genetic determinants appear to be particularly relevant, as they cause large interindividual variability in the activity of enzymes that metabolize PIs and NNRTIs [28], as well as differences in the activity of proteins that transport these drugs through cellular membranes [29]. Genetics and other determinants of pharmacokinetic variability are held responsible for the strong interindividual variability in plasma levels of PIs and NNRTIs that remains when patients are strictly

adherent to antiretroviral therapy without drug interactions [30-34]. In contrast to variability due to pharmacokinetic interactions, these intrinsic types of pharmacokinetic variability can not be corrected. However, abnormally low or high plasma levels in individual patients could be detected by measurement of drug concentrations (Therapeutic Drug Monitoring, TDM).

Emergence of viral resistance

The occurrence of resistance is associated with specific mutations in viral genes. Expression of these mutated genes will affect the interaction of drugs with their target enzymes. It is assumed that (marginally) resistant viral strains pre-exist at low frequencies in antiretroviral drug-naive, predominantly wild-type virus populations. In addition, mutations can be generated *de novo*, but only if a virus is actively replicating.

Emergence of resistance requires the presence of an antiviral drug in partially suppressive concentrations [35,36]. Under these circumstances, active replication of wild-type virus could occur and favor the chance of *de novo* formation of mutated viruses. In addition, pre-existing or new resistant viruses will compete favorably with (and outgrow) the wild-type virus in the presence of suboptimal drug levels; the drug provides "selection pressure". These events will not occur if a patient does not take drug at all (plasma concentration = 0), since the wild-type virus is then permitted to flourish (no selection pressure). If the plasma concentrations are above certain thresholds, the patient will tend to remain mutant-free as well, since the viral replication of wild-type and pre-existing mutant viruses is completely blocked, and *de novo* generation of mutations is prevented.

Emergence of resistance may limit the remaining treatment options for a patient, considering that extensive cross-resistance exists between antiretroviral agents of the same class. The concept of HAART is to combine multiple drugs as a means to limit the emergence of resistance. Any virus resistant to one drug in a regimen is suppressed by other drugs. The most likely scenario for selecting resistant viruses when using multiple drugs is one in which the patient does not have three or more active drugs present all of the time. This can be due to either inadequate adherence, the occurrence of pharmacokinetic interactions or large variability in plasma levels.

Adverse events to HAART

Apart from insufficient efficacy, antiretroviral therapy is also complicated by a high incidence of drug-related adverse events [37]. This may affect adherence, and contribute to high rates of treatment discontinuations and switches in the treatment of HIV-infection.
The etiology of many adverse events remains an area of research. Several adverse events appear to be drug class specific, and some are drug specific. In addition, the incidence or severity of some adverse events appears to be associated with antiretroviral plasma concentrations. Relationships have been found between plasma concentrations and toxicity of indinavir [38], ritonavir [39], amprenavir [40], nelfinavir [41] and efavirenz [33].

Optimization of dosing recommendations for antiretroviral drugs

Clinical pharmacology is defined as the scientific study of drugs in man [42]. The discipline of clinical pharmacology was added to the discipline of *pharmacology* (the study of drugs) to provide a scientific basis of *therapeutics* [43], which is the application of drugs to treat diseases.
Clinical pharmacology is traditionally divided into two disciplines, pharmacokinetics and pharmacodynamics. *Pharmacokinetics* is the study of the relationship between the dose administered and the (time course of the) plasma or blood concentration achieved, and

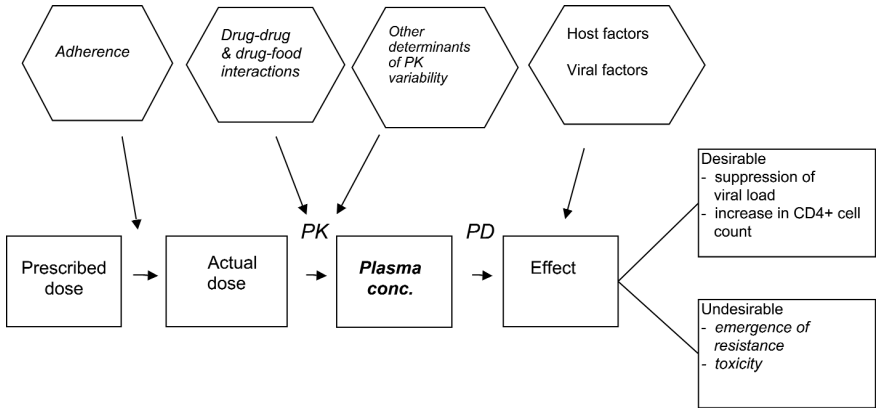

Figure 1. Conceptual model for the treatment of HIV infection.
Abbreviations: PK: pharmacokinetic(s), PD: pharmacodynamic(s), conc.: concentration.

pharmacodynamics is the study of the relationship between concentration and effect. The interest of these two disciplines corresponds to two phases that can be discerned after administration of a drug, the pharmacokinetic phase and the pharmacodynamic phase. Figure 1 shows these two phases in a conceptual model for the treatment of HIV infection (adapted from [44,45]), viewed from a clinical pharmacological perspective. The model shows the plasma drug level as a connecting link between major reasons for inadequate response to HAART. Nonadherence, pharmacokinetic interactions and pharmacokinetic variability increase the risk of suboptimal (low or high) drug levels. In turn, these may cause the emergence of resistance and concentration-related toxicity.

One of the major goals of clinical pharmacology is to identify (and provide a basis for) the optimum dosage regimen for a given type of patient and disease state [46]. Optimization of antiretroviral drug dosing clearly represents a challenge for clinical pharmacology. Dosing regimens of antiretroviral drugs should facilitate adherence, account for pharmacokinetic interactions, prevent the emergence of resistance and reduce the risk for adverse events.

Optimization of dosing recommendations can often be achieved by relating response to the dose administered. Thus, for drugs with a clear dose-response relationship, the actual concentrations associated with response need not be known. However, from the previous discussion it is evident that plasma concentrations of PIs and NNRTIs are worth knowing, since they are associated with major reasons for treatment failure in HIV infection. In fact, there is convincing evidence for PIs [47-68] and accumulating evidence for NNRTIs [33,34,69] to conclude that these drugs belong to a relatively small group of drugs for which the plasma concentration is a better correlate of response than dose. For PIs, available evidence suggests that these drugs exert time-dependent viral inhibition [70,71]; this means that minimum antiviral concentrations are required throughout the whole dosing interval. In turn, this implies that the trough level (Cmin) is the most important pharmacokinetic determinant for the efficacy of PIs.

The association between plasma concentrations of PIs and NNRTIs and the response to these drugs provides a clear rationale to carefully evaluate the pharmacokinetic characteristics of these agents, and to incorporate this information into the design of drug dosing regimens. In this way pharmacokinetically-optimized, fixed dosing regimens can be developed. Further optimization may be achieved by individualization of the dose, based on plasma drug measurements in individual patients (TDM).

Objective and outline of the thesis

The overall objective of the studies in this thesis is to contribute to the optimization of dosage regimens for antiretroviral drugs (PIs and NNRTIs) by the assessment and interpretation of pharmacokinetic characteristics of these agents, i.e. by the application of pharmacokinetics. The central principle underlying these studies is that the intensity and duration of the effect (either desired or undesired) of PIs and NNRTIs is related to their plasma concentrations. Optimized dosage regimens are expected to improve the response to antiretroviral drugs.

Chapter 2 presents a *review* of published high-performance liquid chromatographic (HPLC) methods for analysis of PIs in human biological matrices and a *review* of the prospects and limitations of TDM for antiretroviral drugs. Bio-analytical methods are a prerequisite for the use of pharmacokinetics to optimize drug dosing. TDM could be considered as the ultimate application of pharmacokinetics in the management of HIV-infected patients.
Chapter 3 is devoted to *optimization of bio-analytical methods* for measurement of PIs and NNRTIs in plasma. This chapter describes the development and results of an international interlaboratory quality control program as a means to monitor and improve antiretroviral drug measurements.
The studies in *chapter 4* aimed to provide *pharmacokinetically-based dosing recommendations for some existing antiretroviral drugs* (or drug combinations) when used alone, in combination, or administered with certain co-medicated agents. The studies in this chapter evaluated pharmacokinetic drug-drug and drug-food interactions of PIs and NNRTIs that were potentially undesirable.
Chapter 5 focuses on the *development of new PI-based dosing regimens that should offer ease of adherence.* The studies in this chapter also evaluated pharmacokinetic interactions, but these were desirable and intentionally used to achieve once-daily administration of PIs.
In *chapter 6*, the findings of the previous chapters are discussed and some methodological issues are considered that are relevant to several studies in the thesis.

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Chapter 2.

Review of the literature

Chapter 2.1

High-performance liquid chromatography of HIV protease inhibitors in human biological matrices

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Abstract

Methods for HPLC analysis of protease inhibitors (PIs) in human biological matrices were reviewed. Assays have been developed for analysis of single PIs or for simultaneous measurement of multiple PIs in plasma/serum, saliva, cerebrospinal fluid and semen. Liquid-liquid extraction was most often applied for sample pretreatment, but solid-phase extraction and protein precipitation were used as well. Reversed-phase or ion-pair chromatography have been used to separate PIs. Detection of PIs should be sensitive enough for quantitation of plasma concentrations below trough levels of single PIs, or below proposed therapeutic thresholds for PIs. The large majority of assays employs UV detection. As the potential for interferences is large, the selectivity of every method should be evaluated properly. The available HPLC methods have been applied in clinical pharmacokinetic studies and for Therapeutic Drug Monitoring of PIs. Participation in an interlaboratory quality control program is recommended for every laboratory engaged in the bioanalysis of PIs.

Contents

1. Introduction

Contemporary treatment of HIV infection and AIDS is a complex and long-term undertaking, unavoidably entailing polypharmacy. Three therapeutic classes have been developed for inhibition of viral replication: protease inhibitors (PIs), nucleoside reverse transcriptase inhibitors (NRTIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs). In order to provide optimal efficacy and to prevent viral resistance, available antiretroviral drugs should be administered in combination regimens, which are generally referred to as highly active antiretroviral therapy (HAART). According to current guidelines, HAART should consist of two NRTIs combined with either one or two PIs, or with an NNRTI.
Especially the introduction of PIs (since 1995) has dramatically decreased mortality and morbidity in HIV infection [1]. These drugs interfere with viral replication by inhibiting the HIV protease enzyme [2,3]. This results in production of non-infectious virions and prevents consecutive infection of other cells. To date six PIs have been approved by the Food and Drug Administration (FDA): indinavir, nelfinavir, ritonavir, saquinavir and, more recently, amprenavir and lopinavir.
Since the advent of PIs there has been increasing interest in the bioanalysis of these drugs. Numerous high-performance liquid chromatographic (HPLC) assays have been published for each individual PI [4-24] and for simultaneous determination of several PIs [25-39]. This review describes the rationale for the large interest in bioanalytical methods for PIs and gives a survey of current applications of these methods (section 2). Essential elements of published methods will be described subsequently (section 3), followed by a more detailed evaluation of methods for simultaneous measurement of PIs (section 4). Finally, some conclusions and future perspectives will be described (section 5).

Published HPLC methods were retrieved using the Medline database (January 1994 to July 2001, keywords “HPLC” or “high-performance liquid chromatography”, combined with the names of the individual approved PIs) or Analytical Abstracts (using names of the individual PIs as keywords). Methods cited from these articles were also checked. Only papers written in English and with a full, detailed description (including method validation results) of an HPLC method were included. Methods briefly described in reports of pharmacokinetic studies or in abstracts were therefore not

considered. Furthermore, the review confined to methods intended for analysis of PIs (not their metabolites) in human plasma/serum, urine, saliva, cerebrospinal fluid (CSF) and semen. However, a few articles that did not meet the criteria were cited often and contained valuable information; accordingly these articles were included as well [40,41].

2. Application of HPLC analysis of protease inhibitors in pharmacokinetics and Therapeutic Drug Monitoring

Development of new drugs such as the PIs inevitably leads to interest in the bioanalysis of the compounds involved, as drug development traditionally includes various stages that require analytical input. Moreover, research into clinical pharmacology (especially clinical pharmacokinetics) of PIs typically extends beyond the formal approval of these drugs. This may be explained by the accelerated FDA approval conditions for these drugs, which may have called for additional research, but is certainly due to the suboptimal response to PI-based regimens as well. Despite the remarkable antiviral potency of PIs, only about 50% of patients commencing treatment will achieve and maintain adequate antiviral response in the long term. The unfavorable and variable pharmacokinetics of PIs and their large potential for drug interactions are to a large extent responsible for this heterogeneity in antiviral response. Therefore several types of clinical pharmacokinetic studies have been set up in the last years. Accurate and sensitive analytical methods are a prerequisite for all these studies:

2.1 Description of plasma pharmacokinetics of PIs

Many studies have concerned the description of the pharmacokinetics of PIs and combinations of PIs in various populations. These studies have complemented data from the pharmaceutical industry and have shown the unfavorable pharmacokinetics of PIs. This regards to their poor and variable bioavailability and short elimination half-lives. Poor bioavailability necessitates food restrictions for intake of most PIs, whereas short half-lives result in inconvenient twice or thrice daily dose regimens. Both may negatively influence compliance to treatment regimens and cause inadequate exposure to the drugs.

2.2 Evaluation of drug interactions

Apart from variable bioavailability and fast elimination, drug interactions are of major concern when PIs are being prescribed. The drugs are primarily metabolised by CYP3A, one of the cytochrome P450 iso-enzymes, and therefore interact with other drugs that inhibit or induce this enzyme. Furthermore, PIs also have capacity to inhibit CYP3A themselves. Especially ritonavir is a potent inhibitor of CYP3A and interacts with a long list of other drugs. The large interaction potential of PIs has been established in numerous drug interaction studies [42].

2.3 Evaluation of PI-based regimens with a better pharmacokinetic profile

New PI-based regimens are being evaluated in order to obtain combinations with pharmacokinetics that are more favorable. More specifically, interactions between PIs are being exploited to overcome the pharmacokinetic shortcomings of PIs as single agents. Combination of low doses of ritonavir with other PIs often leads to higher drug levels of the latter PIs ("pharmacokinetic enhancement"), better bioavailability and a reduction in dose and dose frequency [43-45]. Other pharmacokinetic studies aim at developing once daily PI dosing regimens, again by exploiting interactions with ritonavir [46-48].

2.4 Drug-transporting proteins and sanctuary sites

PIs are to varying degrees substrates for drug transporting proteins P-glycoprotein and MRP. Affinity for these proteins may prevent penetration of the PIs in some body compartments, such as the central nervous system and semen. As a result, these body compartments could harbour reservoirs of poorly tractable HIV, and are therefore designated as sanctuary sites for HIV [49,50]. Pharmacokinetic studies are being untertaken to study the penetration and retention of PIs in CSF [51] and semen [52] and in other putative sanctuaries. These studies require assays that are validated for these purposes.

2.5 Studies relating pharmacokinetic parameters to observed clinical effect

Over the past four years data have emerged demonstrating an important link between PI drug concentrations and efficacy or toxicity of these drugs [53]. As a result, the issue of Therapeutic Drug Monitoring (TDM) for PIs has risen [54]. PIs appear to be appropriate candidates for TDM indeed, as there is also large interindividual variability in their plasma pharmacokinetics, a narrow range between therapeutic and toxic drug concentrations, and no direct measure for the pharmacological effect of PIs applied in combination therapy. However, therapeutic ranges or target values have not been defined unequivocally. Clinical trials have been started to validate such target values and to assess the value of TDM for PIs. So far, only preliminary results of these studies have been presented [55,56]. Nevertheless, TDM for PIs has already been applied and four indications for TDM have evolved from practice. It can be used (a) to prevent treatment failure, (b) to explain or prevent drug toxicity, (c) to manage drug interactions and (d) to document non-compliance to medication schedules. It appears that nonadherent patients can be identified using drug level measurements of PIs in plasma [57,58] or saliva [59,60].

3. HPLC analysis of protease inhibitors: essential elements

3.1 Physico-chemical and pharmacokinetic properties of protease inhibitors

Physico-chemical properties and pharmacokinetic parameters of PIs are valuable clues for the choice of conditions for HPLC separation, as well as for evaluating existing methods. Although the PIs are pharmacologically related, they differ structurally (figure 1). PIs are compounds of medium polarity with weak basic properties due to ionizable substituent groups. Table 1 summarizes solubility data, UV absorption maxima and pKa values, as far as these data could be retrieved [34,35,61-65]. Complete UV spectra of PIs have been depicted in the literature [34,35]. These spectra show that all PIs have high absorbances in the lower wavelength range (200-220 nm). For indinavir, lopinavir, ritonavir and nelfinavir, absorbances in this range are significantly higher than their respective peak absorbances at higher wavelengths. For amprenavir

and saquinavir, absorptivities at their maxima approximate those at lower wavelengths [34,35]. The aqueous solubilities of indinavir and nelfinavir are strongly dependent on pH. At pH values above 3.5 these drugs show a sharp decline in solubility [61,64]. Amprenavir, indinavir, nelfinavir, ritonavir and saquinavir appear to be very stable drugs [27]. Whole blood or plasma samples containing these drugs can be kept at room temperature for several days and at -20°C for several months. Repeated freeze-thaw cycles do not affect the stability of the drugs. Stock solutions of these drugs in methanol are stable at -20°C for several months. No such stability data have been published for lopinavir so far, but our own experience with this drug indicates that its stability is comparable to other PIs.

All PI plasma concentrations are expressed as the free base. Knowledge of the concentration range of PIs is important for an estimation of the required upper and lower limits of quantitation for their measurement (see table 2, [63,66-76]). It should be noted that large variability exists in minimum (trough) and maximum concentrations of PIs in plasma. Furthermore, these concentrations can increase when PIs are combined with ritonavir. PIs exhibit strong (≥ 90%) binding to plasma proteins, except for indinavir which is about 60% bound to proteins. All PIs are extensively metabolised to numerous metabolites. The major metabolite of nelfinavir (M8) shows in vitro activity and in vivo protein binding comparable to nelfinavir [70,77]. M8 concentrations are roughly about 30% of nelfinavir concentrations [78].

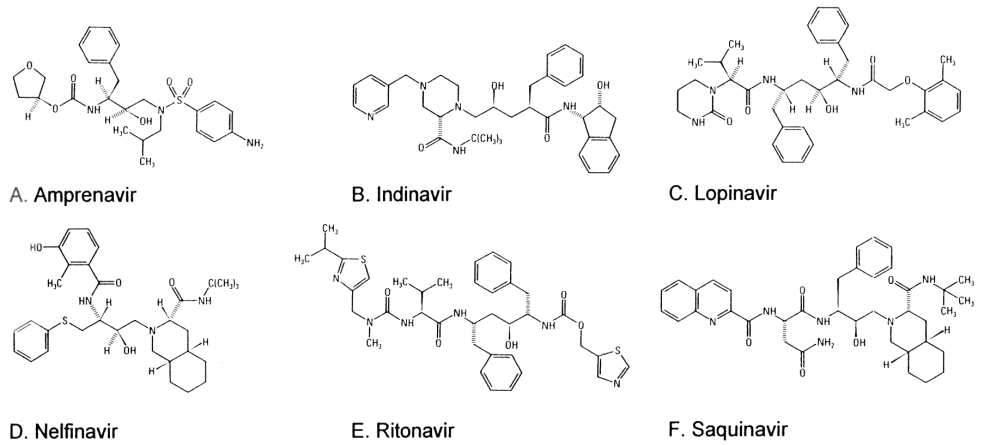

Fig. 1. Chemical structures of amprenavir, indinavir, lopinavir, nelfinavir, ritonavir and saquinavir

Table 1. Physico-chemical properties of protease inhibitors[a]

Protease inhibitor	Solubility in water	UV max (nm)	pK_a value	Ref.
Amprenavir	na	265	na	[34,35]
Indinavir	100 mg/ml (sulfate) 60 mg/ml (pH 3.5), 0.3 mg/ml (pH 4.8)	260	3.7	[61,62]
Lopinavir	practically insoluble	259[b]	na	[63]
Nelfinavir	4.5 mg/ml (mesylate)	252	6.00, 11.06	[62,64]
Ritonavir	practically insoluble	239	na	[62]
Saquinavir	2.2 mg/ml (mesylate)	239	7.01	[62,65]

[a] *See Nomenclature for abbreviations.*
[b] *Recorded in ACN - phosphate buffer (40:60 v/v).*

Table 2. Concentration range and proposed therapeutic thresholds of protease inhibitors[a,b]

Protease inhibitor	Proposed plasma threshold (ng/ml)	Steady state C_{min} concentration (ng/ml)	Steady state C_{max} concentration (ng/ml)	Refs.
Amprenavir	na	280	5360	[66]
Indinavir	100-110	130	6840	[67-69]
Lopinavir/ritonavir	na	5500	9600	[63]
Nelfinavir	0.25-0.45 or 0.77	1000[c], 700[d]	3000[c], 4000[d]	[70-72]
Ritonavir	2100	4000	11000	[73,74]
Saquinavir (HGC)	50	38	198	[75,76]
Saquinavir (SGC)	50	70	2181	[75,76]

[a] *See Nomenclature for abbreviations.*
[b] *All PIs dosed without ritonavir, except for lopinavir which is coformulated with ritonavir.*
[c] *Regimen: 750 mg three times daily.*
[d] *Regimen: 1250 mg two times daily.*

3.2 Protease inhibitor analysis in biological matrices

Bio-analytical assays for PIs have almost exclusively been developed and validated for plasma and serum samples. However, some HPLC methods have been published for analysis of these drugs in other biological fluids that are of interest for clinical pharmacokinetic studies, or TDM; urine, saliva, CSF and seminal fluid [4,6,13,14,19,23,40]. From an analytical point of view these matrices are relatively free of interferences compared to plasma. Authors reporting analytical methods in these fluids do not seem to have experienced large problems in applying or adapting

existing methods for measurement of PIs in plasma or serum, provided that these methods are sensitive enough.

3.2.1 Analysis in urine

Analysis in urine may be particularly relevant for indinavir, which is excreted in urine for up to 20% (much more than other PIs), and causes urological complaints by crystallisation of indinavir in the urinary tract. Since urine is generally free of protein and lipids we ourselves just acidify urine samples containing indinavir to a pH below 3.5 with orthophosphoric acid, in order to dissolve indinavir that may have precipitated. After centrifugation, urine is then diluted and injected in the HPLC system. Recovery using this methodology is 101%. However, Woolf et al [6] and Svensson et al [14] describe a more extensive sample pretreatment method for analysis of indinavir in urine, using the same liquid-liquid extraction procedures and separation conditions for urine and plasma or serum. In the assay by Woolf, recovery of indinavir from urine was less (68%) as compared to plasma (81%), but it remained constant over the range of the standard curve.
Interestingly, Woolf et al also developed another method for analysis of indinavir in plasma, consisting of liquid-liquid extraction followed by HPLC and tandem mass spectrometric (MS) detection with a turbo ion spray interface ([7], see also table 3). They attempted to apply this plasma method for measurement of indinavir in urine as well, omitting the liquid-liquid extraction step [40]. In theory, the highly specific nature of LC-MS should allow for minimal sample clean up (just dilution) and short chromatographic analysis times. However, after dilution and injection of urine samples, a high degree of variability in MS/MS responses was observed. Both sample clean up and better chromatographic separation (increasing k') improved the instrument response and reproducibility of ionization, thus potentially improving sensitivity and precision of the method. These findings illustrate that analysis of urine with MS detection may require sample pretreatment and adequate separation of analytes from co-eluting species that are unseen by the detector.

3.2.2 Analysis in saliva

Analysis of PIs in saliva has been studied as an alternative for plasma or serum in TDM, as use of saliva offers several advantages (e.g. easy and non-invasive sample

collection, and diminished risk of HIV transmission). Hoetelmans et al used a special device (Salivette®, a cotton wool swab impregnated with citric acid) as a standardized manner to collect stimulated saliva for the measurement of ritonavir and saquinavir [19,23]. They applied the same sample pretreatment as for plasma samples. Only very low concentrations of ritonavir and saquinavir were measured in saliva samples from HIV-infected patients who took these PIs. This is probably due to extensive protein binding of these PIs in plasma, which restricts the amount of drug that can diffuse into saliva. Based on protein binding data, only indinavir can be expected in saliva (see section 3.1). Hugen et al analysed indinavir in stimulated saliva using the same separation conditions as for plasma samples [8,59]. Adsorption of indinavir to the Salivette® was 40%. Salivary indinavir concentrations correlated well with plasma levels, but a large intra-and inter-individual variation in saliva/plasma concentration ratios was found. It was concluded that salivary indinavir concentrations can not be used to predict plasma concentrations, but may be applied for monitoring of compliance. Wintergerst et al also found good agreement between indinavir concentrations in plasma and (unstimulated) saliva, particularly at the end of the dose interval [60]. Saliva and plasma samples were analysed using the same LC-MS/MS assay.

3.2.3 Analysis in CSF and semen

Analysis of PIs in CSF and semen requires higher sensitivity than measurement of PIs in plasma. Limits of quantitation for plasma assays are often in the 10-50 ng/ml range, but drug levels behind blood-brain and blood-testes barriers can be significantly lower. Furthermore, only small volumes of CSF are mostly available.
Sparidans et al extracted amprenavir from small samples (100 µL) of CSF or semen using liquid-liquid extraction [4]. Recovery of amprenavir was more than 95%. Fluorescence detection enabled measurement of amprenavir in the low nanogram range (see table 3). In order to facilitate the use of calibration samples in plasma for measurement of CSF and semen samples, plasma was added to these matrices prior to further treatment.
Zhong et al report a method for measurement of indinavir in both plasma and CSF samples (see table 3, [13]). Recovery of indinavir from CSF was more than 90% and the lower limit for quantitation of indinavir in CSF was 2 ng/ml. Likewise, Svensson et

Table 3. Summary of published HPLC methods: measurement of single PIs[a]

PIs[b]	Matrix	Sample pretreatment	Column	Mobile phase	Run time[c]	Limit of quantitation[d]	Detection[e]	Refs.
APV	plasma, CSF, semen	LLE chloroform	C_{18} (100x4.6 mm, 3.5 µm) 50°C	Isocratic 25 mM sodium phosphate buffer pH 6.8 / ACN (60:40 v/v) 1.5 ml/min	18.5	1 (plasma, semen) 0.5 (CSF)	FL Ex 270 nm Em 340 nm	[4]
APV	serum, plasma	LLE at basic pH diethylether	C_{18} (50x2.0 mm, 3 µm)	Isocratic ACN / water (1:1, v/v) + 0.1% formic acid 0.15 ml/min	< 5	50	MS/MS	[5]
IDV	plasma, urine	LLE at pH 9.5 MTBE Backextr. in 10 mM HCl Re-extr. MTBE pH 9.5	column switching I: cyano (80x4 mm, 5 µm) II: C_{18} (150x4.6 mm, 5 µm)	Isocratic I: ACN / water (34:66 v/v) II: ACN / water (38:62 v/v) Both in 10 mM orthophosphoric acid, pH 7.5, 1.2 ml/min	20	5	UV 210	[6]
IDV	plasma	LLE at pH 9.5 MTBE	C_8 (50x2 mm, 3 µm)	Isocratic ACN / water (40:60 v/v) + 7 mM ammonium acetate, pH 4.9 0.2 ml/min	6	1 (at least)	MS/MS	[7]
IDV	plasma	protein precipitation ACN	C18 (150x4.6 mm, 5 µm)	Isocratic ACN / 50 mM phosphate buffer pH 6 + 4 g/l TMACl (40:60 v/v) 1 ml/min	12	50	UV 210	[8]
IDV	plasma	LLE at pH 9.0 diethylether	C_4 (150x3.9 mm, 5 µm)	Isocratic 10 mM $NH_4H_2PO_4$ + 1 mM 1-heptanesulfonic acid sodium pH 4.8 / ACN (65:35 v/v) 0.6 ml/min	30	10	UV 210	[9]
IDV	urine	Dilution with ACN or LLE at pH 9.5 MTBE	C_8 (50x2.0 mm, 3 µm)	Isocratic ACN/ 7 mM ammonium acetate (40:60 v/v), pH 4.9 or Isocratic ACN/ 7mM ammonium acetate (30:70 v/v), pH 4.9 Both 0.2 ml/min	6 12	na	MS/MS	[40]
IDV	plasma	SPE (C_{18})	C_8 (250x4.6 mm, 5 µm)	Isocratic ACN / 10 mM KH_2PO_4 pH 3.1 (40:60 v/v) 1.5 ml/min	19	25	UV 210	[10]

Table 3. Continued

PIs[b]	Matrix	Sample pretreatment	Column	Mobile phase	Run time[c]	Limit of quantitation[d]	Detection[e]	Refs.
IDV	plasma	LLE pH 10.4 Dichloro-methane Hexane wash	C_{18} (150x4.6 mm, 5 µm)	Isocratic ACN / 25 mM phosphate buffer + 0.2% triethylamine in water, pH 7 (34.5:65.5 v/v) 2 ml/min	15	25	UV 210	[11]
IDV	plasma	SPE (Oasis® HLB)	C_8 (150x4.6 mm, 5 µm)	Isocratic Water / ACN / 5.9 M orthophosphoric acid / triethylamine (73:27:0.5:0.02 v/v), pH 3.2 0.8 ml/min	12	10	UV 210	[12]
IDV	plasma, CSF	SPE (strong cation-exchange, SCX benzene sulfonic acid)	Column switching I: cyano (80x4 mm, 5 µm) II: C18 (150x4.6 mm, 5 µm) Columns at 28°C (see [6])	isocratic I: ACN / water (34:66 v/v) II: ACN / water (38:62 v/v) Both in 10 mM orthophosphoric acid, pH 7.5 1.2 ml/min	20	5 (plasma) 2 (CSF)	UV 210	[13]
IDV	cell culture	Protein precipitation (ACN)	C_{18} (250x3 mm, 5 µm)	Isocratic 10 mM NaH2PO4 pH 6.3 / ACN (65:35 v/v) 0.6 ml/min	19	4	ED First el.: + 400 mV Second el.: + 750 mV	[41]
IDV	Serum, urine, CSF	LLE at basic pH Diethylether Back extr. in acid aqeous phase	C_{18} (75x4.6, 3.5 µm)	Isocratic 50 mmol/L acetic acid buffer (pH 4.8) / ACN (52:48 v/v) 1.5 ml/min	3.5	na (LOD: 6 ng/ml)	UV 260	[14]
IDV	plasma	96-well SPE (mixed phase cation exchange)	C_{18} (30x3.0 mm, 3 µm) 35°C	Isocratic ACN / 10 mM ammonium acetate + TFA 0.5 ml/L (42.5:57.5 v/v) 0.6 ml/min	Very short (high throug put)	1	MS/MS	[15]
NFV	plasma	LLE at pH 10 Ethyl acetate-ACN (90:10 v/v)	C_{18} (250x4.6 mm, 5 µm)	Isocratic 25 mM monobasic sodium phosphate buffer pH 3.4 / ACN (58:42 v/v) 1.3 ml/min	12	50	UV 220	[16]
NFV, M8	plasma	LLE at pH 9.5 MTBE/hexane (90:10) Hexane wash	C_{18} (250x4.6 mm, 5 µm)	Isocratic 0.1% TFA / ACN / MeOH (49:46:5 v/v), pH 5 1.5 ml/min	15	25 (NFV, M8)	UV 220	[17]

Table 3. Continued

PIs[b]	Matrix	Sample pretreatment	Column	Mobile phase	Run time[c]	Limit of quantitation[d]	Detection[e]	Refs.
RTV	plasma	LLE Ethyl acetate-hexane (9:1 v/v) Hexane wash	C_{18} (50x4 mm or 50x4.6 mm , 3 µm)	Isocratic ACN / MeOH / 0.01M TMAP in 0.1% aqueous TFA (40:5:55 v/v) 1 ml/min	15	12	UV 205	[18]
RTV	plasma, saliva, CSF	Protein precipitation (ACN)	C_{18} (75x4.6 mm, 3.5 µm)	Isocratic ACN / 25 mM sodium acetate + 25 mM hexane-1-sulfonic acid, pH 4 (44:56 v/v) 1 ml/min	20	50	UV 239	[19]
RTV	plasma	LLE Ethyl acetate-hexane (9:1 v/v) Hexane wash	C_{18} (300x3.9 mm, 10 µm)	Isocratic ACN / 0.05 M monobasic ammonium phosphate (pH 3) (52.5:47.5 v/v) 2.0 ml/min	12	na	UV 210	[20]
SQV	plasma	SPE (C_2)	ODS (20) guard column (30x4.6 mm, 5 µm)	Isocratic Aqueous ACN (80:20 v/v) with 0.0025M ammonium acetate pH 6.5 1.5 ml/min	1.5	0.4	MS/MS	[21]
SQV	plasma	hexane wash, LLE with diethylether	C_8 (125x3 mm) 45°C	Isocratic 5 mM sulfuric acid / ACN (75.5:24.5 v/v) containing 10 mM TBA pH 3.5 1 ml/min	10	10 (at least)	UV 240	[22]
SQV	plasma, saliva, CSF	SPE (C_2)	C_{18} (75x4.6 mm, 3.5 µm)	Isocratic ACN / 25 mM sodium acetate + 25 mM hexane-1-sulfonic acid, pH 4 (40.5:59.5 v/v) 1 ml/min	35	2.5	UV 239	[23]
SQV	plasma	protein precipitation (monochloracetic acid) SPE (C_8)	phenyl (4 µm)	Isocratic methanol / 0.01M ammonium acetate / glacial acetic acid (90:9.75:0.25) 2 ml/min	15	1.0	UV 239?	[24]
SQV	plasma	LLE at basic pH diethyl ether hexane wash	C_8 (250x4.6 mm, 5 µm)	Isocratic aqueous ACN (37:63) 1 ml/min	30	20 (at least)	UV 239?	[24]

[a] *See Nomenclature for abbreviations.*
[b] *References listed by PI, then chronologically.*
[c] *Run time in minutes.*
[d] *Limit of quantitation in ng/ml.*
[e] *Wavelength of detection in nm.*

al [14] developed a method that can be applied for both serum and CSF, as well as urine. The methods reported by Hoetelmans et al for measurement of ritonavir and saquinavir in plasma and saliva can also be applied to CSF samples [19,23]. Recoveries from CSF were 99-101% for ritonavir and 60-61% for saquinavir.

3.3 Heat treatment to inactivate HIV

Samples from HIV-infected persons obviously pose a health hazard. Chemical treatments can be used to inactivate HIV, but such treatments may influence the HPLC analysis, or degrade the PIs to be measured. For example, Marzolini et al evaluated viral inactivation by Triton X-100, but this detergent perturbed UV detection at 201 nm and influenced the peak shape of the PIs [33].
Heat treatment is another effective means for deactivation of HIV. Deactivation has been performed at 56-60°C, using a variety of heat treatment durations (from 30 min to 4 hours). Somewhat conflicting data have been reported with regard to stability of PIs under these circumstances. Whereas several authors have assessed that 30 min to one hour at 56°C or 60°C did not affect concentrations of amprenavir [4,32,33,37], indinavir [12,15,28,32,33,37], nelfinavir [28,32,33,37], ritonavir [19,28,32,33,37] and saquinavir [28,32,33,37], others have found slight degradation (less than 15%) for indinavir [9] and ritonavir [18], larger decreases for saquinavir after 2 h at 60°C (mean decrease 18% [24]), and even an increase in indinavir concentrations after 4 h at 58°C [10]. Therefore, heat treatment may have a slight affect on PI concentrations. This implicates that calibration and quality control samples should be heat treated as well, or that heat treatment should be avoided.
If sample pretreatment consists of an extraction step using organic solvents, this may be sufficient to deactivate biological hazards such as HIV [79].
Irradiation has been suggested as an approach for deactivation of HIV in biological samples [79], but this methodology has not been applied in any of the described analytical methods.

3.4 Sample pretreatment

Sample pretreatments that have been applied for analysis of PIs in liquid biological matrices include protein precipitation, liquid-liquid extraction and solid-phase extraction.

3.4.1 Protein precipitation

Protein precipitation reagents used in the analysis of PIs are acetonitrile and monochloracetic acid. Protein precipitation with acetonitrile has been used as sole sample pretreatment method in the analysis of indinavir in plasma [8] and cell cultures [41] and in the analysis of ritonavir in plasma [19]. Monochloracetic acid has been used as a prelude to solid-phase extraction of saquinavir from plasma [24].

3.4.2 Liquid-liquid extraction

Liquid-liquid extraction of PIs has been used in the majority (56%) of the assays, both for extraction of single PIs as well as for simultaneous extraction of several PIs. Most extractions were performed in one single step. Before extraction, samples have been alkalinized, thereby allowing PIs to exist in an uncharged form, being more readily extracted by organic solvents. Using this methodology, PIs have been extracted using methyl tert.-butyl ether (MTBE) [6,7,27,29-31,36,40], MTBE/hexane [17], diethylether [5,9,14,22,24,26], ethylacetate/hexane [18,20,28,38], ethylacetate/acetonitrile [16], chloroform [4] or dichloromethane [11]. MTBE and diethylether have been used most often. As a consequence of their low densities, these solvents can be easily collected after extraction, as the upper layer in a tube. Freezing the lower aqueous layer in a dry ice-aceton bath may facilitate collection of the organic solvent. The solvents are then evaporated to dryness and the residue is reconstituted for injection in the chromatographic system [5,7,9,29,31,40]. Alternatively, the reconstituted aqueous phase can be washed with hexane if lipophilic co-elutants have to be removed or if quantitation in the lower range is desired [17,22,24,26,27,30,36]. Hexane washing has been applied in the same way after extraction with other solvents [11,18,20,38]. As an alternative to hexane washing, the extraction step into MTBE or another solvent may be followed by back extraction of PIs into acid [14], if necessary with subsequent pH adjustment to high values and reextraction into MTBE. Woolf et al demonstrated the latter reextraction strategy for indinavir [6]. However, this procedure resulted in low recoveries of nelfinavir and M8 compared to washing with hexane [17].

3.4.3 Solid-phase extraction

Solid-phase extraction of PIs was first applied by Knebel et al, who extracted saquinavir on C2 solid-phase cartridges, obtaining more than 95% recovery [21]. Hoetelmans et al modified this procedure slightly and applied it for extraction of saquinavir [23] and for simultaneous extraction of five PIs [32].
C8 columns have been used for extraction of saquinavir [24], whereas C18 cartridges have been applied for extraction of indinavir [10] and for simultaneous isolation of multiple PIs [25,33,35]. Using C18 columns, Marzolini et al [33] and Simon et al [39] extracted PIs together with NNRTIs, and Aymard et al used C18 cartridges to isolate PIs, NNRTIs as well as NRTIs from one single plasma sample [35].
Oasis® HLB cartridges (Waters) have been applied to extract indinavir alone [12] or five PIs simultaneously [34,37]. Poirier et al choose this polymeric sorbent because its hydrophilic properties prevent the wettability problem encountered with C18 packings, and because reproducible results can be obtained even when the cartridges run dry [12,34].
The potential for separation of PIs based on cation-exchange is suggested by the presence of nitrogen in multiple functional groups in the PI molecules. Zhong tested cartridges with weak cation-exchange functional groups (carboxylic acid) for extraction of indinavir, but strong cation-exchange functional groups (benzenesulfonic acid) showed the best separation and recovery [13].
Rose et al developed an assay for high throughput analysis of indinavir in plasma, using semi-automated 96-well solid-phase extraction in the mixed phase cation exchange format (MPC), in conjuction with LC-MS/MS [15]. This allowed for analysis of 288 samples (three 96-well plates) in one overnight run.

3.5 Separation conditions

Reversed-phase or ion-pair chromatography appear to be the most appropriate HPLC methods for analysis of ionizable drugs such as PIs in an aqueous biological matrix. Separation conditions described in most publications are fairly straightforward, derived from initial conditions that have been proposed for systematic HPLC method development [80].

Therefore frequently chosen stationary phases were C8 or C18, whereas other columns (C4 [9,29], cyano [6,13], phenyl [24,30]) have been applied occassionally. Woolf et al [6] and Zhong et al [13] used a column switching system for measurement of indinavir in plasma and urine [6] or plasma and CSF [13]. The column switching configuration was designed to separate indinavir from endogenous interferences. By combining the different selectivities provided by the first (cyano) and second (C18) column, the analytes could be detected under interference-free conditions at 210 nm. Some authors have thermostatted column temperature above ambient temperature, apparently in order to influence selectivity, to reduce the variation in retention times, or to improve peak efficiency [4,13,15,22,29,34,35,39].

Mobile phase frequently consisted of acetonitrile and a buffer, most often a phosphate buffer. Because of the ionic character of PIs, buffering the aqueous phase of the solvent is imperative. When the mobile phase pH is close to the pKa values of one of the PIs, small pH changes can have a major impact on band spacing. Variation in mobile phase pH is thus a powerful way to influence selectivity when separating PIs. However, the exact pH conditions that favor maximum resolution of PIs may not favor method ruggedness. Several authors stress the exact setting of mobile phase pH for adequate and reproducible separation [17,23,27,30,33].
Besides changes in pH, several other mobile phase characteristics have been varied in order to optimize band spacing and peak shape. Apart from changes in solvent type selectivity, several authors have used additives (diethylamine, triethylamine, trifluoracetic acid) in the mobile phase, presumably to improve the peak shape of the basic PIs (less tailing) or to act as weak ion-pairing reagents [11,12,15,17,18,31,34]. Furthermore, 10 methods describe the addition of strong ion-pair reagents to the mobile phase as an additional way to vary band spacing [8,9,18,19,22,23,32,33, 35,38]. As mobile phases were generally slightly acidic, alkylsulfonates have been applied to provide retention of the basic PIs in their protonated form [9,19,23,32, 33,35]. Tetraalkylammonium salts have been used in slighly acidic mobile phases to avoid tailing [8], probably by blocking silanols, or to obtain a clean baseline by retention of negatively charged interferences [22].

All HPLC methods for single PI assay involve isocratic separation conditions. If isocratic conditions are applied for simultaneous chromatography of several PIs, this may result

in a wide retention range of the drugs. This may be reflected in inadequate resolution of the most polar PIs from early eluting interferences on the one hand, and peak broadening of the late-eluting (less polar) PIs on the other. Such problems may be present in some of the methods for simultaneous measurement of PIs [31,32]. Gradient elution has been applied in 7 out of 15 methods for simultaneous measurement of PIs [26,27,33,34,37-39].

Figures 2 to 5 show typical separations that have been obtained in methods for simultaneous analysis of PIs.

3.6 Sensitivity

Most of the HPLC methods for PIs have been developed for application in pharmacokinetic studies and TDM. Pharmacokinetic studies require lower limits of quantitation below expected trough levels of PIs, in order to be able to accurately calculate important pharmacokinetic data (e.g. half-life) from the terminal phases in drug elimination. TDM demands a similar or better sensitivity; limits of quantitation should be below population trough levels for single PIs, or preferably below threshold values that have been proposed for PIs (table 2). Data in table 2 refer to trough levels after administration of PIs as single agents. Co-administration of two PIs results in higher plasma levels and is becoming increasingly popular. Analytical methods that have been

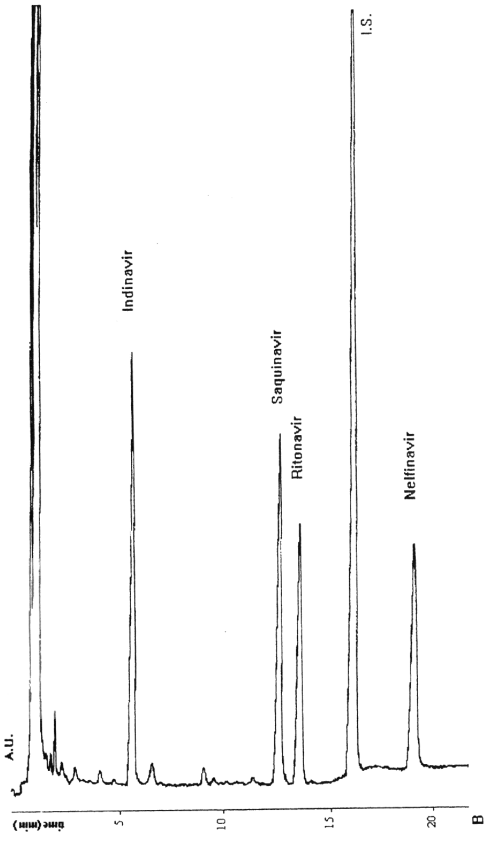

Figure 2. Typical chromatogram of a spiked plasma sample containing 1050 ng/ml of indinavir, nelfinavir, ritonavir, and saquinavir, and internal standard (IS) (from Hugen et al. [27])

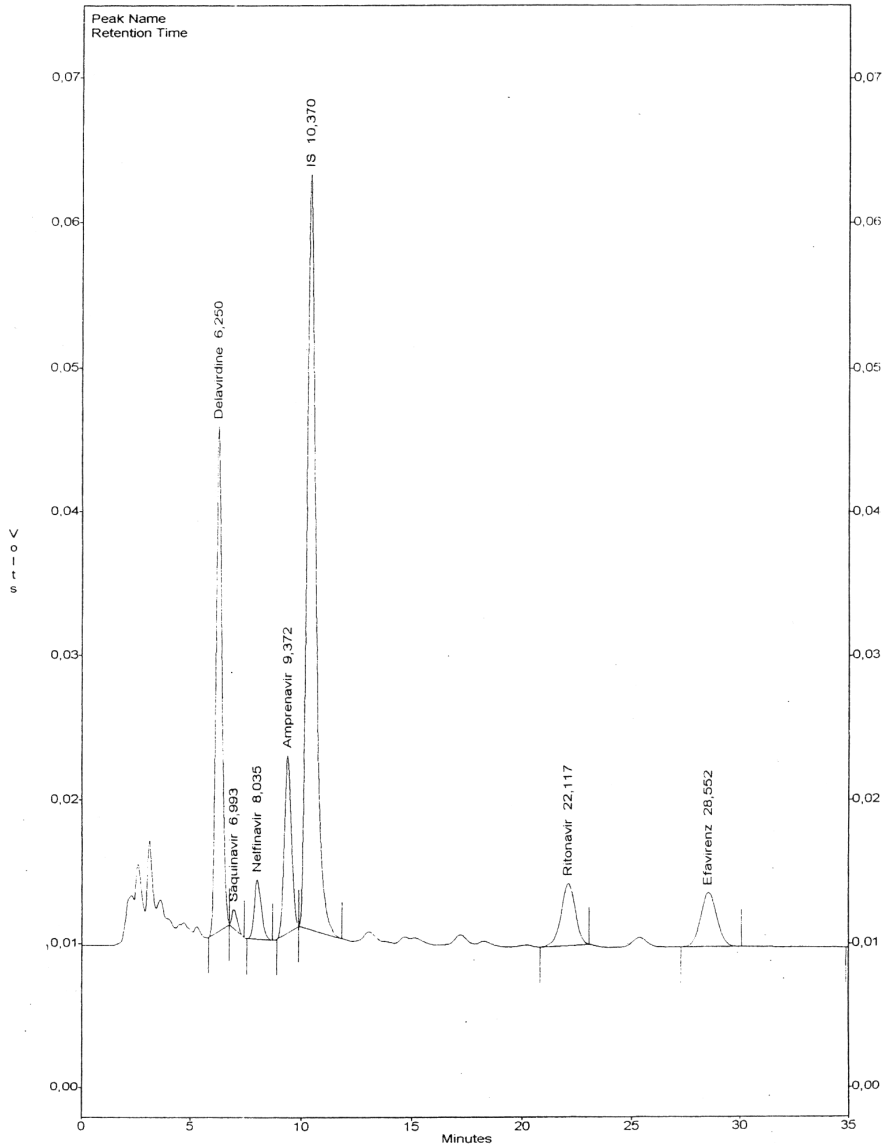

Figure 3. Typical chromatogram of a spiked plasma sample containing four PIs, delavirdine, efavirenz and internal standard (from Proust et al. [31])

validated to measure such higher drug levels only (thus being less sensitive) have restricted applicability, as many patients still take single PIs.

Clearly, trough levels for saquinavir are relatively low compared to other PIs (table 2), whereas ritonavir trough and threshold levels are in the µg/ml range. Therefore, ritonavir will not pose sensitivity problems. Moreover, quantitation of this drug is not

indicated when it is applied in low doses as pharmacokinetic enhancer for other PIs (which is most often the case nowadays).
There is a large variation amongst assays in lower limits of quantitation for measurement of PIs (see tables 3 and 4). A number of assays is certainly not sensitive enough to measure concentrations below population trough levels or below proposed threshold limits (table 2).

3.7 Selectivity

The selectivity of the methods for PI assay is another major item in method validation. Apart from endogenous substances, the potential for drug interferences is enormous, due to the large number of co-administered drugs used by HIV-infected patients, as well as the formation of a large number of PI metabolites. Interferences are especially troublesome to the development of methods for simultaneous analysis of PIs. To assure selectivity, many authors have analyzed several samples of blank plasma. Furthermore, co-administered drugs have been tested for interference. The number of drugs tested varied from antiretroviral drugs only to a tremendous amount of comedications. Metabolites have generally not been available so far and their influence has been

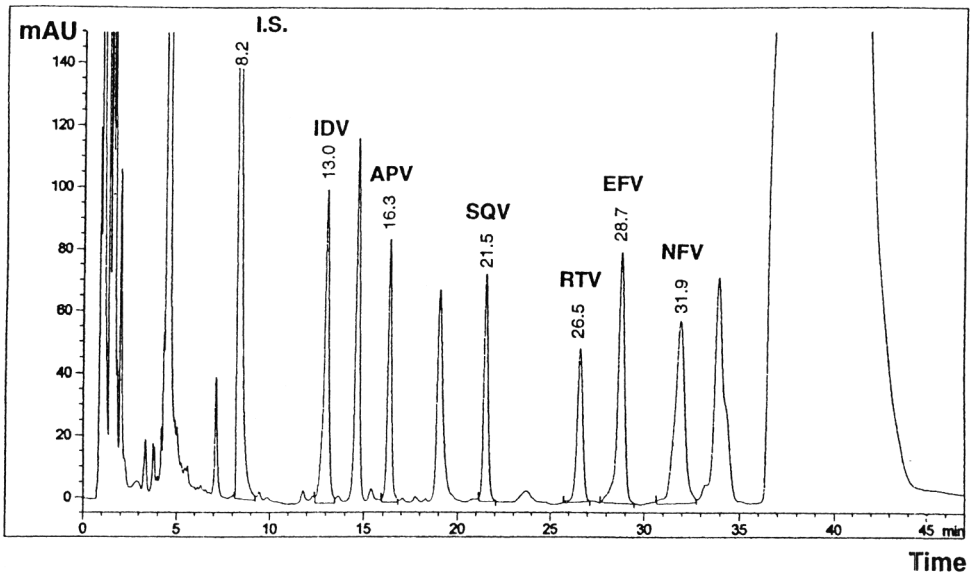

Figure 4. Typical chromatogram of a spiked plasma sample containing 3000 ng/ml of PIs, efavirenz and internal standard (IS) (from Marzolini et al. [33])

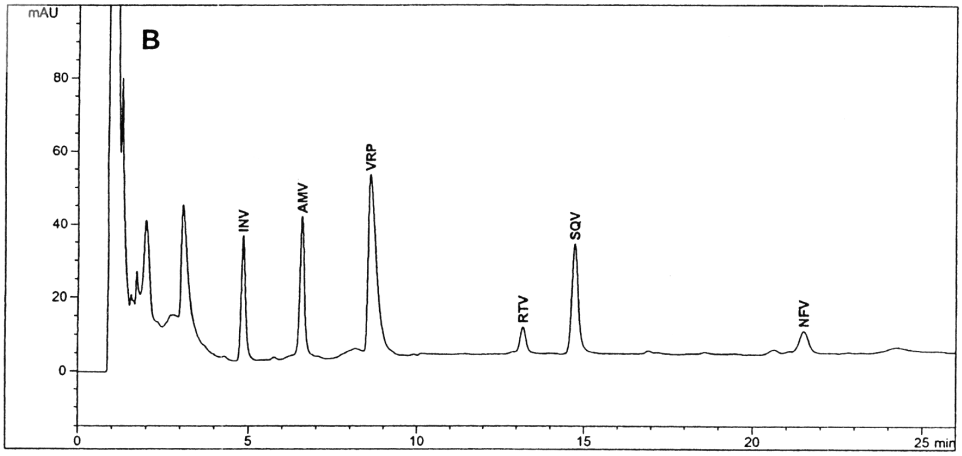

Figure 5.Typical chromatogram of a spiked plasma sample containing 400-500 ng/ml of amprenavir (ANV), indinavir (INV), nelfinavir (NFV), ritonavir (RTV), saquinavir (SQV), and internal standard (VRP) (from Sarasa-Nacenta et al. [37])

evaluated in a small number of studies only, by analysis of samples from treated subjects. Some authors apply peak purity techniques built into diode array UV systems to show that the spectra of peaks are consistent [12,17,34,38]. It should be noted that methods developed before 1998 (mainly for measurement of single PIs) have not been evaluated for interference by new antiretroviral compounds.

3.8 Detection

Detector type and operation can affect the response of PIs and interferences, thus influencing both sensitivity and selectivity. Concerning sensitivity, molar absorptivities of PIs appear to be sufficiently high to meet the sensitivity requirements for measurement of PIs in plasma. Therefore the large majority of methods use UV-detection. In order to obtain maximum sensitivity, many authors have chosen the lower wavelength range for detection of single or multiple PIs. As a consequence of increased sensitivity, this may also permit the use of small plasma volumes. However, low wavelengths are rather non-specific and many endogenous interferences or drugs will absorb in this region. Accordingly, detection at lower wavelengths demands careful investigation of selectivity. As an alternative, PIs can be measured at higher wavelengths in order to minimize interference rather than maximize response. For example, Poirier et al measured four PIs at 210 nm and amprenavir at 265 nm [34]. The latter PI eluted

together with an endogenous peak, which was detected at 210 nm, but not 265 nm, the wavelength of maximum absorbance for amprenavir.

Alternative detectors may be selected when samples are to be analyzed with low PI concentrations, such as in CSF and semen (section 3.2), or when high sensitivity and specificity are required for other reasons.
Sparidans et al used fluorescence detection to enable them to measure low concentrations of amprenavir (lower limit of quantitation: 0.5 ng/ml) in small sample volumes of semen and CSF [4]. No derivatization was required. Other PIs have not been measured using fluorescence detection. Indinavir was reported not to exhibit fluorescence [6], whereas saquinavir demonstrated only minor fluorescence (wavelengths of excitation and emission are 325 and 375 nm respectively [23]).
Fizzano et al used electrochemical detection for sensitive and specific measurement of low indinavir concentrations (lower limit of quantitation 4 ng/ml) in cell cultures [41]. A hydrodynamic voltammogram of indinavir showed a voltage dependent increase starting from + 500 mV before reaching a final plateau after + 750 mV. The first electrode potential was set at + 400 mV, to remove compounds with lower oxidation potentials than indinavir. For detection, the second electrode was set at + 750 mV. Under these conditions detection of indinavir was twice more sensitive than that obtained with an UV detector set at 210 nm.
MS detection has been applied for measurement of amprenavir [5], and it allowed for measurement of indinavir at 1 ng/ml in plasma [7,15]. Likewise, measurement of saquinavir in plasma can be performed with a lower limit of quantitation of 0.4 ng/ml, using HPLC with MS detection [21].

Quantitation of PIs has mostly been performed with use of internal standard calibration. Some methods did not use an internal standard, for reasons of inavailabity of a suitable internal standard, or because satisfactory validation results were obtained without the use of one [14,19,23,32,35,36,39].

3.9 Intralaboratory and interlaboratory quality control

Important decisions are taken based on data obtained with bioanalytical methods for PIs. Therefore application of these methods requires quality control (QC) procedures,

usually including intralaboratory method validation, intralaboratory QC procedures (e.g. use of internal QC samples), and participation in an interlaboratory QC program.

Concerning *inter*laboratory QC, only two methods were tested against reference methods [24,29]. Furthermore, interlaboratory QC programs for measurement of antiretroviral drugs have not been available until recently. However, an international interlaboratory QC program for both PIs and NNRTIs was initiated lately [81], and two national programs have been started in France [82,83]. Results of the three programs have been similar, demonstrating that intralaboratory QC procedures need to be improved in a substantial number of laboratories participating in these programs. For example, 17 laboratories in the USA, Canada, Europe and Australia participated in the second round of the international program and measured varying concentrations of four PIs and two NNRTIs [81]. Twenty percent limits around the weighed-in concentrations of the drugs were considered to be appropriate thresholds for a satisfactory measurement. Measurements of indinavir, nelfinavir, ritonavir and saquinavir yielded satisfactory results in 69%, 78%, 78% and 94% of the analyses, respectively. Only two laboratories performed all measurements (including those of NNRTIs) within 20% limits [81].

The findings of the three QC programs demonstrate both the need for and utility of ongoing QC programs in this area of bioanalysis. Inaccurate analysis of patient samples within the scope of TDM may result in inappropriate dose adjustments, or the advice not to adjust doses where it might be desirable. Both may lead to unnecessary toxicity or to inadequate drug levels, causing resistance development. Inaccurate measurements in pharmacokinetic studies may, for example, lead to incorrect evaluation of drug interactions, which may affect the treatment response of many patients. However, by participating in a QC program, laboratories are being alerted to possible undetected problems in their QC procedures. This enables them to optimize their methods.

4. HPLC methods for simultaneous measurement of protease inhibitors

Development and use of one HPLC method for measurement of several PIs saves time and costs compared to several methods for single PIs. Fifteen methods for simultaneous

analysis of PIs have been published so far. All these methods have been developed (and will most likely be employed widely) for pharmacokinetic studies and TDM. Therefore criteria for deciding on which method to choose should be derived from these applications.

Firstly, the method of choice should be applicable for as many PIs as possible, since a large variety of HAART schemes is being prescribed in every patient population. The possibility to simultaneously measure other antiretroviral drugs (especially NNRTIs, such as efavirenz and nevirapine) is an advantage.

Secondly, a method for simultaneous measurement of PIs should be sensitive enough to measure both trough levels of single PIs and proposed threshold limits (section 3.6, table 2).

Thirdly, high specificity should be ensured because of the large number of co-administered drugs in HIV infection (section 3.7).

Concerning the choice of sample pretreatment and separation conditions, it seems that available HPLC equipment and expertise, as well as personal preferences, may well direct the choice of these method characteristics.

With regard to the choice between liquid-liquid and solid-phase extraction, the costs of disposable cartridges may be an additional criterium [36,38].

With respect to separation conditions, it appears advantageous to choose conditions that are as simple as possible, thus avoiding the use of ion-pair reagents as well as amine modifiers. Generally, ion-pair chromatographic methods are more complicated to use and are subject to additional experimental problems [80]. Furthermore, the use of strongly retained additives in the mobile phase (ion-pair reagents, amine additives) can complicate the use of gradient elution [84]. Gradient elution may also appear more complicated than isocratic separation, but it is often required or preferred for samples with many analytes and a wide retention range. Some may have a strong bias against the use of gradient elution for several reasons [84]. Certainly, gradient methods do not always transfer well to other laboratories. This may be particularly true for non-linear gradients.

Run time is another important criterion when there is pressure on the laboratory to perform large numbers of assays. This may even be relevant when samples are being processed automatically. Run times for simultaneous analysis of PIs varied from 11 [25] to 52 minutes [39].

With regard to detection conditions, it should be noted that all published methods for

simultaneous measurement of PIs use UV detection. The (in)availability of a switchable UV-detector or photo-diode array detector may influence the choice of a method.

The published methods for simultaneous measurement of PIs are summarized in table 4 and will be briefly commented upon below.
Frappier [25] and Langmann [26] measured only few PIs. The lower limit of quantitation for ritonavir in the method by Frappier is high. The method applied by Langmann et al may be considered lengthy (49 min).
The method by Hugen [27] has recently been extended (and slightly modified) for measurement of amprenavir, lopinavir and nelfinavir metabolite M8 as well (data in press). This method demands very pure HPLC quality water and extra pure MTBE. Peroxides in MTBE can cause decomposition of nelfinavir. Furthermore, pH of the mobile phase should be set exactly at the desired level.
Moyer [28] applied an unusual gradient, not for mobile-phase strength, but for flow rate. Flow rate increased during the run, apparently aiming at a shorter run time. The limit of quantitation for indinavir (100 ng/ml) is high. Several significant interferences were noted among a very large amount of drugs evaluated for interference.
Remmel et al [29] report adequate limits of quantitation, but inspection of the chromatogram of a low concentration QC sample shows only small peaks for indinavir, nelfinavir and ritonavir at concentration levels far above their limits of quantitation.
Bouley et al [30] separated PIs in only 15 minutes. However, from the chromatograms it appears that there is just baseline resolution between bands of the internal standard and ritonavir, whereas saquinavir and nelfinavir elute close together as well. The limit of quantitation for indinavir is high (100 ng/ml), despite a relatively large volume of plasma (1 ml) to be used in this method. Possible interferences by other than antiretroviral drugs were not reported.
Proust et al describe a method which includes efavirenz, but unfortunately not indinavir [31]. The limit of quantitation of saquinavir is high for measurement of this drug when administered without ritonavir.
In the isocratic method by Van Heeswijk et al [32], indinavir and amprenavir elute early, on the solvent front and close to endogenous interference peaks. The last band of nelfinavir shows peak broadening and is non-symmetric, which may result in sensitivity problems at lower concentrations.

Table 4. Summary of published HPLC methods: simultaneous measurement of PIs[a]

PIs	Matrix	Sample pretreatment	Column	Mobile phase	Run time[b]	Limit of quantitation[c]	Detection[d]	Refs.
RTV, SQV	serum	SPE (C_{18})	C_8 (150x4.6 mm, 5 µm)	isocratic ACN / 5 mM potassium phosphate monobasic buffer pH 8 (55:45 v/v), 1 ml/min	11	RTV:800 SQV:50	UV 240	[25]
IDV, RTV, SQV	plasma	LLE at pH 9.4 diethyl ether hexane wash	C_{18} (150x2 mm, 5 µm)	gradient a. 67 mM KH2PO4 pH 4.6 b. ACN 0.2 ml/min	49	IDV: 75 RTV: 45 SQV: 10	UV 258 (IDV) 240 (RTV, SQV)	[26]
IDV, NFV, RTV, SQV	plasma	LLE at basic pH MTBE Hexane wash	C_{18} (150x4.6 mm, 5 µm)	gradient a. ACN b. 50 mM KH2PO4 (pH 5.63) 1.5 ml/min	30	all PIs: 40	UV 215	[27]
IDV, NFV, RTV, SQV (+ DLV)	serum	LLE at basic pH Ethyl acetate-hexane (1: 1)	C_8 (150 mm)	isocratic ACN / MeOH / 15mM phosphate (pH 7.5) (45:5:50 v/v) gradient flow rate 0.8-1.5 ml/min	25	IDV,NFV, RTV: 100 SQV: 10	UV 254	[28]
IDV, NFV, RTV, SQV	plasma	LLE at basic pH MTBE	C_4 (250 x 3 mm, 5 µm) 40^{0}C	Isocratic ACN / 50 mM sodium formate buffer (52:48, v/v) pH 4.10, 0.5 ml/min	16	IDV: 49 NFV: 43 RTV: 50 SQV: 22	UV 218 (IDV, NFV, RTV) 235 (SQV)	[29]
IDV, NFV, RTV, SQV	plasma	LLE at pH 10.8 MTBE Reconstitution in TMAP-sol Hexane wash	phenyl (250x4.6 mm, 5 µm)	isocratic 0.04 M ammonium acetate / ACN (48:52 v/v), pH 7.5 1.0 ml/min	15	IDV, NFV: 100 RTV: 250 SQV: 25	UV 260	[30]
APV, NFV, RTV, SQV (+ DLV, EFV)	plasma	LLE at pH 10 MTBE	C_{18} (5 µm)	Isocratic Sodium phosphate 25 mM / ACN (55.2:44.8 v/v) + diethylamine 0.9% + THF 1%, pH 3.0 0.5 ml/min	35	APV: 50 NFV: 150 RTV,SQV: 100	UV 260	[31]
APV, IDV, NFV, RTV, SQV	plasma	SPE (C_2)	C_{18} (75x4.6 mm, 3.5 µm)	isocratic ACN / 25 mM sodium acetate +25 mM hexane-1-sulfonic acid, pH 6.0 (40.5:59.5 v/v) 1.5 ml/min	20	APV, IDV, SQV: 25 NFV,RTV: 50	UV 210 (APV, IDV, NFV) 239 (RTV, SQV)	[32]

PIs	Matrix	Sample pretreatment	Column	Mobile phase	Run time[b]	Limit of quantitation[c]	Detectione[d]	Refs.
APV, IDV, NFV,RTV, SQV (+EFV)	plasma	SPE (C_{18})	C18 (125x4 mm, 5 µm)	Gradient a. ACN b. H_3PO_4+ sodium heptane sulphonate in water, PH 5.15 C. 0.3% acetic acid in ACN 1 ml/min	47	APV,SQV: 100 IDV,RTV, NFV: 250	UV 201	[33]
APV,IDV, NFV,RTV, SQV	plasma	SPE (Oasis® HLB)	C^{18} (150x2.1 mm, 4 µm) 24°C	Gradient a. 0.5% 5.8 mol orthophosphoric acid + 0.02% triethylamine, pH 5.0 b. ACN c. MeOH 0.4 ml/min	45	APV,IDV: 5 NFV,RTV, SQV: 10	UV 265 (APV) 210 (IDV, NFV, RTV, SQV)	[34]
APV,IDV, NFV,RTV, SQV (+EFV)	plasma	SPE (C_{18})	C_{18} (250x4.6 mm, 5 µm) 37°C	Isocratic 0.04 M Na_2HPO_4 + 4% v/v OSA / ACN (50:50 v/v) 1.3 ml/min	32	APV: 25 IDV,NFV, RTV: 50 SQV: 5	UV 261 (APV, IDV) 241 (RTV, SQV) 254 (NFV)	[35]
APV,IDV, NFV,RTV, SQV	plasma	LLE at basic pH MTBE Hexane wash	C_{18} (150x4.6 mm, 5 µm)	Isocratic ACN / 50 mM KH2PO4 + 50 mM NaHPO4 (pH 5.6) (43:57 v/v) 1.5 ml/min	40	all PIs: 50	UV 215	[36]
APV,IDV, NFV,RTV, SQV	plasma	SPE (Oasis®)	C_{18} (150x3.9 mm, 5 µm)	gradient a. 15 mM potassium phosphate pH 5.75 B. ACN 1 ml/min	25	APV: 50 IDV: 40 NFV: 85 RTV: 100 SQV: 44	UV 210 (APV, IDV) 240 (RTV, SQV) 220 (NFV)	[37]
APV,IDV NFV,RTV, SQV (+ NVP)	plasma	LLE Ethyl acetate hexane (9:1 v/v) Hexane wash	C_{18} (250x4.6 mm, 5 µm)	gradient a. ACN + 0,025M TMAP in 0.2%TFA b. MeOH + 0,025M TMAP in 0.2%TFA 0.9 ml/min	35	APV: 50 IDV,NFV, SQV: 200 RTV: 400	UV 239 (RTV) 254 (APV, NFV,SQV) 259 (IDV)	[38]
IDV,NFV, RTV,SQV, (+ DLV, EFV,NVP)	serum	SPE (C^{18})	C_{18} (150x4.6 mm, 5 µm) 60°C	gradient a. ACN b. 0.004 M sulphuric acid 0.85 ml/min	52	IDV: 210 (e) NFV: 400 RTV: 510 SQV: 100	UV 265 (IDV) 240 (NFV, SQV,RTV)	[39]

[a] See Nomenclature for abbreviations.
[b] Run time in minutes.
[c] Limit of quantitation in ng/ml.
[d] Wavelength of detection in nm.
[e] Limits of detection instead of limits of quantitation

The assay by Marzolini [33] uses a rather complex, non-linear gradient and requires careful control of one of the solvents' pH for reproducible separation. These features may complicate the transfer of this method to other laboratories. Reported limits of quantitation for indinavir and saquinavir are high. However, as the authors mention, accuracy and precision at the lower limits of quantitation are well below 20% allowances. Therefore, it may be possible to decrease these limits.
Poirier et al [34] use a photodiode array detector for measurement of amprenavir at 265 nm and other PIs at 210 nm. An automatic switchable UV detector may not be applicable as an alternative, since the small difference in retention time between amprenavir and indinavir may not allow for programmed wavelength changes.
Aymard et al developed a method for measurement of 12 antiretroviral drugs, PIs, NNRTIs and NRTIs [35]. One solid-phase extraction procedure was coupled with two separate reversed-phase HPLC systems, one for 5 PIs and efavirenz, and one mainly for NRTIs.
Yamada et al used a sample preparation procedure identical to that described by Hugen [27], but they chose isocratic (instead of gradient) separation conditions [36]. The method was not tested for interference by other antiretroviral drugs or other medications.
In the method by Sarasa-Nacenta et al, good separation of 5 PIs depends on accurate setting of pH and ionic strength of the mobile phase [37].
Dailly et al measured nevirapine together with 5 PIs [38]. Separation is achieved by gradient elution, combined with a hydrophobic ion-pair reagent in the mobile phase. Limits of quantitation for indinavir, ritonavir and saquinavir are high.
Simon et al were able to measure 4 PIs and 3 NNRTIs in one run [39]. Run time may be considered long (52 min). Possible interference by other drugs was not reported. The limits of detection are high.

5. Conclusions

Since the introduction of PIs for treatment of HIV infection, numerous HPLC methods have been developed for analysis of these drugs in plasma and serum, saliva, CSF and semen. Fifteen methods described so far have concerned the simultaneous analysis of several PIs in one run. Heat treatment for deactivation of HIV may lead to slight degradation of PIs in plasma samples. Liquid-liquid extraction was most often applied

for sample pretreatment, but solid-phase extraction and protein precipitation were used as well. Reversed-phase or ion-pair chromatography have been used to separate PIs. Isocratic conditions have been applied for measurement of single PIs, and gradient elution has been used in 7 of the 15 methods for simultaneous measurement of PIs. Detection of PIs should be sensitive enough for quantitation of concentrations below trough concentrations of single PIs, or below presumed therapeutic thresholds for PIs. The large majority of assays employs UV detection. As the potential for interferences is large, the selectivity of every method should be evaluated properly.
The available HPLC methods have been applied in clinical pharmacokinetic studies with PIs and have provided the basis for important developments in the clinical pharmacology of antiretroviral drugs. New interests, such as in free (non-protein bound) plasma concentrations and intracellular PI drug levels, also require application and development of reliable assays. Furthermore, studies relating pharmacokinetics to clinical effects have raised large interest in TDM for PIs. The promising perspective of TDM to optimize the clinical use of PIs may really spread the use of HPLC methods, as TDM requires that measurement of PIs is not confined to a small number of research laboratories, but can be applied in hospital laboratories as well. Fortunately, most HPLC assays for PIs are quite straightforward and can be performed with equipment that is available or affordable in most hospitals. Assays for simultaneous measurement of PIs appear to be most convenient for the purpose of TDM. It is recommended that any laboratory engaged in the analysis of PIs, whether as a routine service or as part of a research project, joins an interlaboratory QC program in addition to establishing its own QC procedures.

6. Nomenclature

ACN	acetonitrile
APV	amprenavir
Cmax	maximum (peak) concentration
Cmin	trough concentration
CSF	cerebrospinal fluid
DLV	delavirdine
ED	electrochemical detection
EFV	efavirenz
El.	electrode
Em	wavelength of emission
Ex	wavelength of excitation
FL	fluorescence
HAART	higly active antiretroviral therapy
HGC	hard-gelatin capsule
IDV	indinavir
LLE	liquid-liquid extraction
LOD	limit of detection
MeOH	methanol
MTBE	methyl tert.-butyl ether
na	not available (or not reported)
NFV	nelfinavir
NNRTI	non-nucleoside reverse transcriptase inhibitor
NRTI	nucleoside reverse transcriptase inhibitor
NVP	nevirapine
OSA	octane sulphonic acid
PI	protease inhibitor

QC	quality control
RTV	ritonavir
SGC	soft-gelatin capsule
SPE	solid-phase extraction
SQV	saquinavir
TBA	tetrabutylammonium hydrogen sulphate
TDM	Therapeutic Drug Monitoring
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TMACl	tetramethylammonium chloride
TMAP	tetramethylammonium perchlorate

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Chapter 2.2

Therapeutic Drug Monitoring: an aid to optimising response to antiretroviral drugs?

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Abstract

Therapeutic Drug Monitoring (TDM) has been proposed as a means to optimise response to highly active antiretroviral therapy (HAART) in HIV infection. Protease inhibitors (PIs) and the non-nucleoside reverse transcriptase inhibitors (NNRTIs) efavirenz and nevirapine satisfy many criteria for TDM. Nucleoside reverse transcriptase inhibitors (NRTIs) are not suitable candidates for TDM, since no clear plasma concentration-effect relationships have been established for these drugs. Several important limitations to the application of TDM for antiretroviral drugs should be recognised, including uncertainty about the best pharmacokinetic predictor of response and insufficient validation of target concentrations for individual PIs and NNRTIs. Data from two clinical trials support the use of TDM in treatment-naive HIV-infected patients who start with an indinavir- or nelfinavir based regimen. TDM either prevented virological failures (presumably by preventing the development of resistance) or treatment discontinuations due to concentration-related toxicity. Application of routine TDM in other patient groups (treatment-experienced patients) or for drugs other than indinavir or nelfinavir (NNRTIs, other PIs, combination of PIs) is speculative at this moment. However, TDM can be used in selected patient groups (children, pregnant women, patients with renal or hepatic dysfunction) to confirm adequate drug levels, and for management of drug-drug interactions. TDM in treatment-experienced patients may be optimally used in conjunction with resistance testing. The integration of pharmacological and virological measures in the inhibitory quotient (IQ) needs to be standardised and elaborated further. TDM should be accompanied by careful assessment of adherence and can itself help identify non-adherence, although a drug concentration only reflects the last few drug doses taken by a patient. Additional clinical trials are needed before routine TDM can be adopted as standard of care in the treatment of HIV infection.

1. Introduction

The use of highly active antiretroviral therapy (HAART) is associated with marked improvement in the clinical outcome of patients with HIV infection [1]. However, this success is tempered by rates of virological failure which reach 50% in therapy-naive patients who have been using HAART for one year [2,3]. This can be ascribed to a number of interactive factors related to the patient (host), the virus, or the antiretroviral drugs used [4]. As long as these factors result in inhibitory concentrations of multiple drugs at the sites of HIV replication, the highly error-prone replication process can be constrained almost completely. Development of new drug-resistant mutants will then be rare [5,6]. High drug concentrations are also believed to suppress the outgrowth of drug-resistant viruses which pre-exist in drug-naive wild-type virus populations. In contrast, suboptimal drug concentrations will confer selective pressure, which ultimately results in the emergence of mutant viral isolates with reduced susceptibility to one or more drugs [5-7]. This will compromise the response to the treatment regimen and may also jeopardize the efficacy of remaining therapeutic options for an individual, given the potential for cross-resistance between antiretroviral agents of the same class. Moreover, the increased potential for resistant virus to be transmitted to other persons has important public health implications [8].

Within this context, it has been speculated that Therapeutic Drug Monitoring (TDM) may have a role in optimising antiretroviral therapy. In contrast to administering the same dose to all patients ("one dose fits all"), TDM seeks to individualise drug dose, guided by measurement of plasma drug concentrations. The aim is to bring (and keep) a patient's plasma concentration within a target concentration (therapeutic) range [9-12].

The objective of this article is to present an overview of the current status of TDM for antiretroviral drugs by; (i) reviewing its rationale and the arguments for and against the use of TDM in HIV infection; and (ii) discussing clinical trial results and recent developments that integrate drug level measurements and viral resistance testing.

2. Rationale for Therapeutic Drug Monitoring in HIV infection

The concept of TDM to individualise drug dosage to attain certain target plasma concentrations is currently applied to a small number of drugs, e.g. gentamicin, digoxin and theophylline. This concept seems suitable for antiretroviral drugs as well, since the exposure obtained from an identical antiretroviral drug dose varies greatly between patients. By identifying and adjusting drug exposures that are suboptimal, TDM could possibly aid in preventing the development of viral resistance, which occurs as outlined in the first paragraph of this article. In addition, TDM might be instrumental in overcoming moderately decreased viral susceptibilities, by increasing exposure to an antiretroviral drug. Apart from preventing or overcoming decreased viral susceptibility, TDM may also be useful in avoiding inadequate response that is not mediated by resistance development [13]. More specifically, TDM may prevent suboptimal drug levels which limit the response to HAART even in the absence of resistance development [14-17]. It may be helpful for management of drug interactions and in identifying non-adherence, both of which are major problems in the treatment of HIV infection [18,19]. An additional major cause of treatment discontinuation is the occurrence of adverse events [20,21], which may be associated with excessively high drug concentrations. By identifying and reducing unduly high drug concentrations, TDM may prevent avoidable toxicity and non-adherence related to toxicity.

3. Criteria for TDM to be useful: the case for TDM

Whereas there is a clear rationale for TDM of antiretroviral drugs, these drugs need to fulfill certain criteria for TDM to be useful. The most important criteria for TDM [9-12]

Table 1. Currently licensed protease inhibitors (PIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs) and nucleoside reverse transcriptase inhibitors (NRTIs)

PIs	NNRTIs	NRTIs
amprenavir	delavirdine	abacavir (ABC)
indinavir	efavirenz	didanosine (ddI)
lopinavir (coformulated with ritonavir)	nevirapine	lamivudine (3TC)
nelfinavir		stavudine (d4T)
ritonavir		zalcitabine (ddC)
saquinavir		zidovudine (ZDV, AZT)

are discussed in the following sections for the three major classes of antiretroviral drugs, the protease inhibitors (PIs), nucleoside reverse transcriptase inhibitors (NRTIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs) (table 1).

3.1 A more direct intermediate measure of patient response is not available

TDM is a specific form of a general strategy in which an intermediate endpoint is employed rather than a clinical endpoint. Antiretroviral treatment is already being evaluated using intermediate endpoints such as viral load and CD4+ cell count measurements, but these parameters are not sufficient for optimal prevention of resistance development. By the time increased HIV replication is observed using viral load measurements, drug resistance may already have developed. In contrast, measuring and adjusting plasma drug concentrations early in treatment may provide some assurance that resistance development will not occur or will be delayed.

3.2 There is large interindividual variability in pharmacokinetic parameters

If the interpatient variability in pharmacokinetics were small, concentrations of drugs in plasma could be predicted adequately from drug dose, and dose would be as good as drug concentrations in predicting response. However, it is well documented that all antiretroviral drugs exhibit wide inter-patient variation in plasma trough concentrations (Cmin), peak concentrations (Cmax) and area under the time versus concentration curve (AUC) [22-28]. Interpatient concentrations obtained by the same dose often vary up to 10-fold. This also applies to PIs when they are co-administered with low-dose ritonavir, which increases PI drug concentrations through inhibition of the cytochrome P450 (CYP) isoenzyme CYP3A (so called "boosting" [29]). The wide interpatient pharmacokinetic variability can be explained by large variability in the activity of CYP isoenzymes which catalyse the metabolism of many antiretroviral drugs [30], genetic variability in drug-transporting proteins [31,32], and the large potential for drug-drug and drug-food interactions among antiretroviral drugs [18,33].

3.3 A good relationship exists between plasma drug concentrations and therapeutic or toxic effect

If the relationship between antiretroviral plasma concentration and drug response was unpredictable as well, TDM would be useless. Indeed this appears to be the case for NRTIs (table 1), which are prodrugs that require intracellular activation to active triphoshate derivatives. No clear relationships have been established between plasma concentrations of the parent NRTIs and antiviral response. Measuring triphosphate derivatives in peripheral blood mononuclear cells may be more useful for predicting response [34,35], but such measurements are technically difficult and not widely available. Consequently NRTIs do not appear to be suitable candidates for TDM at this time.
In contrast to NRTIs, PIs do not require metabolic conversion to achieve antiviral activity. An abundance of studies supports an association between PI plasma concentrations and antiviral response, especially in treatment-naive patients [14,17,36-56]. In addition, it has been reported that the rate at which PI resistance mutations appear is inversely related to plasma PI concentrations [37]. Relationships have also been found between pharmacokinetics and toxicity of indinavir [57-62], ritonavir [63], amprenavir [50], and nelfinavir [64].
Similarly, relationships have been demonstrated between plasma concentrations and antiviral efficacy of the NNRTIs efavirenz [26,65,66] and nevirapine [27]. Theoretically, TDM might be especially useful for these drugs, as only one single mutation can engender resistance to these agents. High efavirenz concentrations have been linked to central nervous system adverse effects in some studies [26,67], but not in all [68]. High nevirapine Cmin values have been associated with hepatotoxicity [69].

3.4 There is a narrow range of concentrations that are effective and well tolerated

On the basis of the studies discussed in the previous sections, it is possible to outline plasma "target" concentrations for several PIs and NNRTIs (table 2). Therapeutic thresholds are sometimes close to median population pharmacokinetic parameters. Unfortunately, patients can not simply be treated with very large doses (as is commonly done with penicillins) since toxicity can be expected for several antiretrovirals, demonstrating their narrow therapeutic ranges.

Table 2. Therapeutic target concentrations for wild-type viral isolates and maximum (toxic) concentrations of protease inhibitors (PIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs) [a]

Drug	Therapeutic target concentration (mg/L)	Maximum concentration (mg/L)	References
PIs			
amprenavir	0.228	-	[50]
indinavir	0.10	8-10 [b]	[42,47]
lopinavir (coformulated with ritonavir)	-	-	
nelfinavir	0.80	-	[53,80]
ritonavir	2.1	-	[36]
saquinavir	0.05	-	[45]
NNRTIs			
delavirdine	-	-	
efavirenz	1	4	[26]
nevirapine	3.4	-	[27]

[a] Therapeutic target concentrations for PIs refer to plasma trough concentrations (Cmin). The therapeutic range for efavirenz refers to samples obtained between 8 and 20 hours post-dose administration; the therapeutic threshold for nevirapine relates to samples taken at random times post-dose administration.
[b] Indinavir-associated nephrotoxicity has been related to peak plasma concentrations (Cmax), but no clear threshold value for nephrotoxicity has been found. Such a value may vary dependent on the dosage regimen (indinavir with or without co-administration of low-dose ritonavir).
" - " indicates no data available at this time

3.5 Drug assays are available

Numerous accurate, precise and specific high-performance liquid chromatographic (HPLC) assays with ultraviolet or mass spectrometric detection have been described for measurement of PIs and NNRTIs in the plasma [70]. Laboratories can now participate in an international interlaboratory proficiency testing (quality control) program for measurement of PI and NNRTI concentrations [71,72].

4. Theoretical and practical limitations of TDM: the case against TDM

Several limitations of TDM for antiretroviral drugs should be considered [25,73,74]. Some of these limitations apply to TDM in general, others specifically relate to TDM for PIs and NNRTIs.

4.1 TDM results are confounded by non-adherence

Adequate in-office measurements of antiretrovirals may not always reflect out-of-office concentrations, considering that patients may adhere more to their dosage schedules before appointments with their physician. Conversely, an *in*adequate drug level may not reflect an insufficient dose for a particular patient, but non-adherence. These issues are an argument for use of TDM in conjunction with proper adherence assessments (preferably by a combination of methods [75,76]), and for measuring drug concentrations after observed intake of drugs. If this is not possible, low drug concentrations identified by TDM still alert physicians to some unrecognised problem, enabling them to take action.

4.2 Intra-individual variability in the pharmacokinetics of antiretroviral drugs

Whereas a large *inter*-individual variability in pharmacokinetics is one of the prerequisites for TDM, large *intra*-individual variability limits the value of a single drug measurement. Fortunately, it appears that intra-individual day-to-day variations in PI and NNRTI drug concentrations are modest (coefficients of variation in drug levels are about 30 to 45%) [24,26,77], but this issue has not been fully evaluated. Additional blood samples may in any case be required to obtain reliable estimates of individual pharmacokinetic parameters, especially if an advice for dose adjustment is considered. Large intra-individual fluctuations in plasma concentrations are not expected for efavirenz and nevirapine, as these NNRTIs have long elimination half-lives [26,28].

4.3 TDM does not measure unbound drug concentrations

Measurements of most drugs in TDM comprise the total drug concentration and not the unbound fraction, which is free to exert an effect or to be distributed to tissues. This also applies to measurement of PIs and NNRTIs, most of which are highly bound to plasma proteins, specifically the acute-phase alpha-1-acid glycoprotein (AAG) [25,28]. Since AAG levels fluctuate with intercurrent ilnesses, blood for PI and NNRTI concentrations should be drawn in stable clinical conditions or otherwise be interpreted cautiously. Concern has been raised by the considerable inter-patient

variability in protein binding demonstrated for indinavir, as well as the variable indinavir protein binding over the dosage interval [78]. Still, it should be noted that relationships between plasma PI and NNRTI concentrations and response have all been established by measuring total drug concentrations.

4.4 Presence of active metabolites renders interpretation of drug concentrations difficult

Nelfinavir is the only current antiretroviral drug which forms an active metabolite (termed M8) which circulates at appreciable concentrations in plasma [79]. M8 is as active as nelfinavir in vitro [79]. There is no agreement among studies as to the need to measure M8 in addition to nelfinavir [54,80,81]. However, M8 levels should be measured (and added to nelfinavir values, assuming additive virological efficacy) when ritonavir is co-administered to increase nelfinavir levels, since ritonavir strongly enhances the M8-to-nelfinavir ratio [82-84].

4.5 Target concentrations

Several important but unresolved issues relate to the target concentrations to be used for TDM of antiretroviral drugs [25,73,74].
Firstly, it has not been demonstrated beyond doubt which of three interrelated pharmacokinetic parameters (Cmin, Cmax, or AUC) is the best predictor of the antiviral activity of PIs and NNRTIs. For PIs, most evidence points to Cmin as the most critical parameter, especially when considering studies with indinavir [85-87]. This means that minimum antiviral concentrations would be required during the entire dosage interval to prevent viral breakthough (i.e. time-dependent viral inhibition). In a recent study, the Cmax of indinavir was uniquely associated with increases in CD4+ cell count in treatment-naive patients who used indinavir, lamivudine and zidovudine, and who had an undetectable viral load. This suggests that distinct pharmacokinetic-pharmacodynamic relations exist for effects of PIs on CD4+ cell count (immune reconstitution) and HIV viral load [56].
As a second issue, it can not be excluded that target concentrations for PIs and NNRTIs vary to some extent dependent on the background antiretroviral drugs, due to additivity or synergy between components of an antiretroviral drug combination.

Thirdly, proposed target concentrations (table 2) have not yet been validated sufficiently in clinical trials (see section 5).
Finally, many treatment-experienced patients have HIV isolates with reduced susceptibility. This implies that higher, individualised target concentrations should be considered for these patients (see section 6).

4.6 Sampling times

It is rather complex to assess Cmin accurately, as this requires patients and providers to be available at a particular time during a dosage interval. Similarly, it is not easy to assess Cmax, which has been related to nephrotoxicity of indinavir [57-61]. These sampling problems can be circumvented by using simple (figure 1) or more sophisticated (Bayesian) population pharmacokinetic approaches. Pharmacokinetic data on the various antiretroviral treatment regimens (and accompanying concentration-time curves) were summarised recently [88].
For PIs, it should be considered that Cmin values in the morning may be higher than Cmin values in the evening as a result of diurnal variation in the pharmacokinetics of

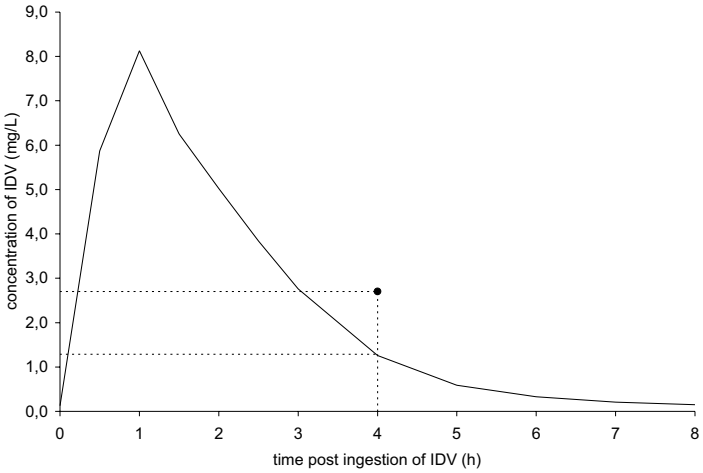

Figure 1. Calculation of the concentration ratio (CR) as a simple method for interpretation of drug concentrations from randomly drawn samples. The figure displays the reference population curve of indinavir (IDV) 800 mg three times daily. A patient sample drawn 4 h post-ingestion contained 2.7 mg/L IDV; the population value at 4 h is 1.3 mg/L. Therefore the CR can be calculated as 2.7/1.3 = 2.1. Dependent on the CR, the therapeutic drug monitoring service could propose interventions to the physician in attendance (e.g. a dose adjustment, discussing correct dietary intake requirements, discussing adherence).

these drugs [89]. For the NNRTIs nevirapine and efavirenz, randomly taken samples are sufficient, since the decay in plasma concentrations within the dosage interval is relatively small [26,28,90].

4.7 Logistics, bio-analysis, and interpretation of measurements

TDM is a process involving a series of steps, including a pre-analytic, analytic and post-analytic component [91,92]. The complexity of certain steps and the length and logistics of the whole process may complicate the practical implementation of TDM for antiretrovirals [73,93]. As to the analytic component, available HPLC methods certainly require skilled personel, are not standardised and not widely available at the moment, resulting in a long turn-around time for test results. Moreover, results from an interlaboratory proficiency testing program showed that at least 40% of participating laboratories needed to improve their performance with respect to HPLC measurement of PIs and NNRTIs [71,72]. However, the post-analytic component of drug concentration interpretation, which really transforms a therapeutic drug *measuring* service into a therapeutic drug *monitoring* service [74], may be cause for more concern in the long term. It is challenging to maintain adequate knowledge of the pharmacokinetics and pharmacodynamics of the bewildering choice of antiretroviral drug combinations. Expert advice seems to be required for optimal drug level interpretation and appropriate dose adjustments.

5. Weighing pros and cons of TDM for antiretroviral drugs: results from clinical trials

An essential prerequisite for TDM is the availability of prospective, randomised, controlled clinical trials that validate proposed target values and the utility of TDM [9]. Several such studies have been initiated, focusing on HIV-infected treatment-naive patients (prevention of resistance development and treatment discontinuation) or treatment-experienced patients (overcoming treatment failure and resistance).

A small, randomized, open-label study among treatment-naive patients demonstrated the feasibility, efficacy and safety of a dose adjustment strategy to achieve target concentrations of indinavir, lamivudine and zidovudine simultaneously [94,95]. After

1-year follow-up, 14 of 16 patients in the "concentration-controlled" arm achieved the desired concentration for indinavir, compared with 3 of 17 patients in the control arm, which received fixed-dose therapy ($p<0.001$). The proportion of patients with an undetectable viral load was higher in the concentration-controlled arm (15 of 16 vs 9 of 17 participants, figure 2); drug-related clinical events or laboratory abnormalities were similar in both arms.

Preliminary results have been reported for a larger trial which was set up as part of the Dutch ATHENA (Anti-HIV Therapy Evaluation, The Netherlands) study [96,97]. In this study, 147 treatment-naive patients initiating therapy with either a nelfinavir- or indinavir-based regimen were randomised to a TDM arm and a control arm. A significantly lower proportion of patients in the TDM arm failed after 1 year of therapy compared with patients in the control arm (17.4% versus 39.7%). In patients receiving nelfinavir, this benefit was attributed to fewer treatment discontinuations due to virological failure. TDM recipients in the indinavir arm were less likely to stop treatment because of adverse events. Therefore, TDM of indinavir can be used to prevent concentration-related toxicity and/or to improve the management of adverse effects.

Another large study, the Pharmadapt trial, randomised treatment-experienced patients who failed previous therapy to an arm receiving genotypic resistance testing and an arm receiving genotypic resistance testing plus TDM for PIs [98]. After 12 weeks and 32 weeks, no favourable effect of TDM was demonstrated [98,99]. However, multiple factors limit the interpretation of this study, including target concentrations that were probably too low for treatment-experienced patients. Likewise a similar study did not demonstrate a short-term benefit of TDM for PIs and

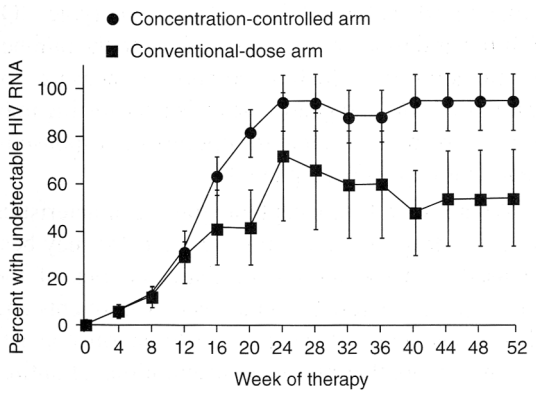

Figure 2. Percentage of patients in the "concentration-controlled" arm and conventional dose arm with less than 50 copies/ml of plasma HIV RNA (undetectable viral load) throughout the 52 weeks of the study by Fletcher et al [95]. The bars indicate 95% confidence intervals. Reprinted with permission from Lippincott Williams & Wilkins.

NNRTIs in patients failing antiretroviral therapy [100].
In summary, only limited and preliminary data from randomised clinical trials have been reported so far. More data are needed to warrant the use of TDM in the routine management of HIV infected individuals.

Apart from these randomised trials, that were specifically designed to assess the value of TDM, other studies have suggested the value of TDM in selected patient groups with altered pharmacokinetics. TDM may be particularly useful in HIV-infected children, as the pharmacokinetics of many antiretroviral drugs in children are different from that of adults and also change over time, as a result of maturation of organ systems involved in drug absorption and disposition. In an overview of clinical trials that evaluated HAART regimens in children, 4 of 23 studies used dosages that were adjusted after pharmacokinetic evaluation. These studies showed superior virological response rates compared with the other studies that applied fixed dosages [101].
Other patient groups with altered pharmacokinetics are pregnant women and patients with liver function disturbances. Limited data suggest that TDM may be indicated in these patients as well [69,102-105].
Pharmacokinetic parameters can also be altered by the many drug interactions that have been described for antiretroviral drugs [18]. By taking baseline antiretroviral drug levels before introduction of an additional drug, the effect of a possible pharmacokinetic interaction can be evaluated.
Finally, studies have evaluated ways to assess adherence based on antiretroviral drug concentrations [106,107] and have described TDM as an objective means to assess non-adherence to PIs [49,76,108,109]. However, it should be realised that a drug concentration merely reflects the last few doses taken by a patient.

6. Further individualization of antiretroviral therapy: the inhibitory quotient

In using a single target concentration (Cmin) for an antiretroviral drug, it is assumed that all patients have viral isolates with the same susceptibility. However, the majority of treatment-experienced patients will harbour resistant viral isolates, i.e. viruses with reduced susceptibility to drugs. Drug susceptibility can be expressed quantitatively by

using a phenotypic resistance assay, which assesses the concentration of a drug required to inhibit 50% (IC_{50}), 90% (IC_{90}) or 95% (IC_{95}) of viral replication *in vitro*. It is unknown what concentration of a particular drug is necessary to fully suppress the replication of a viral strain with reduced susceptibility, but it is likely to be higher than the IC_{50} for that drug. This realisation has led to the revitalization of the Inhibitory Quotient (IQ) [110], which in the field of antiretroviral therapy has been defined loosely as the ratio of a drug's Cmin value to an inhibitory concentration (usually IC_{50}) which is corrected for protein binding (figure 3). Using the IQ concept, it may become possible to predict Cmin values required to overcome reduced viral susceptibility [111]. Such information could aid in the selection of optimal therapy, in terms of the drugs chosen and the doses applied [112]. In the case of PIs, individualised Cmin and IQ values that are both achievable and tolerable can be targeted by co-administration of low-dose ritonavir (boosting [29]). First evidence suggests that IQ is a good predictor of virological response to PIs in treatment-experienced patients [86,112-115], better than Cmin or IC values alone [86,113]. Moreover, it appears possible to

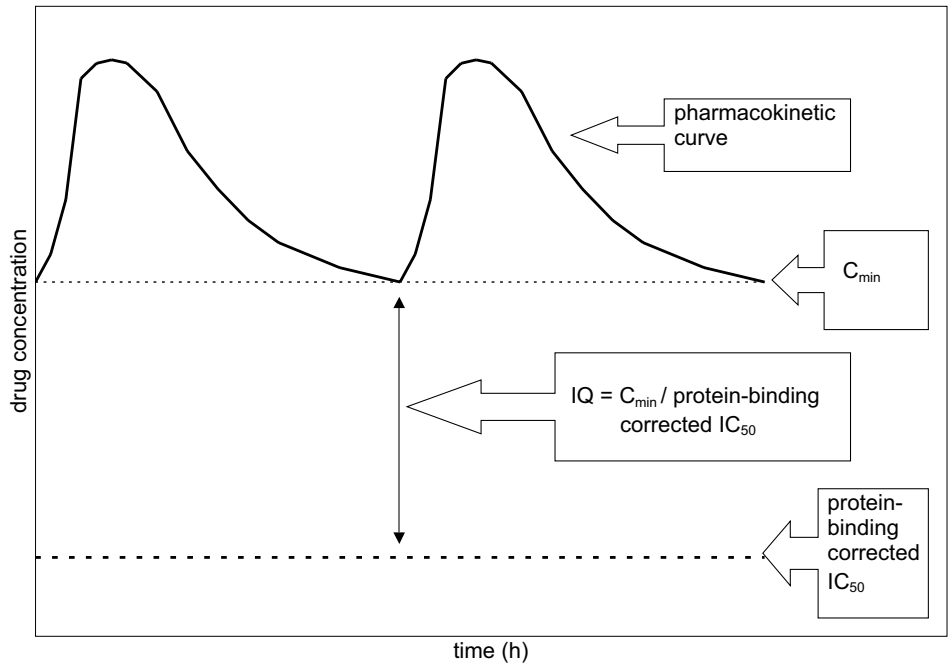

Figure 3. The concept of the Inhibitory Quotient (IQ), the ratio of Cmin to a protein binding corrected IC_{50}. A higher IQ presumably results in more complete suppression of viral replication. Cmin = minimum plasma concentration; IC50 = concentration required to inhibit 50% of viral replication.

overcome early virological failure with boosted PIs and this may be explained by the IQ concept [112,113]. However, initial studies using the IQ concept also highlighted large variation in the definition and assessment of IQ values; these need to be standardised if the IQ is to be used in the clinical setting [116-119]. Much of this variability in IQ values can be attributed to a lack of standardization in performing *phenotypic* resistance testing. Recent studies focussed on TDM combined with *genotypic* resistance testing (i.e. evaluating mutations in the virus' genetic material) and found that plasma PI concentrations and the number of mutations (as well as certain specific mutations) predict the response to PIs in treatment-experienced patients [120-123]. The "genotypic IQ" (GIQ) was defined as the ratio of Cmin to the number of mutations [120]. Such a composite measure for results of TDM and genotypic resistance testing may prove to be most suitable for optimising response in treatment-experienced patients [120].

Whereas the IQ concept integrates characteristics from the drugs and the virus, future TDM may best be used in association with characteristics of the host as well, i.e reliable adherence assessments and pharmacogenetic information. As to pharmacogenetics, it appears that plasma concentrations of antiretroviral drugs may vary according to variations in genes coding for P-glycoprotein and CYP iso-enzymes [32]. Although these data should not be over-interpreted at this moment, pharmacogenetic information may eventually be applied for initial dose stratification or identification of patients where certain drugs are simply not effective enough [124].

7. Conclusions

There is a clear rationale for TDM of antiretroviral drugs to prevent and overcome resistance and optimise treatment response in HIV infection. The PIs and NNRTIs (efavirenz and nevirapine) satisfy many criteria for TDM, but NRTIs are not suitable candidates for TDM. Several limitations of TDM for antiretroviral drugs should be recognised and restrict its application at this moment. Data from two clinical trials have provided "proof-of-concept" for TDM of PIs, supporting its use in treatment-naive HIV-infected patients who start with an indinavir- or nelfinavir-based treatment regimen. Other applications of routine TDM beyond this patient group (i.e. treatment-experienced patients) or beyond these antiretroviral drugs (other PIs, ritonavir-boosted

PIs, NNRTIs) are speculative at this time. However, TDM can be used in selected patient groups with altered pharmacokinetics to confirm adequate drug concentrations, and for management of drug interactions. TDM in treatment-experienced patients aims to overcome low-level resistance and may be optimally used in conjunction with resistance testing. The calculation of the IQ value needs to be standardised and prospective clinical trials are warranted to document its value. TDM should be accompanied by careful assessment of patient adherence. TDM itself can help identify non-adherence but has its limitations in this respect. More clinical trials are needed before TDM can be adopted as standard of care in the treatment of HIV infection. All current endeavors for TDM should preferably be performed in clinical studies to extend available data. There remains a strong need for development of new drugs and strategies which achieve predictably high plasma drug concentrations with less toxicity.

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Chapter 3.

Interlaboratory quality control for measurement of antiretroviral drugs

Chapter 3.1

International interlaboratory quality control program for measurement of antiretroviral drugs in plasma

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Abstract

An international interlaboratory quality control program for measurement of antiretroviral drugs was initiated. The first round was confined to protease inhibitors and showed large variability in the performance of participating laboratories. The results demonstrate the need for and utility of an ongoing quality control program in this area of bioanalysis.

There has been increasing interest in bioanalysis of protease inhibitors (PIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs) since the advent of these drugs for treatment of human immunodeficiency virus (HIV) infection. Numerous analytical methods have been published, describing the quantitation of PIs and NNRTIs in human plasma and other body fluids [1]. These methods have been used to study the pharmacokinetics and interactions of these drugs [1,2]. Furthermore, it has been suggested that analysis and interpretation of plasma levels can be applied to individualise drug dosage of antiretrovirals, especially PIs [3,4]. Randomized clinical trials have been started to determine the value of Therapeutic Drug Monitoring (TDM) for these drugs [5,6]. Anticipating the final results of these trials, many laboratories already offer a TDM service for antiretroviral drugs.
The wide application of analytical methods for antiretroviral drugs requires quality control (QC) procedures to ensure that these methods have sufficient accuracy, precision and specificity. Such procedures usually include intralaboratory (internal) method validation, intralaboratory QC procedures, and participation in an interlaboratory (external) QC program. So far there has been no interlaboratory QC program for antiretroviral drugs. Therefore such a program was initiated in order to enable laboratories to assess and improve their performance with respect to measurement of these drugs [7,8].

The first round of the program was confined to measurement of PIs. First, QC samples were prepared by spiking drug-free plasma from HIV negative volunteers with indinavir, nelfinavir, ritonavir, and saquinavir. All protease inhibitors were obtained from pharmaceutical industries and had a very high (> 99%) and specified purity. Drug-free human plasma was obtained from the regional blood bank.
PIs were weighed out on an independently calibrated balance. They were dissolved in methanol and diluted with blank plasma to obtain three different QC samples. Each of the three samples contained all four PIs in variable concentrations. For every PI, there was a sample with a low concentration, a sample with an intermediate concentration and a sample with a high concentration (table 1).
The QC samples were dispensed in polypropylene vials that were kept at -20°C. Stability under these and other conditions had been assessed before [9].
All weighed-in concentrations were considered true values. Three vials of every QC sample were analysed with our own validated high-performance liquid

Table 1. Concentration levels in QC samples (expressed as free base and in milligrams per liter)

Drug	Low concn	Intermediate concn	High concn
Indinavir	0.15	1.98	8.49
Nelfinavir	0.20	2.86	8.00
Ritonavir	0.20	2.87	11.04
Saquinavir	0.087	2.06	4.80

chromatographic (HPLC) method [5] as a confirmative check (< 5% deviation from true concentrations) before the samples were released for the QC program.
Nine laboratories from six West European countries and one North American country participated in the first round of the program. They were asked to analyze the samples, and to return their results (with concentrations expressed as free base) within six weeks after dispatch.
Descriptive statistics were calculated after standardization of all laboratory results to percentages with reference to the true value. By subtraction of 100% from these percentages, the percentage bias from the true concentration (inaccuracy) was calculated. Twenty percent limits around the true values were considered to be appropriate threshold values for satisfactory measurements.
Multifactorial analysis of variance (ANOVA) was used to evaluate the simultaneous effect of two factors, the PI to be measured and the concentration level, on the absolute inaccuracy. Results for different concentration levels of the same PI were considered to be related to each other, and the concentration level was therefore included as a within-subjects (repeated-measures) factor. The PI to be analysed was a between-subjects factor.
All participants were informed about their performance within two months after reporting of their results. All results were interpreted briefly in words.

Five of the nine participating laboratories were able to measure all four PIs. Three laboratories were not able to determine nelfinavir. One laboratory measured indinavir only. All laboratories measuring more than one PI used an assay for simultaneous determination of PIs.
Six laboratories used HPLC with UV detection to quantify the PIs and three laboratories used liquid chromatography with mass (or tandem mass) spectrometry detection (LC-MS or LC-MS/MS). Because of the small number of participants in this first round of

Table 2. Measurements of QC samples, subdivided by drug and concentration level [a]

Drug	No. of measure-ments	Concn Level	Measured concn relative to true value (%)	Absolute inaccuracy (%)[b]	No. and % of measurements with acceptable accuracy[c]	
			Median (min-max)	Median (min-max)		
Indinavir	7	Low	107 (96-142)	7 (0.7-42)	5/7	
	9	Intermediate	106 (85-133)	12 (4-33)	7/9	80%
	9	High	106 (91-124)	9 (0.2-24)	8/9	
Nelfinavir	6	Low	94 (50-150)	28 (7-50)	2/6	
	6	Intermediate	86 (70-126)	18 (12-30)	4/6	67%
	6	High	92 (83-118)	12 (6-18)	6/6	
Ritonavir	6	Low	84 (48-184)	30 (1-84)	1/6	
	8	Intermediate	88 (32-134)	18 (2-68)	4/8	36%
	8	High	85 (39-144)	22 (5-61)	3/8	
Saquinavir	7	Low	100 (60-138)	15 (0-40)	5/7	
	8	Intermediate	86 (69-137)	15 (2-37)	6/8	74%
	8	High	89 (75-142)	13 (4-42)	6/8	

[a] *Abbreviations: min, minimum value; max, maximum value.*
[b] *Inaccuracy is percentage bias from the true concentration, i.e., inaccuracy = (100 * measured concentration / true concentration) - 100%.*
[c] *Acceptable measurements are within 20% limits from the true concentration.*

the program, no valid comparison could be performed between HPLC-UV and LC-MS/MS methods.

Two laboratories reported being unable to measure some low concentrations of PIs with sufficient accuracy, since these concentrations were below the lower limits of quantitation of their methods. Results for these measurements were not included in the analyses.

Table 2 and figure 1 summarize the results for the participating laboratories. Four laboratories used analytical methods that appeared to have a large systematic error in one direction, as all measured concentrations of at least one PI were either above or below the assigned 20% threshold for acceptable measurements.

Mean absolute inaccuracies for measurement of the four PIs (in percentage deviation from true values) were not significantly different: $F(3,22)=1.40$, $p = 0.27$. This may be due to the small number of laboratories and measurements in this first round of the program, and/or the large variability in results. However, conversion of the results to a dichotomous scale (acceptable accuracy or not) suggested a much worse performance

for ritonavir (table 2, last column). This could be attributed to a relatively large number of ritonavir measurements with an inaccuracy of just more than 20%.
A significant effect due to the concentration level to be analyzed was assessed: $F(2,44) = 5.04$, $p = 0.01$. Mean inaccuracy over all PIs was 25.3% for low concentrations and decreased to 17.1% for high concentrations. The PI-by-concentration level interaction was not statistically significant.

The initial results of this program show large variability in the performance of laboratories with regard to measurement of PIs in plasma. The magnitude of observed inaccuracies may have important implications for the interpretation of pharmacokinetic studies and may lead to inappropriate dose adjustments in TDM or the advice not to adjust doses where it might actually be desirable. For example, if it was assumed that the small number of participants represented all laboratories for PI analysis and that 20% deviations from true concentrations could negatively affect patient management, then a physician would have 35% probability to receive such an incorrect result after submission of a random sample to a random laboratory. If 50% inaccuracy would be

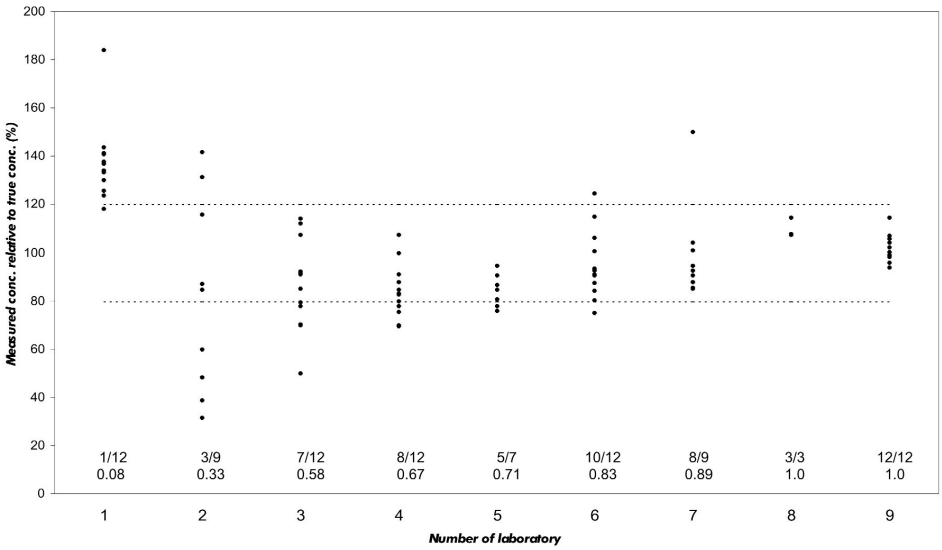

Figure 1. Performance of individual laboratories. Diagram shows the results for all measurements (all four PIs and three concentration levels combined), arranged by laboratory. Results for individual measurements are depicted by points (note that some points are superimposed). Accuracy (y-axis) is expressed as percentage relative to the true concentration (100%). The dotted lines represent the thresholds for measurements with acceptable accuracy (80 to120%). Proportions of measurements with acceptable accuracy are placed on the x-axis, above the number of each laboratory.

considered relevant within the context of TDM, the probability of receiving such a result would still be 6%.

Fortunately, by participating in the program, laboratories were alerted to possible inaccuracies and to previously undetected problems, such as systematic errors and high limits of quantitation that restrict the applicability of analytical methods. Such information may enable and encourage them to optimize their methods and intralaboratory QC procedures; this would confirm the role of interlaboratory QC testing as a means to achieve improvement in laboratory performance [7,8]. Accordingly, the first round of this program highlights both the need for and utility of an ongoing quality control program. The program will be extended to measurement of more PIs (amprenavir, lopinavir) and to analysis of NNRTIs (efavirenz, nevirapine), and will be open for more laboratories to participate.

We are indebted to Merck Sharp & Dohme, Agouron, Hoffmann - La Roche, and Abbott for the supply of indinavir, nelfinavir, saquinavir and ritonavir, respectively.

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Chapter 3.2

Evaluation of antiretroviral drug measurements by an interlaboratory quality control program

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Abstract

An international interlaboratory quality control program for measurement of antiretroviral drugs in plasma has been ongoing since 1999. Results of the third round of this program are presented.
Quality control samples were prepared by spiking drug-free plasma with varying concentrations of the currently available protease inhibitors and the nonnucleoside reverse transcriptase inhibitors efavirenz and nevirapine. Thirty-three laboratories participated in the program and were requested to analyze the quality control samples.
Results were available from 30 laboratories. Of all measurements, 82% were performed within 80-120% accuracy limits. Only 3 laboratories performed all their measurements within these limits, and 12 participants reported at least 90% of their analyses within the acceptance range. Mean accuracy for low drug concentrations was worse than for medium and high concentrations. The percentage of satisfactory measurements for the 6 laboratories that participated for the third time in the program increased from 54% in the first round to 85% in the third round.
The program revealed a large variability in the laboratories' ability to measure antiretroviral drugs accurately. This variability may have important implications for Therapeutic Drug Monitoring of these drugs and for pharmacokinetic studies. Interlaboratory testing is useful to alert laboratories to previously undetected analytical problems.

Introduction

There has been increasing interest in the bioanalysis of protease inhibitors (PIs) and nonnucleoside reverse transcriptase inhibitors (NNRTIs) in recent years. Many high performance liquid chromatographic (HPLC) assays have been published for quantitation of these drugs in plasma [1]. These analytical methods are used to study the pharmacokinetics and interactions of these drugs, and drug level measurements of PIs and NNRTIs are applied to individualize drug dosing (Therapeutic Drug Monitoring, TDM) [2].
In view of the wide application of bioanalytical methods for antiretroviral drugs and the clinical relevance of these applications, our department has initiated the International Interlaboratory Quality Control Program for Therapeutic Drug Monitoring in HIV infection. The aim of this program is to alert laboratories to deviating results with respect to the analysis of PIs and NNRTIs, and thereby enable them to improve their performance.
The first round of the program was performed in 1999 and was limited to nine laboratories and to the measurement of four PIs (indinavir, nelfinavir, ritonavir and saquinavir) [3]. The present report describes the results of the mature program, as reflected in the third round that took place in 2001. This third round of the program included 33 participating laboratories. In addition, the program was extended to the measurement of the PIs amprenavir and lopinavir, and to the NNRTIs efavirenz and nevirapine.

Materials and methods

Materials

Indinavir was obtained from Merck & Co., Inc (Rahway, NJ), ritonavir and lopinavir from Abbott Laboratories (North Chicago, IL), saquinavir mesylate from Hoffmann - La Roche (Basel, Switzerland), nelfinavir mesylate from Agouron Pharmaceuticals, Inc. (San Diego, California), amprenavir from Glaxo Wellcome (Stevenage, Hertfordshire, UK), nevirapine from Boehringer (Mannheim, Germany), and efavirenz was provided by Du Pont Pharmaceuticals (Wilmington, DE). All drugs had a high purity (>97%). PIs were kept at room temperature and NNRTIs were stored at 4^0C. Methanol and

dimethyl sulfoxide were purchased from Merck (Darmstadt, Germany). Drug-free plasma was obtained from the regional blood bank and was stored at –20^0 C.

Preparation and dispatch of the quality control plasma samples

PIs were dissolved in methanol, nevirapine and efavirenz in dimethyl sulfoxide. These solvents were used because the antiretroviral drugs were soluble and stable in these fluids.
Three quality control (QC) samples were prepared by spiking plasma with three different concentrations of the PIs amprenavir, indinavir, lopinavir, nelfinavir, ritonavir, and saquinavir. Three other QC samples contained the NNRTIs efavirenz and nevirapine. All concentrations (table1) related to the active part of the chemical compound, not to the salt or esterificated form.
QC samples were dispensed in polypropylene tubes and were stored at – 20^{0}C. Stability under these and other conditions was assessed and reported previously [4,5 and Droste et al, unpublished data]. QC samples were analyzed in duplicate with our own validated HPLC methods [4-6] as a confirmative check before samples were released for the QC program. Measurements were not allowed to deviate more than 5% from the true values.
The samples were packed on dry ice and dispatched to 33 laboratories in the United States, Europe, Canada, and Australia. Transit time of samples was 4 days at most. The laboratories were requested to analyze the QC samples within 6 weeks and return their results with details about their assays.

Data analysis

Descriptive statistics were calculated after standardization of all laboratory results to percentages with reference to the true value. By subtracting 100% from these percentages, the percentage bias from the true concentration (inaccuracy) was calculated. Twenty percent limits around the true values were considered to be appropriate threshold values for satisfactory measurements.
Analysis of variance (ANOVA) was used to evaluate the simultaneous effect of two factors, the drug to be measured and the concentration level (low, medium, and high), on the absolute inaccuracy. Measurements of different drugs within the same

laboratory were regarded as related to each other, and measurements of the different concentration levels of the same drug were also considered to be associated. Therefore, both drug to be measured and concentration level were repeated-measures (within subjects) factors in the analysis of variance. All statistical evaluations were performed using SPSS for Windows (v. 10.0; SPSS Inc., Chicago, IL). A p-value of < 0.05 was considered statistically significant in all analyses.

Reporting of results and sources of error

All participants were informed about their own performance and about the performance of all participants, as median inaccuracy and the range of inaccuracies were presented anonymously for all separate measurements. Results of all participants were also presented graphically.
Together with the results, an error evaluation form was sent to laboratories that reported unsatisfactory results in one of their measurements. They were asked to complete one form for every measurement with a deviating result. The form categorized errors as follows (derived from similar inquiries [7,8]): methodological problems (M), technical problems (T), clerical problems (T), survey problems (S), and other (O).

Results

Laboratories and analytical methods

Results were received from 30 out of 33 laboratories. Two laboratories did not report a reason for not returning results; one laboratory no longer measured antiretroviral drugs. Of the 30 responding laboratories, 28 were hospital laboratories and 2 were commercial laboratories. Of the 30 participants, 30% were from the USA, 60% from Europe, and the remaining 10% from Canada and Australia. All participants that provided details about their assays reported using HPLC.
Three laboratories were not able to measure the low concentrations of amprenavir, indinavir, or ritonavir, as their lower limits of quantitation were too high. Another participant reported an inability to measure the high concentration of saquinavir, because this concentration was not within the range of the method.

Accuracy of measurements

A maximum number of 24 measurements (3 for each drug) were performed by the laboratories. Table 1 presents the results arranged by drug and concentration level. The performance of individual laboratories is displayed in figure 1.
Only three laboratories reported all their results within the acceptance range (80-120 % accuracy). One laboratory (nr 30, see figure 1) did not report any satisfactory result. Twelve of 30 participants reported at least 90% of their results within the acceptance range. Twelve laboratories used analytical methods that appeared to have a large systematic error in one direction, as all measured concentrations of at least one drug were either above or below the 80-120% accuracy limits.

Table 1. Results, subdivided by drug and concentration level.

Drug	N	Concentration level (mg/l)		% inaccuracy, median (min-max)	N and % within 80 -120% acceptance range	
Amprenavir	21	Low	0.24	13 (0-100)	17/21	
		Intermediate	2.2	8 (1-65)	18/21	83%
		High	7.2	8 (2-49)	17/21	
Indinavir	27	Low	0.13	15 (1-2218)	16/27	
		Intermediate	2.3	12 (1-334)	20/27	70%
		High	11.7	11 (0-98)	21/27	
Lopinavir	23	Low	1.2	7 (1-70)	17/23	
		Intermediate	4.7	5 (1-46)	19/23	80%
		High	11.7	6 (0-44)	19/23	
Nelfinavir	28	Low	0.32	9 (2-92)	24/28	
		Intermediate	2.1	8 (0-207)	23/28	85%
		High	6.4	8 (1-61)	24/28	
Ritonavir	26	Low	0.24	7 (0-41)	16/26	
		Intermediate	2.4	9 (1-28)	23/26	81%
		High	9.7	16 (1-148)	24/26	
Saquinavir	27	Low	0.11	9 (0-446)	19/27	
		Intermediate	1.4	6 (0-35)	25/27	85%
		High	5.1	5 (0-28)	25/27	
Efavirenz	23	Low	0.46	12 (1-80)	18/23	
		Intermediate	3.7	9 (0-71)	19/23	81%
		High	6.6	8 (0-78)	19/23	
Nevirapine	18	Low	0.50	8 (2-71)	16/18	
		Intermediate	3.2	9 (1-19)	18/18	94%
		High	6.9	9 (0-24)	17/18	

Abbreviations: N, number of measurements; min, minimum value; max, maximum value.

Effect of drug to be measured and concentration level on accuracy

Descriptive analysis did not suggest large differences in mean absolute inaccuracies for measurements of the 8 antiretroviral drugs (table 1). This was confirmed by an ANOVA, which was performed for those laboratories that were able to measure all 8 drugs (n=13). There was no significant main effect of the drug to be measured on the absolute inaccuracy [F(2.783, 33.392)=0.955, p=0.42]. However, the concentration level to be analyzed had a significant effect on the absolute inaccuracy [F (1.035,12.421)=7.447, p=0.02]. The mean absolute inaccuracy over all drugs for all 13 laboratories was 20.0% for low concentrations, 11.4% for medium concentrations and 11.1% for high concentrations. Pairwise comparisons were performed at a Bonferroni-adjusted significance level for each separate test, keeping the overall type I-error rate at 0.05. These comparisons showed a significant difference between the absolute inaccuracies for measurements of the low drug levels versus the medium drug levels (p=0.041), and a trend towards a significant difference (p=0.06) between low-level versus high-level measurements. However, no significant differences between measurements of the medium and high drug levels were observed

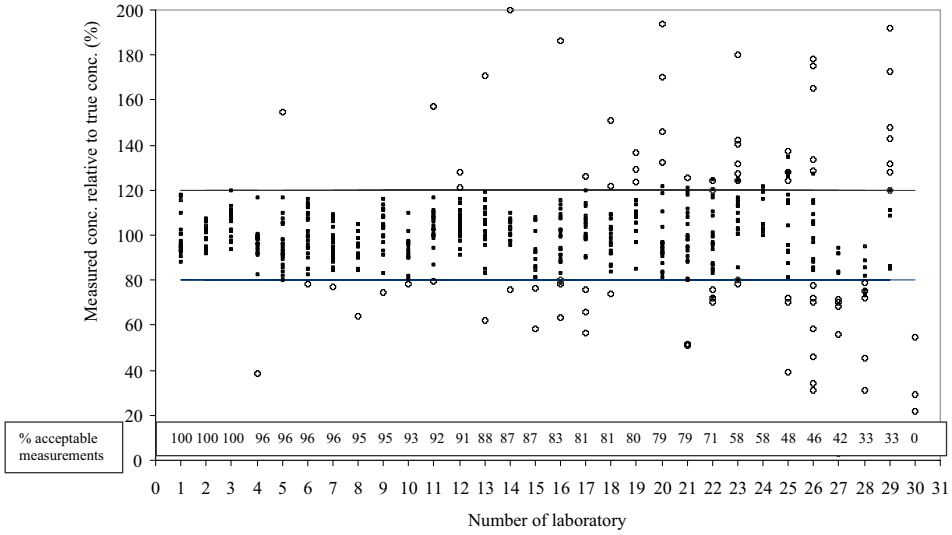

Figure 1. Performance of individual laboratories. Diagram shows the results for all measurements arranged by laboratory. Results for individual measurements are depicted by points (some points are superimposed); the dotted lines represent the thresholds (80-120%).

(p=1.0). The interaction between drug to be analyzed and drug level was not significant [F (1.941, 23.294)=1.419, p=0.26].

Sources of error

Twenty-seven participating laboratories reported at least one measurement with an inaccuracy of more than 20%. Nineteen laboratories returned their error forms. Reported errors are presented in table 2. Every inaccurate measurement for which an explanation was reported was included in the table. There was a wide variability in explanations for deviating results. Frequent sources of error were the use of an analytical method that was not (or not properly) validated and the use of ageing stock solutions.
Five of six laboratories that participated for the third time in this quality control program improved their performance in time. The overall percentage of acceptable measurements for these six laboratories increased from 54% in the first round to 83% in the second round and 85% in the third round.

Discussion

The results of this program show large variability in the ability of laboratories to measure antiretroviral drugs accurately. Measurement of these drugs needs to be improved in a number of laboratories that participated in the program.

The quality control program was designed to represent the reality encountered in the laboratories as close as possible. Therefore it was decided not to use lyophilized plasma that should be reconstituted. Furthermore, no reference substances or reference plasma samples were distributed. The major difference between the QC samples and routine samples related to the presence of other drugs or metabolites, which were absent in the QC samples. As a result of the similarities between QC samples and real samples, it can be inferred that the results of this quality control program provide a measure of the rigor (or effectiveness) of the regular intralaboratory (internal) quality assurance in the participating laboratories. On the other hand, it cannot be excluded that laboratories made extra efforts to achieve accurate results in this program [9]. This means that the results of the quality control program could also represent the best performance of the participants.

Table 2. Explanations for accuracies outside 80-120% fixed limits

	Number	% of total
METHODOLOGIC PROBLEMS		
M1 Instrument problem	-	
M2 Method change before QC-program	-	
M3 Method not validated for all PIs	18	
M Subtotal	18	23.4
TECHNICAL PROBLEMS		
T1 Dilution error	5	
T2 Incorrect pipetting (other than dilution)	4	
T3 Misidentification of the peak	1	
T4 Calculations performed incorrectly	3	
T5 Run accepted in nonlinear range	-	
T6 Run accepted even though controls were out of range	9	
T7 Aging stock solutions	17	
T8 Stock solutions not made of pure substances	3	
T9 Below quantitation limit	2	
T Subtotal	44	57.1
CLERICAL ERRORS		
C1 Results reported in wrong unit	-	
C2 Decimal point error	1	
C3 Transcriptive error into questionnaire	3	
C Subtotal	4	5.2
SURVEY		
S1 Specimen problem	-	
S2 Criteria for acceptance too narrow	-	
S Subtotal	0	0
OTHER		
O1 Unexplained/ unassigned cause	11	
O Subtotal	11	14.3
Total		
	77	100

In this program, results obtained by a certain laboratory were considered acceptable if they fell within preset 80-120% limits for the accuracy. The 20% threshold was based on guidelines for method validation for bioanalysis of drugs [10], as 20% deviations are often used as a fixed criterion for inaccuracy at the lowest level of quantitation. The 20% limits

are also comparable to maximal allowable error specifications for drug measurements according to the U.S. Clinical Laboratory Improvement Amendments (CLIA) of 1988 [11].

The large interlaboratory variability in performance with respect to antiretroviral drug measurements may have important implications for TDM. Based on the inaccurate measurements, wrong dose adjustments might occur, or patients might be adviced not to adjust doses when an adjustment might be necessary. This may lead to resistance development, therapy failure, and concentration-related adverse events. In this respect, it is of special concern that low concentrations were more difficult to measure than medium or high concentrations. This is because the lowest antiretroviral drug concentrations in a dosing interval (trough concentrations) are particularly useful to measure; adequate trough levels appear to be the most critical predictor for the efficacy of PIs [2].

Fortunately, the quality control program alerted the laboratories to inaccuracies and invited them to inquire possible sources of error. Our findings with respect to the possible explanations for inaccuracies (distribution over the error categories) differed from other studies [7,8,12]. In our program, the category of "technical problems" accounted for 57% of the errors (table 2), whereas the studies of Hoeltge et al. [7], Steindel et al. [8] and Jenny et al. [12] found 19%, 19%, and 17% for this category, respectively. This difference can be probably ascribed to the use of complex assays with difficult sample preparation that are required for analysis of antiretroviral drugs (compared with automatic assays for many other drugs). In the category of "methodological problems", all problems were caused by inappropriate validation of the assays. The participants concerned did not check their assays for interference of PIs other than the one the assay was developed for, while the QC samples contained all the PIs.
It seems that corrective action could prevent many errors in the future, although some failures were unexplained (15% in our study). In fact, it appeared that the laboratories that were participating in the quality control program for the third time had better results in the third round than in the first round. It is expected that at least some of these improvements over time also affect the performance on real samples.

In conclusion, the program revealed a large variability in the performance of laboratories to measure antiretroviral drugs. The program alerted a number of laboratories to previously undetected analytical problems. This will enable them to improve their assays.

In the future more agents will be included in the quality control program (e.g. nucleoside reverse transcriptase inhibitors). All laboratories measuring antiretroviral drugs are invited to participate in this program.

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Chapter 4.

Evaluation of undesirable drug-drug and drug-food interactions

Chapter 4.1

Administration of indinavir and low-dose ritonavir (800/100 mg twice-daily) with food reduces nephrotoxic peak plasma levels of indinavir

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Abstract

Background

The objective of this study was to compare indinavir peak plasma (Cmax) values after administration of indinavir/ritonavir (IDV/RTV) 800/100 mg on an empty stomach or with food. High IDV Cmax values have been associated with IDV-related nephrotoxicity.

Methods

This was an open-label, randomized, two-treatment, two-period, crossover pharmacokinetic study performed at steady-state. HIV-infected patients who had been using IDV/RTV 800/100 mg BID for at least four weeks were randomized to take this combination with a light breakfast (2 filled rolls and 130 ml of fluid) on a first study day, and without food on a second day, or in the reverse order. The pharmacokinetics of IDV and RTV were assessed after plasma and urine sampling during 12 hours.

Results

Data for 9 patients were evaluated. Administration of IDV/RTV 800/100 mg on an empty stomach resulted in a higher IDV Cmax (geometric mean (GM) ratio - fasting/fed and 95% confidence interval (CI): 1.28 [1.08-1.52], p=0.01) and a trend to a shorter IDV tmax (p=0.07) compared to administration with food. The mode of administration of IDV/RTV did not affect plasma IDV Cmin and AUC values, parameters that have been associated with the antiviral efficacy of IDV, nor the urinary excretion of IDV.

Conclusions

Administration of IDV/RTV 800/100 mg on an empty stomach results in a higher IDV Cmax compared to ingestion with a light meal. Stated the other way round, intake with a light meal reduces IDV Cmax, which probably reflects a food-induced delay in the absorption of IDV. It is recommended to administer IDV/RTV 800/100 mg with food, as a possible means to prevent IDV-related nephrotoxicity in patients who start or continue with this regimen.

Introduction

The past years have seen an increase in the use of indinavir (and other protease inhibitors) combined with low-dose ritonavir [1]. A twice-daily (BID) regimen of indinavir (800 mg) and ritonavir (100 mg) is commonly used. It has been stated that this indinavir/ritonavir combination can be administered without regard to food, in contrast to indinavir without ritonavir [2,3]. Indeed it was demonstrated that indinavir/ritonavir 800/100 mg can be taken with a low-fat or high-fat meal [3,4], but the pharmacokinetics of this combination have not been investigated after administration on an empty stomach. We were specifically concerned about an increase in indinavir peak plasma levels (Cmax) after intake of the 800/100 mg combination under fasting versus fed conditions, since intake of drugs with food often results in a blunted Cmax [5-7]. Several studies have pointed to high indinavir Cmax values as a risk factor for indinavir-related asymptomatic and symptomatic nephrotoxicity [8-15], especially nephrolithiasis, which occurs in 19-33% of patients who take indinavir/ritonavir 800/100 mg [16-18]. This study was performed to evaluate indinavir Cmax values after administration of indinavir/ritonavir 800/100 mg on an empty stomach and with food.

Methods

Subjects

HIV-infected patients were recruited from the outpatient clinics of the University of Bonn and the University of Cologne, Germany. Adult patients were eligible for participation if they had received indinavir/ritonavir 800/100 mg BID for at least four weeks. Patients were excluded if they used drugs that are known to affect the pharmacokinetics of indinavir or ritonavir. The patients gave written informed consent before participation in the study, which was approved by the Institutional Review Board of the University of Bonn.

Experimental design and procedures

This was an open-label, randomized, two-treatment (fed versus fasting), two-period, two-sequence, cross-over pharmacokinetic study performed at steady state.
Participants were randomly assigned to (i) ingest indinavir/ritonavir 800/100 mg with a meal on a first study day and without food on a second day (within two weeks of the first day), or (ii) to complete the study in the reverse order.
On both study days participants attended in the morning after an overnight fast. They ingested indinavir/ritonavir with a light breakfast, or on an empty stomach. The breakfast consisted of two filled bread rolls and 130 ml of water, coffee or tea (339 kcal; 14% protein, 32% fat, 54% carbohydrate). Blood sampling was performed pre dose and at 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75, 2.0, 2.5, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 10.0 and 12.0 hours post dose. Participants who ingested indinavir/ritonavir on an empty stomach had breakfast at two hours after administration of the drugs. Other drugs were ingested at their prescribed times of administration.
Urine was sampled in one of the participating centres. Participants voided their bladder before administration of indinavir/ritonavir and urine samples were collected at two hour intervals up to 12 hours post dose. During the day, participants drank three liters of fluid. Plasma and urine samples were stored at -20^{0}C until analysis.

Analytical and pharmacokinetic methods

Concentrations of indinavir and ritonavir in plasma and indinavir concentrations in urine were measured using validated HPLC methods [19,20].
Pharmacokinetic parameters were obtained by non-compartmental methods [21]. The primary pharmacokinetic parameter, the Cmax of indinavir, was defined as the highest observed plasma concentration, with the corresponding sampling time as tmax.
The number of participants with indinavir Cmax values above 8 mg/L and with indinavir concentrations above 9 mg/L at one hour post dose were assessed after administration of indinavir/ritonavir with and without food. The 8 mg/L Cmax value is a threshold for crystallization of indinavir derived from in vitro crystallization experiments [8], and 1-h indinavir concentrations above 9 mg/L have been associated with a 2.3 fold increased risk for persistent leukocyturia [12], a sign of subclinical nephrotoxicity. The time above indinavir concentrations of 8 and 9 mg/L was derived

from the pharmacokinetic curves.
The cumulative amount of indinavir excreted in urine up to time t (Aet) was obtained by summing the amount excreted in each time interval up to that time. Renal clearance (CL_R) of indinavir was calculated using the formula Ae12h/AUC0-12h, where AUC is the area under the plasma concentration versus time curve. The fraction of indinavir excreted unchanged (fe) was calculated using the formula: fe * F = Ae12h/800 mg (F is bio-availability).

Statistical analysis

The study was powered to show that administration of indinavir/ritonavir 800/100 mg on an empty stomach results in a different mean value for indinavir plasma Cmax compared to administration with food. All other parameters besides the Cmax of indinavir were secondary, and results of analyses for these parameters were regarded as exploratory.
An analysis of variance (ANOVA) was performed on the logarithmically transformed pharmacokinetic parameters of indinavir and ritonavir to assess the influence of the mode of administration. The ANOVA model included the effects of subject, period and treatment (fasting or fed condition). The effect of food was expressed in a geometric mean ratio (fasting condition/fed condition) plus 95% confidence interval on the original scale. Tmax and time above 8 mg/L or 9 mg/L indinavir were not log-transformed and were compared using Wilcoxon signed-ranks test.

Results

Study subjects

Paired pharmacokinetic curves were recorded in 11 patients. Data for two patients were not evaluated, as one patient used a drug (carbamazepine) that affects indinavir and ritonavir concentrations, and the other did not ingest the correct dose of indinavir/ritonavir on one study day. The nine evaluable patients were all men, with a median age of 40 years (range 31-54 years), a median weight of 71 kg (range 64-89 kg), and normal renal and hepatic function parameters.

Pharmacokinetics of indinavir and ritonavir

Administration of indinavir/ritonavir 800/100 mg on an empty stomach resulted in a significant 28% increase in the geometric mean Cmax value of indinavir, compared to administration with a light meal (figure 1 and table 1). Six out of nine participants showed a large (29-61%) increase in indinavir Cmax under fasting versus fed conditions, whereas the other three participants showed minimal (<10%) changes. After intake of indinavir/ritonavir on an empty stomach, 8/9 participants had an indinavir Cmax above 8 mg/L and a 1-h indinavir level above 9 mg/L, compared to 5/9 and 3/9 participants, respectively, after administration with a light meal. Time above 8 mg/L or 9 mg/L indinavir increased accordingly, but these did not reach statistical significance.
A trend to a shorter indinavir tmax (p=0.07) and (at most) a trend to a modest increase in indinavir AUC0-12h (p=0.09) were observed after administration of indinavir/ritonavir under fasting versus fed conditions. A posteriori power calculations

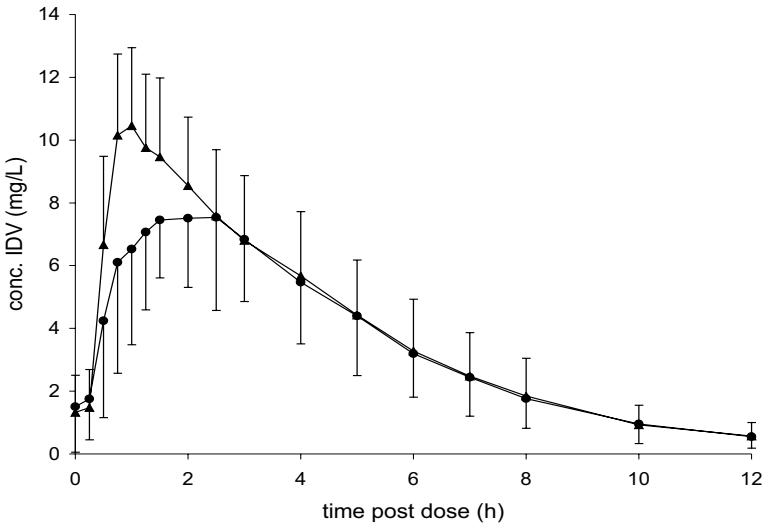

Figure 1. Mean (+ standard deviation) indinavir (IDV) plasma concentrations versus time (n=9). Circles: indinavir/ritonavir 800/100 mg administered with food. Triangles: indinavir/ritonavir 800/100 mg administered without food. [a,b]

[a] Administration with food: intake with a light breakfast (2 filled rolls + 130 ml fluid). Administration without food: intake on an empty stomach after an overnight fast.
[b] Displayed Cmax values differ from geometric mean Cmax values reported in table 1. Concentrations in figure 1 are mean values of concentrations measured at the same time post dose, whereas table 1 shows the geometric mean of Cmax values that were sampled at different times post dose.

Table 1. Pharmacokinetic parameters of indinavir and ritonavir after administration of indinavir/ritonavir 800/100 mg with food or without food (n=9) [a,b]

Parameter	Mode of administration		ANOVA	
	Without food: geom. mean (range)	With food: geom. mean (range)	Point estimate for geom. mean ratio without/with food [95% CI]	P value
Indinavir				
C_{max} (mg/L)	10.6 (5.2-13.8)	8.6 (5.7-14.3)	1.28 [1.08-1.52]	0.01
t_{max} (h) [c]	1.0 (0.75-1.6)	1.5 (0.75-2.5)	-	0.07 [d]
time above 8 mg/L (h) [c]	1.9 (0.0-4.1)	0.4 (0.0-3.0)	-	0.09 [d]
time above 9 mg/L (h) [c]	1.3 (0.0-3.3)	0.0 (0.0-2.5)	-	0.07 [d]
AUC0-12h (h.mg/L)	43.0 (23.4-70.0)	37.7 (22.6-55.2)	1.19 [0.97-1.46]	0.09
C_{min} (mg/L)	0.45 (0.16-1.4)	0.44 (0.18-1.2)	1.16 [0.70-1.92]	0.51
CL/F.kg (L/h.kg)	0.26 (0.18-0.38)	0.30 (0.20-0.42)	0.84 [0.68-1.03]	0.09
Vd/F.kg (L/kg)	0.81 (0.62-1.28)	0.91 (0.56-1.24)	0.87 [0.69-1.10]	0.21
$t_{1/2}$ (h)	2.2 (1.8-2.4)	2.1 (1.8-2.7)	1.04 [0.94-1.16]	0.37
Indinavir-urinary excretion [e]				
Ae_{2h} (mg)	105 (93-131)	103 (85-152)		
Ae_{4h} (mg)	218 (171-260)	209 (145-287)		
Ae_{6h} (mg)	293 (215-342)	310 (239-392)		
Ae_{8h} (mg)	402 (372-427)	378 (287-442)		
Ae_{12h} (mg)	420 (302-528)	406 (303-543)		
CL_R/F.kg (L/h.kg)	0.12 (0.07-0.15)	0.14 (0.09-0.18)		
Fe.F	0.52 (0.38-0.66)	0.51 (0.38-0.68)		
Ritonavir				
C_{max} (mg/L)	1.5 (0.39-3.7)	1.2 (0.70-2.6)	1.33 [0.86-2.06]	0.17
t_{max} (h) [c]	2.5 (0.80-5.0)	3.0 (0.75-5.0)	-	0.40 [d]
AUC0-12h (h.mg/L)	9.0 (3.4-18.4)	7.5 (4.2-15.9)	1.27 [0.80-2.02]	0.27
C_{min} (mg/L)	0.23 (0.10-0.59)	0.23 (0.06-0.51)	1.10 [0.64-1.90]	0.68
CL/F.kg (L/h.kg)	0.15 (0.08-0.37)	0.19 (0.09-0.34)	0.78 [0.49-1.25]	0.26
Vd/F.kg (L/kg)	0.77 (0.34-2.7)	0.85 (0.38-1.5)	0.84 [0.46-1.55]	0.53
$t_{1/2}$ (h)	3.5 (2.1-7.6)	3.2 (2.4-5.5)	1.07 [0.75-1.52]	0.66

[a] *Abbreviations: geom.: geometric, CI: confidence interval.*
Pharmacokinetic parameters: Cmax : highest observed plasma concentration, tmax : sampling time for Cmax, AUC0-12h: area under the concentration-time curve from 0 to 12 h, extrapolated to infinity and corrected for contribution of the predose AUC, Cmin : trough concentration at 12 h, CL/F.kg: total clearance corrected for weight, Vd/F.kg: volume of distribution corrected for weight, t1/2: elimination half life, Aet: cumulative amount excreted in urine up to time t, CL_R/F.kg: renal clearance corrected for weight, fe.F: fraction of dose that is excreted unchanged, F: bio-availability.
[b] *Administration with food: intake with a light breakfast (2 filled rolls + 130 ml fluid). Administration without food: intake on an empty stomach after an overnight fast.*
[c] *median and range.*
[d] *Wilcoxon signed-ranks test.*
[e] *Data from 5 participants; this was considered too few data for a sensible statistical analysis.*

revealed that the statistical power to detect 20% changes in indinavir AUC0-12h and especially Cmin was low (power less than 0.5, using $\alpha = 0.05$).
The pharmacokinetics of ritonavir were not affected by the mode of administration of indinavir/ritonavir.

Urinary excretion data for indinavir were available for five participants, who all had an increase in indinavir plasma Cmax of at least 29% after administration under fasting versus fed conditions (table 1). The results did not suggest differences in the cumulative amount of indinavir excreted at any of the urine sampling times. All five patients showed a small to modest decrease in the renal clearance of indinavir after administration under fasting versus fed conditions.

Discussion

The results of this study show that intake of indinavir/ritonavir 800/100 mg on an empty stomach results in a significant 28% increase in the geometric mean Cmax of indinavir, compared to administration with a light meal. Stated the other way round, administration of indinavir/ritonavir 800/100 mg with a light meal resulted in a decrease in the Cmax of indinavir. This decrease, and the accompanying trend to an increase in indinavir tmax after administration with food, likely reflect a delay in the absorption of indinavir due to a food-induced decrease in the rate of gastric emptying [5-7]. The study did not show significant differences in indinavir Cmin and AUC0-12h values, parameters that have been related to the antiviral efficacy of this protease inhibitor [22]. Geometric mean Cmin values for indinavir were lower than reported previously [3,4,23], but all individual indinavir Cmin values were above the mean Cmin reported for the thrice-daily (TID) regimen of indinavir without ritonavir (0.15 mg/L[2]).

The clinical relevance of the selective effect of food on the Cmax of indinavir depends on the strength of the association between indinavir Cmax values and nephrotoxicity, the relevance of the mean 28% food-effect on Cmax, and on the availability of other means to prevent indinavir-related nephrotoxicity. The critical role of Cmax in the formation of indinavir crystals and kidney stones has been derived from the high urinary excretion of indinavir [2], its poor solubility at physiologic pH-values [24,25], and the concept that intratubular indinavir concentrations depend primarily on the unbound fraction of indinavir

in plasma that is filtered by the glomeruli [8]. The highest unbound concentrations (associated with Cmax) then confer the highest risk of indinavir crystallization in the nephrons, irrespective of the pattern of excretion of indinavir in the lower urinary tract. In vitro crystallization experiments support such a saturation-driven crystallization process with a role for Cmax [8], and pharmacokinetic-pharmacodynamic relationships have shown an association between indinavir Cmax values and nephrotoxicity in HIV-infected patients [9-12]. The most convincing argument for a conclusive role for indinavir Cmax is the low prevalence (even absence) of nephrolithiasis among patients who use indinavir/ritonavir 400/400 mg BID [13-15], a regimen with a lower indinavir Cmax (but a higher Cmin and similar AUC0-24h) compared to the indinavir 800 mg TID regimen without ritonavir [23,26,27].

Considering the observed 28% mean difference in indinavir Cmax, there are only few reference data that provide insight into the clinical relevance of this difference. One study demonstrated an increase in relative risk for indinavir-related persistent leukocyturia with every 1 mg/L increase in Cmax [12]. In addition, evaluation of the observed changes in Cmax in the light of the 8 mg/L [8] and 9 mg/L [12] thresholds for nephrotoxicity (and the time above these thresholds, see table 1) shows that the food-induced decrease in Cmax occurs in a critical concentration window.

As regards to other available means to prevent indinavir-related nephrotoxicity, an indinavir/ritonavir 400/400 mg combination could be considered, but this regimen is associated with other adverse events, related to ritonavir [23,27]. A regimen with low doses of both indinavir and ritonavir (400/100 mg BID) seems promising [28], but experience with this regimen is limited and its indinavir Cmin levels may be considered too low [29]. Dose reductions guided by plasma concentration measurements (TDM) have proven their worth in preventing toxicity to indinavir [30], but TDM is not available everywhere.

Based on these considerations and the results of this study, it is recommended that indinavir/ritonavir 800/100 mg should preferably be administered with food, and not without regard to food, as a possible means to prevent indinavir-related nephrotoxicity in patients who start or continue with this regimen.

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Chapter 4.2

The influence of efavirenz on the pharmacokinetics of a twice daily combination of indinavir and low-dose ritonavir in healthy volunteers

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Abstract

Objective

This study evaluated the effect of multiple-dose efavirenz on the steady state pharmacokinetics of the combination of indinavir (800 mg) and low-dose ritonavir (100 mg) twice a day, in which ritonavir is used to increase indinavir plasma concentrations.

Methods

Eighteen healthy male volunteers participated in this multiple-dose, one-arm, two-period interaction study. They took a combination of 800 mg indinavir and 100 mg ritonavir with food for 15 days. From days 15 to 29, a once-daily administration of 600 mg efavirenz was added to the combination. Pharmacokinetics of indinavir and ritonavir on days 15 and 29 were compared.

Results

Fourteen volunteers completed the study. The addition of efavirenz resulted in significant reductions ($p<0.01$) in indinavir area under the curve (AUC, -25%), trough concentration (Cmin, -50%) and maximum concentration (Cmax, -17%). All indinavir Cmin levels on day 29 remained equivalent to or above the mean Cmin value described for the regimen of 800 mg indinavir three times a day, without ritonavir (0.15 mg/L). Changes in ritonavir AUC, Cmin and Cmax were -36%, -39% and -34%, respectively. Pharmacokinetics of efavirenz on day 29 were comparable with published data.

Conclusions

The addition of efavirenz to a combination of 800 mg indinavir and 100 mg ritonavir twice-daily results in significant decreases in AUC, Cmax, and especially Cmin of indinavir. The dose of indinavir or ritonavir should be increased to maintain similar indinavir drug levels after addition of efavirenz to the indinavir-ritonavir combination. Dose modifications may not be needed in antiretroviral-naive human immunodeficiency virus-infected patients if the reference Cmin of the regimen of 800 mg indinavir three times a day is considered to be adequate.

Introduction

Efavirenz is a member of the class of nonnucleoside reverse transcriptase inhibitors for the treatment of human immunodeficiency virus (HIV) infection and acquired immunodeficiency syndrome (AIDS) [1-3]. The combination of efavirenz and two nucleoside reverse transcriptase inhibitors has shown at least similar antiviral effects compared with indinavir triple therapy [4]. Likewise, the two-drug combination of efavirenz and indinavir shows a potent and durable antiretroviral effect [4]. This two-drug combination can be applied as a nucleoside-sparing regimen in treatment-naive patients or in treatment-experienced persons for whom previous nucleoside therapy has failed, but it can also be combined effectively with nucleoside analogues [4,5].

In the combination of efavirenz and indinavir, the former drug has the advantage of once-daily dosing that is both nonfood dependent and involves few capsules [1-3]. In contrast, indinavir should be taken three times a day under fasting conditions or with a light low fat meal [6]. However, the pharmacokinetic profile of indinavir can be improved by combining it with a low dose (100 mg) of ritonavir [7-11]. Ritonavir is another protease inhibitor and a potent inhibitor of cytochrome P450 3A (CYP3A) and thereby increases the exposure to indinavir, which is primarily metabolized by this isozyme. Inhibition of P-glycoprotein activity may be another explanation for the interaction between indinavir and ritonavir [9]. The combination of indinavir with low-dose ritonavir allows for a more convenient twice-daily administration (800 mg indinavir and 100 mg ritonavir twice a day) and permits concurrent intake with food [9-11]. Furthermore, some HIV strains with reduced susceptibility to indinavir may be more sensitive to this combination because it results in higher exposure to indinavir, particularly higher trough levels [9-11]. The combination of indinavir and low-dose ritonavir is widely used, and available data suggest that its virologic efficacy is at least comparable to 800 mg indinavir three times a day without ritonavir [11,12].

The combination of efavirenz and indinavir combined with ritonavir evidently shows promise to be a potent compact combination regimen that should offer ease of adherence. However, these three drugs cannot simply be combined because mutual drug interactions may alter the pharmacokinetic properties of either of the drugs, potentially resulting in subtherapeutic drug levels of indinavir or efavirenz. For

example, when efavirenz is combined with a regimen of 800 mg indinavir three times a day, it induces a 31% decrease in the exposure to indinavir, which requires an increase in the dose of indinavir from 800 mg three times a day to 1000 mg three times a day [3]. Boosting of indinavir concentrations with low-dose ritonavir may compensate for the effect of efavirenz on indinavir drug levels, but that has not been studied. Therefore this study was designed to determine the effects of multiple-dose efavirenz on the steady-state pharmacokinetics of indinavir combined with low-dose ritonavir.

Methods

Subjects

Volunteers who met the following criteria were eligible for study participation: male sex, age of 18 years or older, and good health (i.e. no acute or chronic illness and not using medications). Female volunteers were excluded because efavirenz has been reported to have teratogenic properties in animals [3].
Volunteers were excluded if they were known to be hypersensitive to indinavir, ritonavir, or efavirenz, if they were seropositive for hepatitis B or C or had any kind of active liver disease, and if they had prespecified abnormal laboratory parameters. Eighteen volunteers provided written informed consent to participate in the study, which was approved by the Institutional Review Board of University Medical Centre Nijmegen, Nijmegen, The Netherlands.

Experimental design and procedures

This was a multiple-dose, one-arm, one-sequence, two-period pharmacokinetic interaction study. During the first 15 days of the study, all participants took indinavir (800 mg, 2 capsules of 400 mg Crixivan®) and low dose ritonavir (100 mg, 1 capsule of 100 mg Norvir®) twice a day with food (at least two slices of bread). They were instructed to ingest the drugs in the morning and evening, with 12-hour intervals. Furthermore, participants had to drink 1.5 L water in addition to normal daily fluid intake to avoid possible nephrotoxicity caused by indinavir. The consumption of grapefruit and grapefruit juice was prohibited because they may influence the

metabolism of protease inhibitors and efavirenz.
At the end of day 15, participants started to take 600 mg efavirenz (3 capsules of 200 mg Stocrin®) once daily in addition to the indinavir-ritonavir combination. Efavirenz was taken at bedtime to attenuate possible central nervous system effects of this drug [1-3]. The combination of indinavir, ritonavir, and efavirenz was taken until day 29.

The study was conducted on an outpatient basis. On days 1, 8, 15, 22, and 29, drug administration was performed under staff supervision. Compliance with study medication at home was evaluated at every study visit by inspection of drug-taking diaries, counting of capsules, measurement of plasma drug concentrations, and electronic measurement of drug-taking behaviour with the Medication Event Monitoring System (MEMS). These are prescription vials with caps that contain microprocessors to record dates and times the vials are opened [13].

Intensive blood and urine sampling was performed during days 15 and 29. On these days participants came to our facility in the morning after an overnight fast. They had taken efavirenz the preceding evening (day 29 only). Each subject voided the bladder, and a pre-dose blood sample was taken. Subjects then ingested indinavir and ritonavir with a standardized medium-fat medium-calorie breakfast (610 kcal in total, 16% of which was attributable to protein and 33% and 51% to fat and carbohydrates, respectively). Serial blood sampling was performed at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 10.0, and 12.0 hours after administration of the indinavir-ritonavir combination. All blood samples were centrifuged within 12 hours. The separated plasma was stored at -20^{0}C. Urine samples were collected at two-hour intervals up to 12 hour after ingestion of the drugs. Urine samples were also stored at -20^{0}C.
Standard meals were served at lunchtime (4 hours after ingestion of the drugs) and dinnertime (10 hours after ingestion of the drugs). During the day, participants drank a total of 3 L fluid (water, tea, or coffee), which is about 1.5 L above normal fluid intake.

Safety and tolerability

Safety and tolerance were assessed by a questionnaire that presented 13 possible adverse events that may occur during treatment with indinavir, ritonavir, or efavirenz. The volunteers were questioned 8 times (on days 3, 8, 11, 15, 17, 22, 25, and 29) about these adverse events. They could also mention adverse events that were not on the list. Participants were asked to grade every complaint as mild (symptoms do not interfere with daily activities), moderate (symptoms may interfere with daily activities) or severe (symptoms interrupt daily activities). On the same 8 days an extensive blood chemistry and hematology screen and urinalysis were performed.

Analytical methods

Plasma samples were analyzed for indinavir, ritonavir, and efavirenz (efavirenz only on day 29), and urine samples were analyzed for indinavir concentrations.
The plasma levels of indinavir and ritonavir were analyzed by a previously described validated reversed-phase HPLC method [14].
Urine samples were analyzed for indinavir with another HPLC method that has been described previously [15], but a modified sample pretreatment procedure was used. Urine was acidified to a pH lower than 3.5 with orthophosphoric acid to dissolve indinavir that precipitated. After centrifugation, the urine samples were diluted 5 times with 0.0625-mol/L dibasic sodium phosphate before injection in the chromatographic system. The lower limit of quantitation was 1 mg/L indinavir, and the standard curve was linear over the concentration range from 1 to 1300 mg/L. Recovery of the method was 101% and accuracy ranged from 103 to 105%, dependent on the urinary concentration level of indinavir. Intraday precision varied between 2.1 and 3.2% and interday precision was between 5.2 and 8.6%.
Efavirenz plasma concentrations were measured by means of protein precipitation followed by reversed-phase HPLC with ultraviolet detection. In brief, 200 µl acetonitrile was added to 100 µl of the plasma sample. The sample was mixed on a vortex mixer during 10 seconds and centrifuged, and 20-µl aliquots of clear supernatant were injected in the chromatographic system. Chromatographic analysis was performed at an ambient temperature with an Inertsil 2 ODS column (250 x 4.6 mm internal diameter, Varian BV, Bergen op Zoom, The Netherlands), protected by a Chromguard

RP stainless steel column (10 x 3 mm internal diameter, Varian BV), with a mixture of acetonitrile (65%) and 0.06-mol/L potassium dihydrogen phosphate (35%) as the mobile phase. Elution was at 1.0 ml/min, and detection was by ultraviolet absorption at 251 nm. Retention time of efavirenz was 8.3 minutes. The efavirenz calibration curve was linear over a range from 0.20 to 20.0 mg/L. Recovery was 106%, and accuracy ranged from 99.0% to 100.5%, depending on concentration level. Intraday and interday precision ranged from 1.8% to 2.6% and from 1.1% to 2.8%, respectively.

Pharmacokinetic analysis

The pharmacokinetic values of indinavir and ritonavir were calculated with noncompartmental methods [16]. The highest observed plasma concentration was defined as Cmax, with the corresponding sampling time as tmax. Cmin was the concentration 12 hours after ingestion of the drugs. The terminal log-linear period (log C versus t) was defined by visual inspection of the last data points ($n \geq 3$). The absolute value of the slope (ß/2.303) was calculated by least-squares linear regression analysis (ß is the first-order elimination rate constant). The elimination half life (t1/2) was calculated by the following equation: 0.693/ß. The area under the concentration-versus-time curve (AUC) was calculated with the trapezoidal rule from 0 to 12 hours. This value was extrapolated to infinity with the following equation: Cmin/ß, in which Cmin is the concentration 12 hours after ingestion of the drugs. The AUC value was corrected for the contribution of the predose AUC by subtraction of C0/ß, in which C0 is the initial plasma concentration. The apparent clearance (CL/F, where F is bioavailability) was calculated by dividing the dose (D) by AUC, and apparent volume of distribution (Vd/F) was obtained by dividing CL/F by ß. Clearance and volume of distribution were corrected for weight of the participant. The cumulative renal excretion of indinavir (Ae) was approximated from volumes of urine produced and indinavir concentrations in urine. Renal clearance (CL_R) of indinavir was calculated with the formula Ae/AUC. The fraction of total amount excreted unchanged to the dose was calculated as follows: $fe * F = Ae/D = CL_R/CL$.

For efavirenz, only the steady state concentrations beyond 8 hours until about 22 hours after ingestion were available because the volunteers ingested this drug at

bedtime in the evening before study day 29. The concentration at 12 hours after intake of efavirenz (average steady-state concentration, Css,av) was derived from the pharmacokinetic curve. The ß and t1/2 could be derived from the log-linear period. Efavirenz trough levels (Cmin) were calculated with use of the first-order equation of the regression line through the last data points and filling in 24 hours. The AUC from 0 to 24 hours [AUC(0-24)] was estimated from Css,av and the dosing interval (τ, 24 hours) with use of the following formula: $Css,av = D/\tau * F/CL = AUC(0\text{-}24) /\tau$.

Data analysis

All statistical evaluations were performed with SPSS for Windows, version 9.0 (SPSS Inc, Chicago, IL). Pharmacokinetic parameters were log-transformed before statistical analysis. Geometric means were calculated for every pharmacokinetic parameter on study days 15 and 29. The effect of efavirenz on the steady-state pharmacokinetics of indinavir and ritonavir was evaluated by comparison of the pharmacokinetic parameters of days 15 and 29 with use of the two-sided Student t-test for paired samples. Furthermore, geometric mean ratios with 95% confidence intervals were calculated for every comparison. The values for Cmax sampling time (tmax) were not transformed and were compared using Wilcoxon signed-rank test. $P \leq 0.05$ was considered to be significant in all analyses.

The incidence of adverse events was calculated separately for the first and second study periods. It was expressed as the percentage of participants that reported a particular adverse event at least one time during the 4 consecutive reporting times in every study period.
Subsequently, every reported mild, moderate, or severe adverse event was ascribed a severity score of 1, 2 or 3 points, respectively. All scores were added up for every participant and were divided by the number of reporting times. In this way the mean toxicity scores for the first and second study periods were obtained for all individual participants.

Correlation between parameters was calculated with the Pearson correlation coefficient (ρ) or Spearman's rho (rank correlation, r_s); the choice between these coefficients was dependent on distributional characteristics of the two variables involved.

Results

Study subjects

Eighteen subjects were included in the study; 14 of those completed the study. Three participants were withdrawn because of adverse events, and one withdrew his consent for personal reasons. The mean age of the 14 volunteers who completed the study was 27 years (age range, 20-55 years) and their mean weight was 75 kg (weight range, 60-89 kg).
The combination of methods for measurement of compliance allowed for a reliable estimation of adherence to study medication. Compliance was good in all volunteers; 9 volunteers showed 100% compliance, and 5 volunteers missed either one or two doses of the indinavir-ritonavir combination or one dose of efavirenz but not in the 3 days before the pharmacokinetic assessments.

Pharmacokinetics of indinavir

Coadministration of efavirenz resulted in a decrease in indinavir AUC and Cmin in all participants (table 1, figures 1 and 2). Marked variability in baseline (day 15) indinavir plasma concentrations was observed, but the absolute ranges of indinavir AUC and Cmin clearly narrowed after coadministration of efavirenz. Values for the highest observed plasma concentration (Cmax) decreased in 9 of the 14 participants. Both total clearance and CL_R of indinavir increased significantly as a result of the interaction with efavirenz. Close examination of the data in table 1 reveals that the absolute increase in CL_R was small compared with the increase in total clearance, which means that efavirenz mainly increased the extrarenal (hepatic) clearance of indinavir.
No significant linear correlation could be shown between AUC or Cmin values of indinavir on day 15 and the respective percentage change in each of these parameters as a result of the coadministration of efavirenz, but baseline Cmax values at day 15 correlated with the decrease in Cmax (ρ= -.596, p=0.024).

Table 1. Summary of results for indinavir and ritonavir; steady-state pharmacokinetic parameters (n=14)

Parameter[a]	Geometric mean (range)		Statistical significance[d]	Geometric mean ratio (period 2 / period 1) and 95% CI[e]
	Study period 1[b]	Study period 2[c]		
Indinavir				
AUC (h.mg/L)	45.9 (30.2-72.6)	34.6 (24.2-44.4)	<0.001	0.75 [0.68-0.84]
C_{min} (mg/L)	0.66 (0.28-1.4)	0.33 (0.15-0.66)	<0.001	0.50 [0.41-0.60]
C_{max} (mg/L)	9.17 (6.31-12.22)	7.65 (5.48-11.45)	0.008	0.83 [0.74-0.94]
t_{max} (h)	2.0 (1.5-4.0)[f]	2.0 (1.5-3.0)[f]	0.262[g]	-
CL/F.kg (L/h.kg)	0.23 (0.17-0.30)	0.31 (0.26-0.46)	<0.001	1.35 [1.19-1.47]
Vd/F.kg (L/kg)	0.73 (0.56-1.0)	0.90 (0.71-1.2)	<0.001	1.23 [1.12-1.34]
$t_{1/2}$ (h)	2.2 (1.6-2.6)	2.0 (1.7-2.6)	0.052	0.91 [0.86-1.00]
Ae (mg)[h]	388 (329-495)	353 (245-484)	0.128	0.91 [0.80-1.03]
CL_R/F.kg (L/h.kg)[h]	0.115 (0.079-0.151)	0.137 (0.079-0.180)	0.005	1.19 [1.07-1.33]
fe.F[h]	0.49 (0.41-0.62)	0.44 (0.31-0.60)	0.128	0.91 [0.80-1.03]
Ritonavir				
AUC (h.mg/L)	15.7 (9.4-32.6)	10.0 (4.0-16.8)	0.005	0.64 [0.48-0.85]
C_{min} (mg/L)	0.33 (0.14-0.81)	0.20 (0.04-0.42)	0.003	0.61 [0.46-0.82]
C_{max} (mg/L)	2.59 (1.4-4.7)	1.72 (0.89-3.1)	0.016	0.66 [0.48-0.92]
t_{max} (h)	1.5 (1.0-5.0)[f]	2.0 (1.0-8.0)[f]	0.133[g]	-
CL/F.kg (L/h.kg)	0.09 (0.05-0.15)	0.13 (0.08-0.35)	0.006	1.44 [1.17-2.11]
Vd/F.kg (L/kg)	0.28 (0.16-0.56)	0.45 (0.26-1.2)	0.005	1.61 [1.18-2.18]
$t_{1/2}$ (h)	2.3 (1.6-3.2)	2.3 (1.6-3.0)	0.645	1.00 [0.92-1.14]

[a] AUC: area under the concentration-time curve, Cmin : trough concentration at 12h, Cmax : highest observed plasma concentration, tmax : sampling time for Cmax, CL/F.kg: total clearance corrected for weight, Vd/F.kg: volume of distribution corrected for weight, t1/2: elimination half-life, Ae: total amount excreted unchanged in the urine, CL_R/F.kg: renal clearance corrected for weight, fe.F: fraction of dose that is excreted unchanged (ie ratio of renal to total clearance), F: bio-availability.
[b] Combination of 800 mg indinavir and 100 mg ritonavir twice a day.
[c] Combination of 800 mg indinavir and 100 mg ritonavir twice a day plus 600 mg efavirenz once a day.
[d] P-value for the difference between pharmacokinetic parameters in the two study periods; 2-sided t-test for paired data.
[e] CI: confidence interval.
[f] median and range.
[g] Wilcoxon signed-rank test.
[h] Based on data from 12 participants.

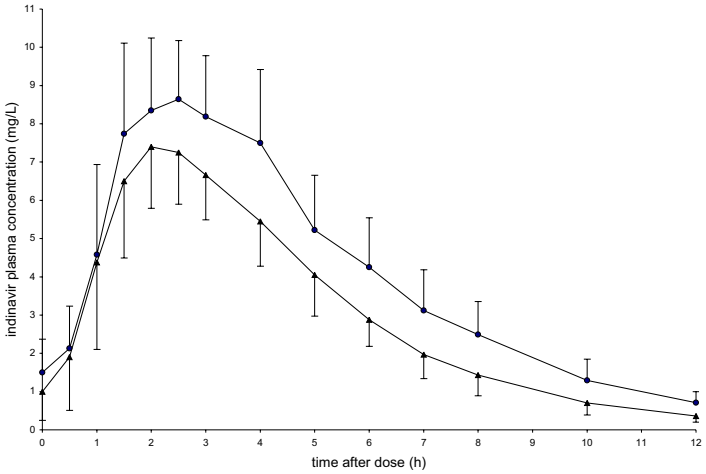

Figure 1. Mean (+ SD) indinavir steady state plasma concentrations versus time (n=14). Circles, combination of 800 mg indinavir and 100 mg ritonavir twice a day; triangles, combination of 800 mg indinavir and 100 mg ritonavir plus 600 mg efavirenz once a day.

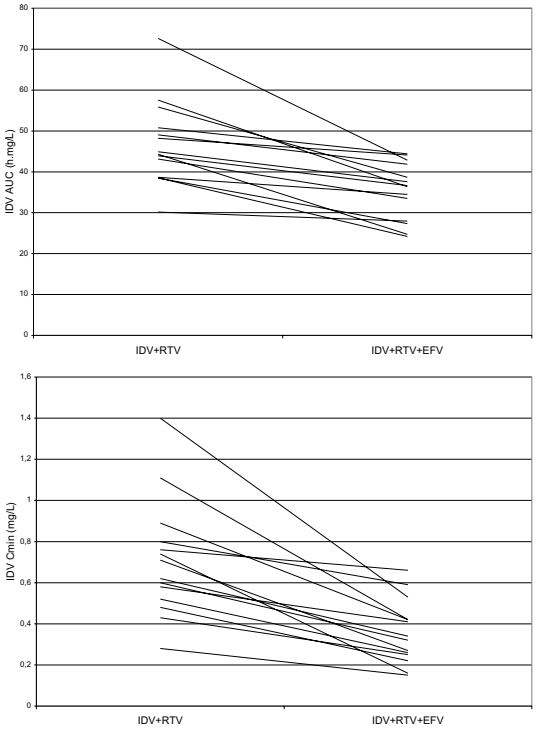

Figure 2. Indinavir (IDV) steady-state area under the curve (AUC) and minimum plasma concentration (Cmin) on day 15 (combination of 800 mg indinavir and 100 mg ritonavir [RTV] twice a day) and on day 29, after coadministration of efavirenz (EFV) for 14 days (combination of 800 mg indinavir and 100 mg ritonavir twice a day plus 600 mg efavirenz once a day) (n=14).

Pharmacokinetics of ritonavir

The effect of efavirenz on the pharmacokinetics of low-dose ritonavir corresponded roughly to the effects on indinavir (table 1; figure 3). A significant linear association (r_s = -.771, p=0.001) was observed between the AUC of ritonavir on day 15 and the percentage change in AUC as a result of interaction with efavirenz. A similar relationship was demonstrated for baseline Cmax and decreases in Cmax (r_s = -.585, p=0.028) but not for Cmin values.
The pharmacokinetic values of indinavir and ritonavir were clearly associated. For instance, significant correlations were found between the AUC values of indinavir and ritonavir on both study day 15 and study day 29. Furthermore, the decrease in indinavir AUC was significantly correlated with the decrease in ritonavir AUC (r_s = .877, p<0.001), and the same applied to decreases in Cmin values.

Pharmacokinetics of efavirenz

The geometric mean of the Css,av for efavirenz was 2.34 mg/L (range, 1.31-5.71 mg/L) and the geometric mean Cmin was 1.60 mg/L (range, 0.87-5.06 mg/L). The

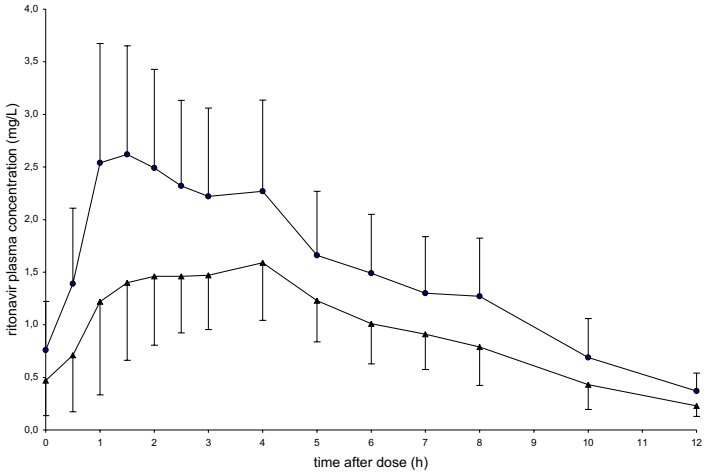

Figure 3. Mean (+ SD) ritonavir steady-state plasma concentrations versus time (n=14). Circles, combination of 800 mg indinavir and 100 mg ritonavir twice a day; triangles, combination of 800 mg indinavir and 100 mg ritonavir plus 600 mg efavirenz once a day.

t1/2 values ranged from 14.6 to 167.6 hours, with a geometric mean of 35.1 hours. The geometric mean of the efavirenz AUC(0-24) was 56.2 h.mg/L (range, 31.5-137.0 h.mg/L). No significant association was found between the exposure to efavirenz (AUC) and the percentage change in indinavir and ritonavir pharmacokinetic parameters.

Safety and tolerability

No serious adverse events (World Health Organization grade 3 or 4) were noticed. Nevertheless, 3 participants were withdrawn because of adverse events. One volunteer had gout in his medical history, and an attack of gout developed in that subject in the first period of the study. He was withdrawn from the study because a relationship with study medication could not be excluded. Another participant was withdrawn after an increase in creatinine values - from 92 mmol/L (baseline) to 188 mmol/L (ninth day of the study) - accompanied by hematuria and flank pain. The third participant was withdrawn from the study because a rash occurred on the chest, back, and legs on the study day 22.

Table 2. Incidence of adverse events (%) [a]

Adverse event	Study period 1[b]	Study period 2[c]
Fatigue /somnolence	50	64
Dizziness	14	50
Headache	50	29
Impaired concentration	7	50
Insomnia	7	29
Diarrhoea	21	29
Nausea	7	7
Vomiting	0	0
Dyspepsia	7	14
Abdominal pain	14	0
Flank pain	21	14
Circumoral paraesthesia	0	0
Skin reactions[d]	14	43
Dry lips[e]	43	43

[a] Incidence in percentage of participants based on active questioning of the 14 participants who completed the study.
[b] Combination of 800 mg indinavir and 100 mg ritonavir twice a day (2 weeks).
[c] Combination of 800 mg indinavir and 100 mg ritonavir twice a day plus 600 mg efavirenz once a day (2 weeks).
[d] Rash, dry skin, or pruritus.
[e] Not in the questionnaire; spontaneously reported.

Table 2 shows the incidence of adverse events as assessed by repeated questioning of the 14 participants who completed

the study. The median toxicity score during the first study period was 1.1 (range, 0.0-4.8). In 12 of 14 participants the severity score increased thereafter, resulting in a median severity score of 1.9 (range, 0.5-6.0) for the second study period. This implies that the average volunteer had an average of two mild adverse events or one adverse event of moderate severity at every instance of questioning in the second period. Significant or near-significant correlations were found between the toxicity score during the first study period and indinavir AUC (r_s = .478, p=0.084) and Cmax (r_s = .589, p=0.027) and ritonavir AUC (r_s = .576, p=0.031), Cmax (r_s = .499, p=0.069), and Cmin (r_s = .589, p=0.027). In the second study period no relationships could be shown between the pharmacokinetics of indinavir, ritonavir, or efavirenz and overall toxicity scores. Toxicity scores for individual central nervous system effects (fatigue, dizziness, headache, impaired concentration, and insomnia) or skin reactions were also not related to the pharmacokinetic parameters of efavirenz.

Analysis of laboratory parameters in the 14 participants who completed the study revealed only a small increase in creatinine values in 8 of 14 volunteers after the four study weeks (median increase, 4 µmol/L; range, 1-12 µmol/L). Total bilirubin increased in all volunteers from baseline (median value, 9 µmol/L; range 5-17 µmol/L) to maximum values (median, 18.5 µmol/L, range 11-46 µmol/L), which generally occurred in the first two weeks of the study. At the end of the study, bilirubin levels had almost declined toward baseline values (median, 10.5 µmol/L; range, 5-21 µmol/L). Elevations in fasting cholesterol levels were observed in all participants (median increase at the end of the study, 1.4 mmol/L; range, 1.0-2.3 mmol/L), whereas fasting triglyceride levels increased in 11 of 14 participants (median change, +0.60 mmol/L; range, -0.30 to +2,6 mmol/L). There was no relationship between increases in cholesterol or triglyceride levels and any pharmacokinetic parameter of indinavir, ritonavir, or efavirenz. The study medication had no relevant effect on other laboratory parameters.

Discussion

The results of this study provide steady-state pharmacokinetic data for the twice-daily combination of indinavir and low-dose ritonavir. A significant effect of multiple-dose efavirenz on the pharmacokinetics of this combination regimen is shown.

The baseline steady state pharmacokinetics of indinavir and ritonavir were determined after 15 days of dosing and are in accordance with the sparse pharmacokinetic data for this dual protease-inhibitor combination [9-11]. Combination of indinavir with low-dose ritonavir clearly enhances the exposure to indinavir, as reflected in higher AUC and Cmin values compared with the same parameters after administration of indinavir three times a day without ritonavir [6]. The addition of efavirenz to the combination regimen resulted in significant decreases in steady-state AUC, Cmin and Cmax of both indinavir and ritonavir. The clinical consequences of these decreases have to be derived from indinavir pharmacokinetics because low-dose ritonavir is meant only as a pharmacokinetic enhancer and will not contribute to the antiviral effect of this indinavir-ritonavir combination. Cmin values appear to be the most important pharmacokinetic parameters in this respect, inasmuch as there is accumulating evidence that Cmin values of indinavir and other protease inhibitors should be kept above certain threshold values to obtain and maintain adequate antiviral efficacy [17]. Because efavirenz halved the mean Cmin value of indinavir (from 0.66 to 0.33 mg/L), the dose of indinavir or ritonavir should be increased in the combination with efavirenz to maintain the initial indinavir Cmin levels. This may possibly be achieved by use of a combination of 800 mg indinavir and a higher dose of ritonavir (200 mg twice a day) because this regimen results in higher indinavir AUC and Cmin values than the twice-daily combination of 800 mg indinavir and 100 mg ritonavir [10]. The pharmacokinetics of this and other adjusted combinations of indinavir, ritonavir, and efavirenz should be evaluated in future pharmacokinetic studies.

According to another approach, it may be argued that mean Cmin values of indinavir still remained higher (despite the effect of efavirenz) than the corresponding value described for the 800 mg indinavir three times a day without ritonavir (0.15 mg/L [6]). Even the lowest indinavir Cmin observed in our study (0.15 mg/L in one volunteer) was equivalent to the mean Cmin in the regimen of indinavir three times a day, and it remains above the presumed therapeutic threshold value of indinavir (0.10 mg/L [17,18]). In addition, the mean indinavir AUC(0-24) value (extrapolated to infinity and corrected for predose AUC; 34.6 * 2 = 69.2 h.mg/L; table 1) remained higher than the corresponding value described for the regimen of indinavir three times a day without ritonavir (56.4 h.mg/L [6]). In accordance with these considerations, no dose modifications may be necessary for treatment-naive patients who prefer the twice-daily indinavir-ritonavir combination regimen for reasons of convenience and

who start with efavirenz. However, this would not apply to treatment-experienced patients because these patients may use the indinavir-ritonavir combination to benefit from a higher exposure to indinavir. Appropriate clinical studies are needed to validate possible dose modifications (or no such adjustments) on addition of efavirenz to indinavir and low-dose ritonavir in different patient categories.

With respect to the mechanism of the pharmacokinetic interaction between efavirenz and the indinavir-ritonavir combination, it seems most likely that efavirenz caused induction of CYP3A, the cytochrome P450 isozyme that plays a major role in the biotransformation of protease inhibitors. Efavirenz is known to act as both an inducer and an inhibitor of CYP3A [1-3]. Induction of metabolic enzymes is in accordance with data from this study showing that the increase in clearance of both indinavir and ritonavir as caused by efavirenz is mainly extrarenal in nature.
The effect of efavirenz on the pharmacokinetics of indinavir may be direct, because it has been shown previously that coadministration of efavirenz and indinavir (without ritonavir) leads to a 31% decrease in the AUC of indinavir [3]. However, data from this study suggest that the effect of efavirenz on the exposure to indinavir is at least partly mediated by a decrease in plasma concentrations of the pharmacokinetic enhancer ritonavir because indinavir and ritonavir pharmacokinetics remained highly correlated throughout the study. AUC, Cmin, and Cmax values of indinavir and ritonavir were associated on study day 15, showed highly correlated decreases after coadministration of efavirenz, and were still associated on study day 29.
The observed decrease in the AUC of low-dose ritonavir is in contrast with the influence of efavirenz on the pharmacokinetics of high-dose ritonavir (500 mg twice a day), which results in a small increase (17%) in the AUC of ritonavir [19]. Efavirenz therefore appears to have a differential influence on ritonavir pharmacokinetics, depending on the dose of ritonavir. An explanation may be that enzyme induction by efavirenz is able to compensate for inhibition of the same enzymes by low-dose ritonavir, whereas higher doses of ritonavir cause more complete enzyme inhibition that cannot be reversed.

This study was not designed to evaluate the influence of indinavir and ritonavir on the pharmacokinetics of efavirenz. However, the Css,av and Cmin of efavirenz could be determined accurately and can be compared with the sparse published data. Efavirenz

AUC values in our study were only estimations because blood samples were not collected until 8 to 10 hours after the dose. The efavirenz Css,av in this study (2.34 mg/L) is comparable to other findings (2.38 [20] and 2.19 mg/L [21]), and the mean Cmin level (1.60 mg/L) also corresponds to reference values (1.77 [3] and 1.64 mg/L [20]). These data do not support the concept of a clinically relevant influence of indinavir and ritonavir on the pharmacokinetics of efavirenz, which also was not expected based on literature data. In contrast, efavirenz drug levels in this study were within the therapeutic ranges for efavirenz proposed in two studies. For the average drug levels of efavirenz, all Css,av values in this study were within a proposed therapeutic range from 1-4 mg/L for middosing interval efavirenz levels [21]. Another study explored the calculated in vivo 90% inhibitory concentration (IC_{90}) for K103N mutant viruses (1.1 mg/L) as threshold Cmin level for efavirenz in HIV-infected patients [22]. The percentage of patients with Cmin values above this threshold was 78%, which is comparable with the results in our study, with 11 of 14 healthy volunteers (79%) having a trough level above 1.1 mg/L.

There are virtually no published data with regard to pharmacokinetic interactions between the combination of 800 mg indinavir with 100 mg ritonavir and other drugs. In addition, only sparse and preliminary data are available about the effect of efavirenz on other protease inhibitors (saquinavir, amprenavir, lopinavir) combined with ritonavir [23-30]. These latter data demonstrate that addition of low-dose ritonavir can compensate for decreases in drug exposure that are induced by efavirenz. Only ritonavir doses of at least 200 mg twice a day appear to be able to prevent any decrease in boosted protease inhibitor concentrations after addition of efavirenz, which corresponds to the results in this study. For example, saquinavir concentrations were not affected by efavirenz when saquinavir (400 mg twice a day) was combined with ritonavir (400 mg twice a day) [23]. In addition, amprenavir concentrations were markedly increased by the addition of ritonavir (200 mg twice a day), and were unaltered by the subsequent addition of efavirenz [24]. In contrast, efavirenz was able to induce decreases in amprenavir AUC and Cmin when this protease inhibitor was combined with only 100 mg ritonavir twice a day [27]. Likewise, when efavirenz was combined with the coformulated lopinavir/ritonavir (400 mg lopinavir and 100 mg ritonavir twice a day), it reduced the AUC and Cmin values of lopinavir by 20 to 25% and 40 to 45%, respectively [29]. These findings are

comparable to the results in our study. Finally, ritonavir has also been applied as a pharmacokinetic booster to explore possible once-daily dosing regimens of indinavir. The addition of efavirenz to these regimens results in decreased indinavir concentrations that appear to be too low to prevent selection of indinavir-resistant viral strains [30].

The combination of indinavir, ritonavir, and efavirenz caused a relatively high incidence of adverse events that were generally mild in severity. That high incidence may be attributable in part to the active and frequent questioning of volunteers that we used to guarantee their safety. In addition, the attention of the volunteers was especially drawn to central nervous system effects that could manifest after administration of efavirenz. This may have elicited a higher response for these adverse events.
The spectrum of adverse events was as we expected, including nephrotoxic adverse events related to indinavir [6] (one withdrawal) and typical central nervous system effects that usually manifest in the two weeks after initiation of efavirenz ([1-3]). The latter were most notably a light feeling in the head or slight dizziness, as well as concentration problems. Because efavirenz causes a rash in up to 28% of HIV-infected patients [3], often occurring within the first two weeks of treatment, it was anticipated that this adverse event would occur in this study. Rash did develop in one volunteer during the second study period, but we cannot exclude that this event was related to indinavir or ritonavir.
Toxicity in the first study period was clearly related to the pharmacokinetics of indinavir and ritonavir, but similar relationships could not be shown in the second study period. The addition of a third agent in the second study period may have confounded any such relationships, but development of tolerance to adverse events may be another explanation.
The most important laboratory abnormalities were significant increases in cholesterol and triglyceride values, probably attributable to ritonavir or efavirenz or both. These increases warrant close monitoring when the indinavir-ritonavir combination and efavirenz are used in clinical practice.

In conclusion, data from this study demonstrate that addition of efavirenz to the combination of indinavir and low-dose ritonavir results in significant decreases in

steady state AUC, Cmax, and Cmin of both indinavir and ritonavir in healthy volunteers. This implies that the dose of indinavir or ritonavir should be increased to maintain similar indinavir drug levels when efavirenz is coadministered with the combination of 800 mg indinavir and 100 mg ritonavir. Additional pharmacokinetic studies are needed to assess the appropriate dose modifications to achieve this. For treatment-naive HIV-infected patients it may be argued that dose modifications may not be necessary after addition of efavirenz to the indinavir-ritonavir combination because all Cmin concentrations of indinavir still remained higher than the mean indinavir Cmin of the conventional three-times-a day regimen without ritonavir. Follow-up pharmacokinetic analyses in a clinical setting are warranted to confirm the findings of this study in healthy volunteers.

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Chapter 4.3

Pharmacokinetics of indinavir/ritonavir (800/100 mg BID) combined with efavirenz in HIV-infected patients in the EASIER study

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AIDS, accepted for publication

Abstract

A pharmacokinetic study was performed in HIV-infected patients who used indinavir/ritonavir (800/100 mg BID) plus efavirenz (600 mg) in the EASIER study. Indinavir plasma concentrations were similar to values previously obtained in healthy volunteers who used the same combination. Efavirenz concentrations were higher than reported before. The pharmacokinetic data suggest that indinavir/ritonavir plus efavirenz (without dose modifications) should be effective in treatment-naive patients, and this was supported by the treatment response of the participants.

The nucleoside-sparing combination of indinavir plus efavirenz has demonstrated a potent antiretroviral effect in HIV-infected patients who have not previously been treated with protease-inhibitors (PIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs) [1]. Indinavir plus efavirenz can also be combined effectively with nucleoside analogues [2]. In the combination of indinavir and efavirenz, the pharmacokinetic profile of indinavir can be improved by combining it with a low dose (100 mg) of ritonavir. This allows for twice-daily (BID) dosing of indinavir (800/100 mg BID) and administration with food. However, indinavir, ritonavir and efavirenz can not be combined without considering the pharmacokinetic interactions between these drugs. A pharmacokinetic study in healthy volunteers showed that addition of efavirenz (600 mg once daily) to indinavir/ritonavir (800/100 mg BID) resulted in a decrease in indinavir concentrations, but indinavir trough (Cmin) values remained sufficiently high to conclude that dose modifications are not required when this combination is used in treatment-naive HIV-infected patients [3]. Subsequently, the European and South American Study of Indinavir, Efavirenz and Ritonavir (EASIER) evaluated the combination of indinavir/ritonavir plus efavirenz (without dose modifications) in PI-, NNRTI-, and stavudine-naive HIV-infected patients, and compared this combination to the same regimen supplemented with stavudine. Preliminary results from EASIER were presented recently [4]. We evaluated the pharmacokinetics of indinavir, ritonavir and efavirenz in patients in the EASIER study, considering that the pharmacokinetics of antiretroviral drugs may differ between healthy volunteers and HIV-infected patients, and to be able to put clinical data from EASIER in perspective.

Steady-state pharmacokinetic data were assessed in six participants in EASIER, three men and three women (median age: 35 years, median weight: 65 kg). Their median viral load and CD4 cell count before entry in EASIER were 104 347 copies/ml (range: 7 923 to 493 798 copies/ml) and 267 cells/µl (range: 139 to 467 cells/µl) respectively. Concomitantly administered drugs were stavudine (2x) and levothyroxine (1x). Fourteen blood samples were drawn at 0.0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 10.0 and 12.0 h after administration of indinavir/ritonavir (800/100 mg) with food in the morning. Plasma samples were analyzed for indinavir, ritonavir and efavirenz using validated high-performance liquid chromatographic methods [3,5]. Pharmacokinetic parameters were calculated using noncompartmental

methods. For indinavir and ritonavir, the area under the concentration-time curve (AUC) was derived from the 14 concentrations measured from 0 h to 12 h post dose (AUC0-12h), using the trapezoidal rule. For efavirenz, the concentrations beyond 8 h until about 22 h post dose were available, as the participants ingested this drug in the evening preceding pharmacokinetic assessments. Efavirenz 24-h Cmin concentrations were calculated by extrapolation of the last concentration (Clast) to 24 h, using the equation $C_{min} = C_{last} * e^{- ß (24 - tlast)}$, where ß is the elimination rate constant of efavirenz and tlast is the sampling time for Clast.

Table 1 presents the results of the pharmacokinetic evaluation. The geometric mean indinavir Cmin for the six patients in the EASIER study was lower than Cmin values that were previously reported for HIV-infected patients who used indinavir/ritonavir (800/100 mg BID) without efavirenz. This difference can be explained by the co-administration of efavirenz, since efavirenz (an inducer of cytochrome 3A4 in the liver) also decreased indinavir Cmin concentrations in healthy volunteers who used indinavir/ritonavir 800/100 mg BID [3]. In effect, the pharmacokinetic data for indinavir in the six HIV-infected patients closely corresponded to the data obtained in healthy volunteers who used the same combination of indinavir, ritonavir and efavirenz. The geometric mean for indinavir Cmin was 0.33 mg/L in healthy volunteers [3] and 0.32 mg/L in the patients in the EASIER study (table 1) and geometric mean values for AUC0-12h were 34.6 h*mg/L in healthy volunteers [3] and 41.0 h*mg/L in the HIV-infected individuals (table 1). It should be noted that the geometric mean Cmin for indinavir in the six patients (0.32 mg/L) remained higher than the mean Cmin (i.e. 0.15 mg/L [6]) for the conventional 800 mg thrice-daily regimen of indinavir without ritonavir, and five out of six patients had a Cmin above 0.15 mg/L.

Considering the pharmacokinetics of efavirenz (table 1), it appeared that the geometric mean Cmin value for efavirenz at t=24 h (2.7 mg/L) was higher than described in the efavirenz product monograph (1.8 mg/L). The mean efavirenz concentrations at 12 h (C12h) and 24 h (Cmin) post dose (table 1) were also higher than values that were previously assessed in healthy volunteers who took the combination of indinavir, low-dose ritonavir and efavirenz (C12h: 2.3 mg/L and Cmin: 1.6 mg/L [3]). All measured efavirenz concentrations in the six patients were above 1 mg/L, which is the lower limit of the proposed therapeutic range for efavirenz (1-4 mg/L) based upon efavirenz

Table 1. Steady-state pharmacokinetics of indinavir, ritonavir and efavirenz in HIV-infected patients using indinavir/ritonavir (800/100 mg BID) plus efavirenz (600 mg once daily) in the EASIER study (n = 6) [a,b]

Parameter	Geometric mean	95% CI	Min.-max.
Indinavir			
AUC0-12h (h.mg/L)	41.0	[29.4-57.2]	(29.6-70.3)
C_{max} (mg/L)	9.4	[7.6-11.6]	(7.6-13.0)
Cmin (mg/L)	0.32	[0.13-0.82]	(0.07-0.98)
t1/2 (h)	1.9	[1.4-2.5]	(1.5-3.0)
Ritonavir [b]			
AUC0-12h (h.mg/L)	11.4	[6.2-21.1]	(4.5-25.1)
C_{max} (mg/L)	2.4	[1.6-3.7]	(1.3-4.4)
Cmin (mg/L)	0.21	[0.07-0.59]	(0.04-0.76)
t1/2 (h)	2.4	[1.7-3.3]	(1.6-3.6)
Efavirenz			
C_{12h} (mg/L)	3.8	[1.9-7.5]	(2.0-11.1)
C_{min} (mg/L)	2.7	[1.3-5.7]	(1.1-7.9)
$t_{1/2}$ (h)	32.5	[26.5-39.9]	(28.4-47.8)

[a] *Abbreviations: CI: confidence interval, min.: minimum value, max.: maximum value, AUC0-12h: area under the concentration-time curve from 0 to 12 h post dose, Cmax : highest observed plasma concentration, Cmin : trough concentration at 12 h (indinavir, ritonavir) or 24 h (efavirenz), t1/2: elimination half life, C12h: concentration at 12 h post dose of efavirenz (mid-dosing interval concentration)*
[b] *Ritonavir is used as a pharmacokinetic enhancer and will not contribute directly to the antiretroviral effect of the combination*

concentrations that are sampled between 8 h and 20 h post dose [7]. Consequently, the efavirenz concentrations in the six patients predicted efficacy for this drug. Two participants had efavirenz concentrations above the upper limit of the therapeutic range (4 mg/L) and one of these had efavirenz C12h and Cmin values of 11.1 mg/L and 7.9 mg/L respectively. There was no obvious explanation for the high efavirenz concentrations in this patient. High efavirenz concentrations can occur as a result of the large interindividual variability in the pharmacokinetics of this drug [7]. It should be recognized that the presence of one such participant with high efavirenz concentrations affects the average (even the geometric mean) efavirenz concentrations in this small study.
The observed pharmacokinetic parameters suggest that the combination of indinavir/ritonavir plus efavirenz without dose modifications should be effective in

treatment-naive patients. Indeed five of the six patients (one of whom also used stavudine) completed the 48 weeks of the EASIER study with an undetectable viral load (< 50 copies/ml) and all had an increase in CD4 cell count. The sixth patient discontinued at week 16, with a viral load of 71 copies/ml, due to adverse events deemed probably related to the study medication. This patient did not have abnormal pharmacokinetic parameters. One of five patients who completed the study had elevated efavirenz concentrations (Cmin 7.9 mg/L, see above) together with two mild to moderate adverse events. However, these adverse events were determined probably not related to study medication.

In conclusion, we performed a pharmacokinetic study in six HIV-infected patients who used a combination of indinavir/ritonavir (800/100 mg BID) plus efavirenz in the EASIER study. The pharmacokinetic data for indinavir were in agreement with data from a previous study in healthy volunteers. Efavirenz concentrations were higher than reported before. The pharmacokinetic data suggest clinical efficacy for the combination of indinavir/ritonavir plus efavirenz in treatment-naive patients, and this was supported by the treatment response of the patients.

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Chapter 4.4

The pharmacokinetics of indinavir/ritonavir 800/100 mg in combination with efavirenz 600 mg in HIV-1 infected subjects

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Abstract

Introduction

Addition of efavirenz (EFV) 600 mg to indinavir/ritonavir (IDV/RTV) 800/100 mg results in significant decreases in drug levels of IDV in healthy volunteers. This study evaluated the steady state pharmacokinetics of IDV/RTV 800/100 mg bid in combination with EFV 600 mg qd in HIV-infected Thai subjects who used this nucleoside-sparing combination in the HIV-NAT 009 study.

Methods

At week 4 of the HIV-NAT 009 study, 12-hour pharmacokinetic profiles for IDV/RTV were obtained from 20 HIV-infected subjects. For EFV, the concentrations at 12 hours (C12h) and 24 hours (Cmin) post dose were assessed.

Results

All subjects (10 male, 10 female) completed the study. The geometric mean [95% confidence interval] AUC, Cmin and Cmax values of IDV were 45.7 h.mg/L [39.8–52.5], 0.32 mg/L [0.24-0.44] and 11.1 mg/L [9.4-13.0] respectively. A greater than 10-fold variation in IDV Cmin was observed. All subjects recorded an IDV Cmin that was at least comparable to the reported mean population Cmin for IDV 800 mg tid without ritonavir (0.15 mg/L). The geometric mean [95% CI] C12h and Cmin values of EFV were 3.1 mg/L [2.5-3.7] and 2.1 mg/L [1.6-2.6] respectively.

Conclusions

Despite the known pharmacokinetic interaction between EFV and IDV/RTV, the combination of IDV/RTV 800/100 mg bid and EFV 600 mg qd results in adequate minimum concentrations of both IDV and EFV for treatment-naive patients.

Introduction

The combination of efavirenz and indinavir given with nucleoside analogues has demonstrated a potent and durable antiretroviral effect in nucleoside-experienced patients [1]. The same combination of efavirenz and indinavir when given in the absence of nucleoside analogues has demonstrated similar efficacy to that achieved with the regimen of indinavir, zidovudine and lamivudine [2].

Efavirenz has the advantage of once daily (qd) dosing with three 200 mg capsules or, more recently, one 600 mg tablet (available in the USA and Europe). In contrast, indinavir must be taken three times daily (tid). The pharmacokinetic profile of indinavir can be improved by combining it with a low dose (100 mg) of ritonavir. The combination of indinavir with low dose ritonavir allows for a more convenient twice daily (bid) administration (800/100 mg bid) and permits concurrent intake with food [3]. This indinavir/ritonavir drug combination is widely used and available data suggest that its virological efficacy is comparable to that of indinavir 800 mg tid without ritonavir [4-6].

Efavirenz and indinavir combined with ritonavir has the potential to be a potent, compact combination regimen that should offer ease of adherence. However, mutual drug interactions between these agents may alter the pharmacokinetic properties of indinavir, ritonavir, or efavirenz, potentially resulting in subtherapeutic drug levels. A study conducted in healthy volunteers demonstrated that the addition of efavirenz to indinavir/ritonavir 800/100 mg bid resulted in significant decreases in drug levels of indinavir [7]. However, all individual indinavir trough (Cmin) levels remained equivalent to or above the mean Cmin value described for the 800 mg tid regimen of indinavir without ritonavir. Therefore it may be argued that dose modifications are not necessary for protease inhibitor-naive patients who use the combination of indinavir/ritonavir 800/100 mg and efavirenz 600 mg qd.

These pharmacokinetic findings have yet to be confirmed in HIV-1 infected subjects, and it cannot be excluded that there may exist differences between HIV-infected subjects and non HIV-infected healthy volunteers with respect to the pharmacokinetics of these antiretroviral drugs. For instance, it has been demonstrated that inflammation

and infection can affect the activity of the cytochrome P450 isoenzymes that are involved in the metabolism of both the protease inhibitor and non-nucleoside analogue reverse transcriptase classes of antiretroviral drugs [8]. Therefore, we undertook this study to evaluate the pharmacokinetics of indinavir, low-dose ritonavir and efavirenz in an open label, single arm, non randomised trial (the HIV-NAT 009 study) designed to evaluate the efficacy, safety and tolerability of indinavir/ritonavir 800/100 mg bid in combination with efavirenz 600 mg qd.

Methods

Subjects

Twenty subjects were sequentially enrolled from a cohort of 61 patients enrolled in the HIV-NAT 009 study. Subjects eligible for the HIV-NAT 009 study were at least 18 years of age, HIV-1 positive by ELISA, and had virologically failed current or previous therapy with nucleoside reverse transcriptase inhibitors (NRTIs) (HIV RNA > 1000 copies/mL). All subjects had serum creatinine < 2 times the upper limit of normal and AST and ALT < 5 times the upper limit of normal. Exclusion criteria included the need, or anticipated need, to use any concomitant medications known to interact with the study medications, an active opportunistic infection or malignancy not under adequate control, and alcohol or substance abuse which would interfere with patient medication adherence or safety. Patients enrolled in the pharmacokinetic study were allowed to participate if they were not suffering from any acute illness on the day of plasma sampling and had not commenced any regular new medications since commencing indinavir, ritonavir and efavirenz.
All included patients gave written informed consent to participate in the study, which was approved by the King Chulalongkorn University Faculty of Medicine Ethics Committee, Bangkok, Thailand.

Study design and procedures

At the HIV-NAT 009 baseline study visit, all patients were instructed to ingest indinavir 400 mg two capsules together with ritonavir 100 mg one capsule in the morning and evening at regular 12 hour intervals. Patients were advised to ingest at least two litres

of water per day in order to reduce the potential for nephrotoxicity from indinavir. Patients were instructed to ingest efavirenz 200 mg three capsules every night before bedtime at regular 24 hour intervals.

At week 4, pharmacokinetic profiles were obtained from the participants on an outpatient basis. On the morning of the study the patients were asked to attend in a fasted state and specifically instructed not to ingest the morning dose of indinavir and ritonavir. After arrival the times of last ingestion of all study medications and concomitant medications were recorded. Subjects then ingested indinavir and ritonavir with a glass of water under the direct supervision of the study staff, and immediately after they were provided with a standardised breakfast which consisted of a rice based meal, a milk or soy milk drink, and a plain bread roll.

At designated time intervals associated with the ingestion of indinavir and ritonavir, 14 blood samples were drawn over a 12 hour period according to the following schedule: pre-dose, and then at 30, 60, 90, 120, 150, 180, 240, 300, 360, 420, 480, 600 and 720 minutes post dose. Blood samples were collected in 5 ml EDTA tubes and plasma was isolated within one hour by centrifugation at 3000 G for 10 minutes. Blood samples were stored at –70 degrees Celsius at the HIV-NAT laboratory until their transfer packaged in dry ice to The Netherlands for analysis.

Analytical and pharmacokinetic methods

Plasma samples were analysed for indinavir, ritonavir and efavirenz by previously described validated reversed-phase high-performance liquid chromatographic (HPLC) methods [7,9]. The pharmacokinetics of indinavir and ritonavir were calculated using noncompartmental methods [10]. The highest observed plasma concentration was defined as Cmax, with the corresponding sampling time as t_{max}. Cmin was the concentration 12 hours after ingestion of the drugs. The terminal, log-linear period (log C versus t) was defined by visual inspection of the last data points ($N \geq 3$). The absolute value of the slope (ß/2.303) was calculated by least squares linear regression analysis, where lz is the first-order elimination rate constant. The elimination half-life (t1/2) was calculated by the equation 0.693/ß. The area under the concentration versus time curve (AUC) was calculated using the trapezoidal rule from

0 to 12 hours. This value was extrapolated to infinity using the equation Cmin/ß and was corrected for contribution of the predose AUC by subtraction of C0/ß. Apparent oral clearance (Cl/F where F is bioavailability) was calculated by dividing dose (D) by AUC, and apparent volume of distribution Vd/F was obtained by dividing Cl/F by ß. Clearance and volume of distribution were corrected for weight of the participant.

Since the ingestion of efavirenz occurred on the evening prior to the pharmacokinetics study day, according to the subjects' regular schedule, we were unable to assess all pharmacokinetic parameters for efavirenz. However, the concentration at 12 hours post ingestion of efavirenz (C12h) was derived accurately from the pharmacokinetic curve and efavirenz trough levels (Cmin) were calculated using the first order equation of the regression line through the last data points and filling in the 24 hour time point.

Data analysis

All statistical evaluations were performed with SPSS for Windows, version 9.0 (SPSS Inc., Chicago, IL, USA). Geometric means with 95% confidence intervals were calculated for each pharmacokinetic parameter. Differences in patient characteristics between subgroups were compared using Wilcoxon rank-sum test and differences in pharmacokinetic parameters were tested using the two-sample t-test on logarithmically transformed data. Correlation between parameters was calculated using Pearson correlation coefficient (ρ) or Spearman's rho (rank correlation, r), dependent on distributional characteristics of the two variables involved. For all analyses, a p-value of 0.05 or less was regarded as significant.

Results

Twenty Thai subjects (10 males, 10 females) were included and all completed the study. Concurrently administered medications on the day of study were co-trimoxazole 480 mg two tablets (n=11), isoniazid 100 mg three tablets (n=2), dapsone 100 mg one tablet (n=1), hydroxyzine 10 mg two tablets (n=1), multivitamin two tablets (n=1), mineral and vitamin supplement three tablets (n=1), and paracetamol 500 mg two tablets (n=1). The characteristics of the patient cohort are summarized in table 1. Table 2 summarises the steady-state pharmacokinetic parameters of indinavir and ritonavir,

together with reference data obtained from two other studies using indinavir/ritonavir 800/100 mg bid. Of note, a greater than 10-fold variation in the Cmin of indinavir (0.14 mg/L to 1.8 mg/L) was observed, emphasizing the great inter-individual variability of this important pharmacokinetic parameter. Ritonavir is used in this regimen only as a pharmacokinetic enhancer and therefore therapeutic concentrations are not achieved. Table 3 summarises the results for efavirenz, using data from the study in healthy Caucasian volunteers for reference [7]. Figure 1 displays the pharmacokinetic curves for indinavir and ritonavir in the presence of efavirenz.

As expected, we observed a strong and significant linear association between the AUC, Cmax and Cmin values of the same drug, either indinavir, ritonavir or efavirenz. We also observed a strong association between the AUC ($r = 0.85$, $p < 0.001$), Cmax ($r = 0.59$, $p = 0.006$), and Cmin ($r = 0.76$, $p < 0.001$) of indinavir and ritonavir. Exposure to efavirenz however was not linearly related to the exposure to indinavir and ritonavir. For example, no significant linear association was found between AUC values of indinavir and efavirenz ($r = 0.20$, $p = 0.40$).

The cohort was stratified for gender and descriptive statistics were calculated for men (n=10) and women (n= 10) separately. The results demonstrated that men were taller (median height 163 cm versus 154 cm, p=0.02), and heavier (median weight 60 kg versus 50 kg, p=0.01) than women. No significant differences between men and women were observed with respect to AUC, Cmin or Cmax values of indinavir,

Table 1. Summary of patient characteristics

Variable	HIV-NAT 009 IDV/RTV 800/100mg bid + EFV 600mg qd[a]
Number of patients	20
Gender [M/F]	10/10
Median age (IQR)[b] [yr.]	39 (34-43)
Median body weight (IQR) [kg]	54.5 (49.1-62)
Median height (SD) [cm]	160 (153-164)
Median CD4 cell count (IQR) [per mm3]	136 (39.5-238)
Median log10 HIV RNA (IQR) [copies/mL]	4.44 (3.95-4.63)

[a] *indinavir 800 mg bid + ritonavir 100 mg bid + efavirenz 600 mg qd*
[b] *inter-quartile range*

Table 2. Summary of results for indinavir (IDV) and ritonavir (RTV) steady state pharmacokinetic parameters for the HIV-NAT 009, Healthy Volunteer and HIV-NAT 005 studies

Parameter[a]	Experimental data		Reference data	
	HIV-NAT 009 (n=20) IDV/RTV[b] 800/100mg bid[c] + EFV[d] 600mg qd[e]		HIV-NAT 005 (n=17) IDV/RTV[b] 800/100mg bid	HV[f] study (n=14) IDV/RTV 800/100mg bid[c] + EFVd 600mg qd[e]
Indinavir	*Geometric mean and 95% CI*	*Range*	*Geometric mean*	*Geometric mean*
AUC (h.mg/L)	45.7 (39.8-52.5)	25.1-86.4	49.20	34.6
C_{min} (mg/L)	0.32 (0.24-0.44)	0.14-1.8	0.68	0.33
C_{max} (mg/L)	11.1 (9.4-13.0)	5.9-19.0	10.56	7.65
t_{max} (h)g	1.0	0.6-3.1	1.98	2.0
CL/F.kg (L/h.kg)	0.32 (0.29-0.36)	0.22-0.49	0.26	0.31
Vd/F.kg (L/kg)	0.85 (0.76-0.94)	0.56-1.29	0.76	0.90
$t_{1/2}$ (h)	1.8 (1.6-2.0)	1.3-2.9	2.12	2.0
Ritonavir	*Geometric mean and 95% CI*	*Range*	*Geometric mean*	*Geometric mean*
AUC (h.mg/L)	13.7 (10.5-17.8)	4.7-40.8	14.01	10.0
C_{min} (mg/L)	0.22 (0.15-0.33)	0.07-1.8	0.40	0.20
C_{max} (mg/L)	2.9 (2.2-3.8)	0.91-7.2	2.24	1.72
t_{max} (h)g	1.0	0.5-5.0	1.76	2.0
CL/F.kg (L/h.kg)	0.14 (0.11-0.17)	0.06-0.38	0.12	0.13
Vd/F.kg (L/kg)	0.49 (0.40-0.61)	0.28-1.19	0.55	0.45
$t_{1/2}$ (h)	2.5 (2.2-2.9)	1.8-4.5	3.14	2.3

[a] *AUC: area under the concentration-time curve, Cmin: trough concentration at 12h, Cmax: highest observed plasma concentration, tmax: sampling time for Cmax, CL/F.kg: total clearance corrected for weight, Vd/F.kg: volume of distribution corrected for weight, t1/2: elimination half life*
[b] *indinavir/ritonavir*
[c] *twice daily dosing*
[d] *efavirenz*
[e] *once daily dosing*
[f] *healthy volunteer study*
[g] *Tmax value is median value*

Table 3. Summary of results for efavirenz (EFV) steady state pharmacokinetic parameters or HIV-NAT 009 and Caucasian Healthy Volunteer study

Parameter[a]	Experimental data		Reference data	
	HIV-NAT 009 (n=20) IDV/RTV[b] 800/100 mg bid + EFV[c] 600 mg qd		HV[d] Study (n=14) IDV/RTV[b] 800/100 mg bid + EFV 600mg qd	
Efavirenz	*Geometric mean and 95% CI*	*Range*	*Geometric mean*	
C_{12h} (mg/L)	3.1 (2.5-3.7)	1.5-7.6	2.34	
C_{min} (mg/L)	2.1 (1.6-2.6)	0.89-6.9	1.60	

[a] *C12h: average steady state concentration at t=12h post ingestion of efavirenz, Cmin: trough concentration at 24h*
[b] *indinavir/ritonavir*
[c] *efavirenz*
[d] *healthy volunteer*

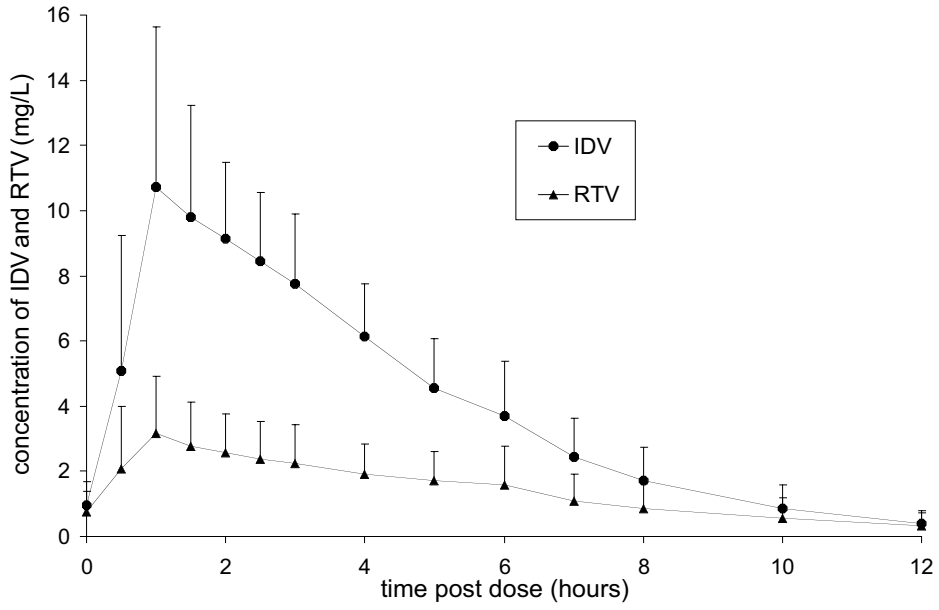

Figure 1. Mean (standard deviation) indinavir (IDV) and ritonavir (RTV) steady state plasma concentrations in subjects receiving indinavir/ritonavir (800/100 mg bid) plus efavirenz (600 mg qd) (n=20). Circles: indinavir 800mg. Triangles: ritonavir 100mg.

ritonavir or efavirenz, although there was a trend towards higher Cmin values for efavirenz in women compared to men (2.5 vs 1.7 mg/L, p=0.097). Men had a significantly longer elimination half-life for indinavir compared to women (geometric mean 2.0 vs 1.6 h, p= 0.005) and a significantly longer elimination half-life for ritonavir (2.9 vs 2.2 h, p=0.04).
Increased weight and an increase in body mass index (BMI) were correlated with a decrease in indinavir AUC (r = -0.52, p = 0.02 and r = -0.54, p =0.015, respectively), but otherwise no significant linear associations were observed between weight or BMI and the AUC, Cmin or Cmax of indinavir, ritonavir or efavirenz.

Discussion

The results from this study provide steady state pharmacokinetic data for the combination of indinavir/ritonavir 800/100 mg bid combined with efavirenz 600 mg qd in HIV-1 infected subjects. In order to provide a perspective on these results it is useful to compare and contrast them with the results of other pharmacokinetic studies of indinavir/ritonavir 800/100 mg. There are a number of studies that have described the pharmacokinetics of indinavir/ritonavir 800/100 mg which could be chosen as comparators [3-7,11,12]. However, we have chosen to compare our results with the results from two selected studies. The first is a study that reported pharmacokinetic data for indinavir/ritonavir 800/100 mg used in combination with zidovudine and lamivudine in HIV-1 infected Thai patients [5]. This study was chosen as a comparator because the data was drawn from a Thai patient cohort enrolled at the same institution (HIV-NAT). The second is a study of indinavir/ritonavir 800/100 mg bid and efavirenz 600 mg qd used in healthy Causasian volunteers from The Netherlands [7]. This study was chosen as it affords a direct comparison with pharmacokinetic data employing the exact same regimen as utilized in the current study, but in Caucasian healthy volunteers. In both cases the data were determined using the same bioanalytical and pharmacokinetic methodology in the same laboratory. The careful selection of these two compator studies should therefore minimize the potential biases that exist when comparing independent cohorts.

Baseline characteristics were similar for the Thai HIV infected patients in the indinavir/ritonavir reference study [5] (median age (IQR) 36 (32-41) years, median

height (IQR) 163 (155-179) cm, and median body weight (IQR) 59.2 (54.2-73.6) kg) (see table 1). The healthy Caucasian volunteers [7] were younger (median age (IQR) 22 (20-29) years), taller (median height (IQR) 183 (176-185) cm), and heavier (median body weight (IQR) 74.6 (71.4-81.3) kg) than the Thai subjects in the current study.

The comparative pharmacokinetics of indinavir and ritonavir are summarised together with the experimental data in table 2.

With regard to indinavir AUC, results are similar for indinavir/ritonavir when given with and without efavirenz in a Thai population (45.7 h.mg/L v 49.2 h.mg/L respectively, see table 2), although the indinavir AUC was decreased in the presence of efavirenz in the Caucasian healthy volunteers (from 45.9 h.mg/L to 34.6 h.mg/L respectively) [7].

For indinavir Cmin, the addition of efavirenz 600 mg to the combination of indinavir/ritonavir 800/100 mg results in a geometric mean indinavir Cmin (0.32 mg/L) that is substantially lower than the indinavir Cmin level achieved in a similar Thai population without the addition of efavirenz (0.68 mg/L, see table 2). The indinavir Cmin value of 0.32 mg/L is comparable to that obtained in the study with healthy Caucasian volunteers who used indinavir, ritonavir and efavirenz (0.33 mg/L) [7]. However, despite the reduction, the geometric mean indinavir Cmin value of 0.32 mg/L value is greater than the mean indinavir Cmin of 0.13 mg/L obtained using the conventional three times daily (tid) regimen of indinavir 800 mg when assessed in Thai subjects [5]. This value also remains above the mean Cmin of indinavir 800mg tid quoted in the product monograph (0.15 mg/L [13]). In addition, all 20 participants in the current study had an indinavir Cmin above a value of 0.10 mg/L that has been derived as a therapeutic threshold for treatment-naive patients using indinavir 800 mg tid [14]. This suggests that despite the reduction in indinavir Cmin with the addition of efavirenz, the regimen should maintain adequate indinavir minimum concentrations in protease inhibitor-naive subjects. The wide inter-individual variations for indinavir Cmin have been noted in other studies.

For indinavir Cmax we noted considerably higher values in both Thai study populations compared to healthy Caucasian volunteers (see table 2), raising the possibility of greater indinavir toxicity in this population. Higher indinavir Cmax levels have been associated with an increased prevalence of indinavir-related nephrotoxicity, particularly nephrolithiasis [15]. The higher indinavir Cmax levels in Thai patients is

consistent with their lower body mass and therefore likely reflects the effect of a smaller volume of distribution for indinavir when compared to the heavier Caucasian subjects. Alternatively, the higher indinavir Cmax values observed in the Thai subjects might be explained by differences in the composition of the standardized meal that was offered with the indinavir/ritonavir [16]. The heavy meal used by Caucasian subjects [7] may have led to a greater delay in gastric emptying and absorption of indinavir, thereby resulting in a lower Cmax and a longer tmax (see table 2) compared to the Thai patients in the current study who were fed a lighter rice-based meal.
While this study provides reassuring pharmacokinetic data with regard to the anticipated efficacy of the use of this drug combination in protease inhibitor-naive subjects, the same cannot be confidently said for patients with previous protease inhibitor exposure, in which case the pharmacokinetic threshold indinavir Cmin may be higher. In this case, dose modifications should be considered. Further studies are needed to explore the role of dose modifications within this regimen when used in patients with previous protease inhibitor exposure.

With regard to efavirenz, we were unable to calculate all pharmacokinetic parameters for this drug in the study, since efavirenz was dosed on the night before the study day. However, the geometric mean concentrations at 12 hours (C12h) and 24 hours (Cmin) could be accurately determined and compared to the published post efavirenz dose data. The geometric mean C12h and Cmin values for efavirenz in this study were 30-35% higher than findings in the healthy volunteer study (see table 3). The best evidence for a minimum efficacy threshold for efavirenz has been provided by Marzolini et al which suggested that the efavirenz level obtained between 8 and 20 hours after ingestion of the drug should be greater than 1 mg/L in order to maintain virological suppression [17]. In this current study, all patients recorded a 20 hour post drug ingestion level for efavirenz greater than 1 mg/L. The Marzolini data also suggested those subjects with an 8-20 h efavirenz level of > 4 mg/L were more likely to experience central nervous system (CNS) toxicity. Five patients (25%) on study recorded an efavirenz level between 8 and 20 h post dose of > 4 mg/L, possibly predisposing these patients to a higher risk of CNS toxicity. The results for efavirenz 600 mg qd in this study therefore predict adequate efficacy but also the potential for toxicity.

The determinants of the pharmacokinetics and pharmacodynamics of the antiretroviral drugs are not well characterised. We investigated the influence of gender, weight and body mass index (BMI) on the results. For gender, no significant differences were observed for AUC, Cmin and Cmax values of indinavir, ritonavir and efavirenz between men and women, but we did observe significant differences in the elimination half-lives of indinavir and ritonavir. However, it should be understood that the absence of some significant differences could possibly be due to the small sample size in the study subgroups, resulting in a lack of statistical power. For weight and BMI we did observe a statistically significant inverse association with the indinavir AUC, but this correlation was not found for other pharmacokinetic parameters of indinavir, ritonavir or efavirenz. Further research in bigger patient samples is needed to determine the predictors of the pharmacokinetics for this regimen.

CYP3A4 is the cytochrome P450 isoenzyme that plays the major role in the biotransformation of the protease inhibitors. Efavirenz is an inducer of liver CYP3A4 in healthy volunteers, but does not appear to induce intestinal CYP3A4 or intestinal P-glycoprotein [18]. The data from both this and the study in healthy Caucasian volunteers [7] is in accordance with an induction effect of efavirenz resulting in an increase in the hepatic clearance of both indinavir and ritonavir. Results from both studies suggest that the effect of efavirenz on the indinavir exposure is indirect and mediated by a decrease in the pharmacokinetic enhancing activity of ritonavir, as indinavir and ritonavir parameters were closely correlated in both studies. However, it is known that coadministration of efavirenz with indinavir (without ritonavir) leads to a 31% decrease in the AUC of indinavir [19]. Consequently, it cannot be excluded that the effect of efavirenz on the pharmacokinetics of indinavir is at least to some extent direct.

In conclusion, the data from this study in HIV-1 infected Thai patients suggests that the combination of indinavir/ritonavir 800/100 mg bid and efavirenz 600 mg qd results in pharmacokinetic parameters for indinavir and efavirenz that should confer adequate antiviral efficacy in patients not previously exposed to these agents. This confirms the data from a study in healthy Caucasian volunteers who were administered the same regimen. However, the indinavir Cmax value was higher in the Thai patients than that observed in the healthy volunteer study, and a number of

patients experienced efavirenz levels that have been associated with clinical toxicity in other populations. A follow-up analysis of the pharmacodynamic responses in these twenty study subjects is planned.

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Chapter 4.5

Effect of low-dose ritonavir (100 mg twice daily) on the activity of CYP2D6 in healthy volunteers

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In preparation

Abstract

Objective

The protease inhibitor (PI) ritonavir is used in a low dose (100 mg BID) to increase plasma concentrations of co-administered PIs in the treatment of HIV infection. This boosting effect is mediated by inhibition of CYP3A4. When applied in a therapeutic dose (600 mg BID), ritonavir also inhibits CYP2D6. This study was performed to assess the effect of low-dose ritonavir on the activity of CYP2D6 *in vivo*.

Methods

This was a one-arm, two-period, fixed order study in 13 healthy male volunteers who were extensive metabolizers for CYP2D6. The first period examined baseline CYP2D6 activity by evaluation of the pharmacokinetics (PK) of a single dose of desipramine, an index substrate for CYP2D6 (primary measure), and by metabolic phenotyping with dextromethorphan (secondary measure). During the second period participants took ritonavir 100 mg BID for 2 weeks, followed by repeat assessment of the PK of desipramine and the dextromethorphan metabolic phenotype in the presence of low-dose ritonavir. Geometric mean (GM) ratios plus 90% confidence intervals (CIs) were calculated for desipramine PK parameters in the second period relative to the first period. Dextromethorphan/dextrorphan urinary metabolic ratios were log-transformed and compared with the paired t-test at the 5% significance level.

Results

The GM ratio and 90% CI for the $AUC_{0-\infty}$ and the Cmax of desipramine were 1.26 [1.16-1.37] and 1.08 [1.00-1.17], respectively. These findings are indicative for a modest effect of low-dose ritonavir on the bio-availability of desipramine. Low-dose ritonavir did not affect the dextromethorphan/dextrorphan urinary metabolic ratio; GM and range were 0.0043 (0.0008-0.1226) in the first period versus 0.0066 (0.0005-0.0676) in the second period ($p=0.28$). Co-administration of low-dose ritonavir did not convert any extensive metabolizer to a poor metabolizer.

Conclusions

Low-dose ritonavir (100 mg BID) exerts a modest inhibitory effect on the activity of CYP2D6 in extensive metabolizers, as assessed with desipramine as index substrate. This effect was not apparent when the dextromethorphan/dextrorphan metabolic ratio was used as indicator for CYP2D6 activity. It is expected that the effect of low-dose ritonavir on CYP2D6 alone will not require standard dose reductions for CYP2D6 substrates, but the influence of ritonavir on other metabolic pathways should be evaluated for drugs that are partially metabolized by CYP2D6.

Introduction

The treatment of HIV infection and AIDS is complicated by the occurrence of many clinically relevant pharmacokinetic drug interactions [1]. This is incited by the large number of drugs that is taken by HIV-infected patients. Apart from at least three antiretrovirals, patients may also be taking drugs for opportunistic infections, other concurrent diseases, and for management of adverse reactions to antiretroviral drugs. Antiretroviral protease inhibitors (PIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs) are especially prone to be involved in pharmacokinetic drug interactions, since these drugs are both substrates as well as inhibitors or inducers of cytochrome P-450 (CYP) isoenzymes [1,2].

The PI ritonavir is a potent inhibitor of CYP3A4 and (to a lesser extent) of CYP2D6 when used in a therapeutic dose of 600 mg twice-daily (BID) [3,4]. The inhibition of CYP3A4 by this agent is now widely used as a means to raise plasma concentrations of other PIs [5]. This "boosting" strategy can reduce the pill burden and the dose frequency of the co-administered PIs, thereby facilitating adherence, and may result in enhanced efficacy against viral strains with reduced susceptibility to PIs. Application of ritonavir as a boosting agent requires only a low dose of this drug, usually 100 mg BID. The PIs amprenavir, indinavir, nelfinavir and saquinavir have all been combined with this low dose of ritonavir, and lopinavir is co-formulated with ritonavir to facilitate simultaneous administration of lopinavir 400 mg plus ritonavir 100 mg.

Whereas it is evident that administration of ritonavir in a low dose still results in strong inhibition of CYP3A4, it has not been investigated whether the same applies to inhibition of CYP2D6. Consequently, it is unknown whether undesirable interactions may occur between low-dose ritonavir and CYP2D6 substrates, including many antipsychotic agents, tricyclic antidepressants, newer antidepressants such as the selective serotonine reuptake inhibitors (SSRIs), beta-blockers and several antiarrhytmics [6].

The aim of this study was to assess the effect of low-dose ritonavir (100 mg BID) on the activity of CYP2D6 *in vivo*. The pharmacokinetic interaction between low-dose ritonavir and desipramine was evaluated as a primary measure for the effect of ritonavir on

CYP2D6. Desipramine is a tricyclic antidepressant that is nearly completely dependent on CYP2D6 for its metabolism [7]. As a secondary measure, the effect of ritonavir on the dextromethorphan/dextrorphan urinary metabolic ratio was assessed, using dextromethorphan as probe for CYP2D6 activity (metabolic phenotyping [8]).

Methods

Subjects

Healthy male volunteers, aged 18-65 years, were eligible for participation. Health status was assessed on the basis of medical history, physical examination, laboratory test results and ECG. CYP2D6 is known to exhibit genetic polymorphism, which divides the population in extensive and poor metabolizers. The study was confined to extensive metabolizers, as determined by genotyping (Xendo Laboratories, Groningen, The Netherlands).
Subjects were excluded if they received any drugs in the two months preceding the study, if they were HIV or hepatitis B or C seropositive, or if they were hypersensitive to ritonavir, desipramine or dextromethorphan.
All participants provided written informed consent to participate in the study, which was approved by the Review Board of the Regional Medical Ethics Committee, Arnhem and Nijmegen, The Netherlands.

Study design

This was an open-label, one-arm, two-period, fixed-order study (figure 1). The first period examined the baseline dextromethorphan/dextrorphan urinary metabolic ratio (days 1-2) and the baseline single-dose pharmacokinetics of desipramine (days 3-6). During the whole second period of the study (days 8-27), participants took ritonavir 100 mg BID with food. After two weeks in the second period, the dextromethorphan/dextrorphan metabolic ratio (days 22-23) and the pharmacokinetics of single-dose desipramine (days 24-27) were evaluated again.
The two-week period for use of low-dose of ritonavir (days 8-22) was applied to achieve steady-state concentrations for ritonavir [4]. Low-dose ritonavir was administered with food as a further means to simulate clinical practice, since it is

First period		***Second period***		
Days 1-2 (overnight 1-2)	*Days 3-6*	*Days 8-22*	*Days 22-23 (overnight 22-23)*	*Days 24-27*
No drugs	No drugs	Ritonavir 100 mg BID	Ritonavir 100 mg BID	Ritonavir 100 mg BID
Metabolic phenotyping	Single-dose PK desipramine		Metabolic phenotyping	Single-dose PK desipramine
				Steady-state PK ritonavir (day 24)

Figure 1. Schematic illustration of the study design. PK; pharmacokinetics

recommended that several combinations of PIs and low-dose ritonavir are taken with food. The pharmacokinetics of ritonavir were assessed simultaneously with the pharmacokinetics of desipramine on day 24.

The study was conducted on an outpatient basis. On days 10, 13, 16, 20, 24, 25 and 26, administration of ritonavir was supervised. Compliance with study medication at home was evaluated at every study visit by inspection of drug taking diaries, counting of capsules, plasma concentration measurements and electronic monitoring of drug taking behaviour, using the Medication Event Monitoring System (MEMS) [9]. Safety of the participants was monitored by repeated questioning on the basis of a standardized inquiry form. Laboratory tests were performed on the same occasions.

Dextromethorphan metabolic phenotyping

In the evening of study days 1 and 22 (figure 1), participants were requested to empty their bladder and take 30 mg of dextromethorphan hydrobromide monohydrate (2 capsules of Dampo® 15 mg) with two cups of water. All urine produced in the subsequent 8-hour (overnight) period was collected in a single container. A 5 ml aliquot of urine was stored at -20^{0}C before analysis.

Pharmacokinetics of desipramine

On study days 3 and 24, participants attended our facility in the morning after an overnight fast (figure 1). A predosing blood sample was taken before they ingested a single dose of 50 mg desipramine hydrochloride (two tablets of Pertofran® 25 mg) with a standardized breakfast. On day 24, participants also ingested ritonavir 100 mg (one capsule of Norvir® 100 mg) and they continued to use ritonavir 100 mg BID until the desipramine pharmacokinetic assessment was completed. Blood sampling was performed at 0.5, 1, 2, 3, 4, 6, 7, 8, 12, 24, 36, 48 and 72 hours after drug administration. Plasma was separated within 12 hours and stored at -20^{0}C until HPLC determination.

Analytical methods

The concentrations of dextromethorphan and its CYP2D6-mediated metabolite dextrorphan were analyzed in the urine samples at Xendo Laboratories, The Netherlands, using a previously described validated HPLC method [10].
Desipramine plasma concentrations were analyzed with another validated HPLC method. Briefly, 100 µl of imipramine 0.1 mg/L (internal standard) and 100 µl natrium hydroxide 2N were added to 1.0 ml plasma before extraction with 5.0 ml of a 98.5:1.5 (vol/vol) mixture of heptane/iso-amylalcohol. The organic phase was evaporated to dryness by a gentle stream of nitrogen and the residue was dissolved in 75 µl of mobile phase. Volumes of 45 µl were injected into the chromatographic system. Separation was performed on a heated (25^{0}C) Chromspher Si 3µ, 4.6 x 100 mm column, with a mobile phase that consisted of dichloromethane/methanol/buffer (800:200:4.5 vol/vol). The flow rate was 2.0 ml/min. Ultraviolet detection was performed at 253 nm. Standard curves were composed of 6 concentrations, from desipramine 2.0 ng/ml to 20 ng/ml. The lower limit of quantitation was 2.0 ng/ml. Recovery of the method was > 90%. Inter-day precision was 6.2% at 2.0 ng/mL and 6.1% at 20 ng/mL.
Ritonavir concentrations were measured in the same plasma samples that were used to assess desipramine pharmacokinetics on study days 24-27, using another validated HPLC method that was described before [11].

Data analysis

Metabolic phenotyping

The urinary metabolic ratio of dextromethorphan to dextrorphan (dextromethorphan (mol/L) / dextrorphan (mol/L)) was calculated after each phenotyping session. Participants with a metabolic ratio > 0.3 were classified as poor metabolizers of CYP2D6 and subjects with a metabolic ratio ≤ 0.3 were classified as extensive metabolizers [8].

Pharmacokinetics of desipramine and ritonavir

The pharmacokinetics of desipramine and ritonavir were calculated using noncompartmental methods. The terminal, log-linear period (log C versus t) was defined by visual inspection of the last data points (n ≥ 3). The value of the slope (- ß/2.303, where ß is the first-order elimination rate constant) was calculated by least squares linear regression analysis. For desipramine, the area under the concentration versus time curve from zero to infinity ($AUC_{0-\infty}$) was calculated using the trapezoidal rule up to the last measurable concentration (Clast) with extrapolation to infinity using Clast/ß. The AUC0-12h for ritonavir was calculated by application of the trapezoidal rule from 0 to 12h. Cmax was the highest observed plasma concentration and tmax was the corresponding sampling time. Cmin for ritonavir was the concentration at 12h after ingestion of the drug. The elimination half life (t1/2) was calculated as 0.693/ß. Apparent clearance (CL/F, where F is bioavailability) was calculated by dividing dose (D) by $AUC_{0-\infty}$ (desipramine) or AUC0-12h (ritonavir), and apparent volume of distribution (Vd/F) was obtained by dividing CL/F by ß.

Statistical analysis

The study was powered based on the anticipated influence of low-dose ritonavir on the single-dose pharmacokinetics of desipramine, and this was the primary measure for the effect of ritonavir on the activity of CYP2D6. The influence of ritonavir on the dextromethorphan/dextrorphan urinary metabolic ratio was regarded as secondary measure for the effect of ritonavir on CYP2D6.

The effect of low-dose ritonavir on the bio-availability of desipramine was evaluated using the confidence interval approach for bioequivalence studies that is also recommended for drug interaction studies [12,13]. Geometric means were calculated for each of the pharmacokinetic parameters of desipramine when administered alone (first study period) or with ritonavir (second study period). Ratios of geometric means (with/without ritonavir) and accompanying 90% confidence intervals were calculated for each of the pharmacokinetic parameters. Equivalence between the administration of desipramine with and without ritonavir (i.e. lack of an effect of ritonavir on the pharmacokinetics of desipramine, no pharmacokinetic interaction) was concluded if the 90% confidence intervals for the geometric mean ratios for $AUC_{0-\infty}$ and Cmax were entirely contained within 80-125% limits. The results indicated *in*equivalence, i.e. the occurrence of a pharmacokinetic interaction, if this condition was not fulfilled. Tmax values of desipramine were not log-transformed and were compared using Wilcoxon signed-ranks test.

Dextromethorphan/dextrorphan urinary metabolic ratios as assessed without and with concurrent use of ritonavir were log-transformed (because these data were not normally distributed) and were compared using the paired t-test at the 5% significance level. This is equivalent to calculation of the geometric mean ratio (with/without ritonavir) plus 95% CI for the dextromethorphan/dextrorphan urinary metabolic ratio.

All statistical evaluations were performed with SPSS for Windows, version 10.0 (SPSS Inc., Chicago, IL, USA).

Results

Thirteen healthy male participants, all extensive metabolizers for CYP2D6 as determined by genotyping, were included. All subjects were Caucasians. The median age of the participants was 26 years (range 20-56 years) and median weight was 84 kg (range 69-93 kg). All participants completed the study.

Table 1 shows the steady-state pharmacokinetic parameters for low-dose ritonavir. The single-dose pharmacokinetic parameters of desipramine when administered alone

Table 1. Steady state pharmacokinetic parameters of ritonavir 100 mg BID (n=13)

Parameter[a]	Geometric mean [95% CI][b]	Range
AUC0-12h (h.mg/L)	6.2 [4.8-7.9]	3.2-10.8
C_{max} (mg/L)	0.89 [0.67-1.18]	0.42-1.8
C_{min} (mg/L)	0.22 [0.16-0.32]	0.08-0.49
tmax (h)	4.0[c]	0.9-7.0
$t_{1/2}$ (h)	4.7 [3.6-6.1]	2.6-12.9
CL/F (L/h)	16.1 [12.6-20.6]	9.3-31.4
Vd/F (L)	108.6 [70.8-166.6]	40.9-504.6

[a] AUC0-12h: area under the concentration-time curve from zero to 12h, Cmax : highest observed plasma concentration, Cmin: trough plasma concentration at t=12h, tmax : sampling time for Cmax, t1/2: elimination half life, CL/F: total clearance, Vd/F: apparent volume of distribution, F: bio-availability.
[b] CI: confidence interval
[c] median

and under the steady-state conditions for low-dose ritonavir are shown in table 2, and figure 2 shows the corresponding concentration-time profiles for desipramine. Marked inter-individual variability in desipramine pharmacokinetic parameters was observed, both with and without ritonavir. Upon co-administration of ritonavir, 12/13 participants had an increase in the $AUC_{0-\infty}$ of desipramine, varying from +4% to +71% (figure 3), resulting in a geometric mean ratio (with ritonavir / without ritonavir) plus 90% CI of 1.26 [1.16-1.37] for $AUC_{0-\infty}$. Based on these data, equivalence could not be concluded for administration of desipramine without and with low-dose ritonavir. Cmax values for desipramine were equivalent (table 2).

The baseline CYP2D6 phenotyping results obtained on days 1-2 were consistent with the results for CYP2D6 genotyping, since all participants had a dextromethorphan/ dextrorphan metabolic ratio below 0.3, which classifies them as extensive metabolizers. The geometric mean metabolic ratio was 0.0043 (range 0.0008-0.1226) at baseline (days 1-2) and 0.0066 (range 0.0005-0.0676) after administration of ritonavir (days 22-23). The paired t-test on the log-transformed

Table 2. Pharmacokinetic parameters of a single oral dose of desipramine 50 mg given alone and concomitantly with ritonavir 100 mg twice-daily (n=13)

Parameter[a]	Geometric mean (range)		Geometric mean ratio (period 2 / period 1) and 90% CI[d]	P-value
	Study period 1[b]	Study period 2[c]		
$AUC_{0-\infty}$ (h.ng/ml)	393 (89-1683)	495 (100-1745)	1.26 [1.16-1.37]	<0.001
C_{max} (ng/ml)	14.5 (4.2-25.2)	15.7 (4.0-28.6)	1.08 [1.00-1.17]	0.11
t_{max} (h)	6.0 (2.1-8.1)[e]	6.0 (2.0-12.0)[e]	-	0.42[f]
$t_{1/2}$ (h)	17.1 (10.3-44.2)	22.3 (14.5-43.2)	1.31 [1.17-1.45]	0.001
CL/F (L/h)	127 (30-564)	101 (29-501)	0.80 [0.73-0.87]	<0.001
Vd/F (L)	3137 (1654-8646)	3253 (1785-10495)	1.04 [0.93-1.16]	0.57

[a] $AUC_{0-\infty}$: area under the concentration-time curve from zero to infinity, Cmax : highest observed plasma concentration, tmax : sampling time for Cmax, t1/2: elimination half life, CL/F: total clearance, Vd/F: apparent volume of distribution, F: bio-availability.
[b] Desipramine single dose 50 mg
[c] Desipramine single dose 50 mg in the presence of steady-state ritonavir 100 mg BID
[d] CI: confidence interval
[e] median and range
[f] Wilcoxon signed-ranks test

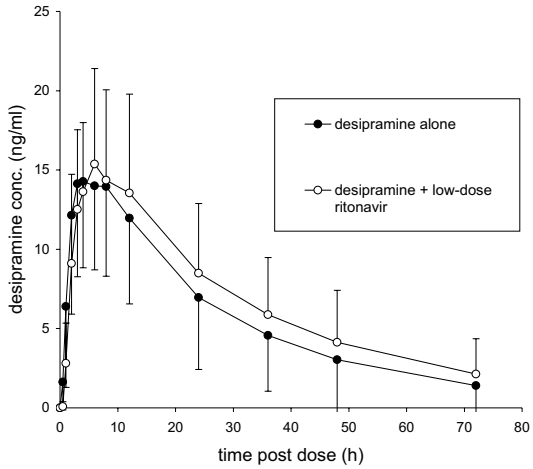

Figure 2. Plasma concentration-time curves of desipramine administered alone (50 mg) and concurrently with low-dose ritonavir (100 mg BID) in extensive metabolizers for CYP2D6 (n=13, mean and standard deviations).

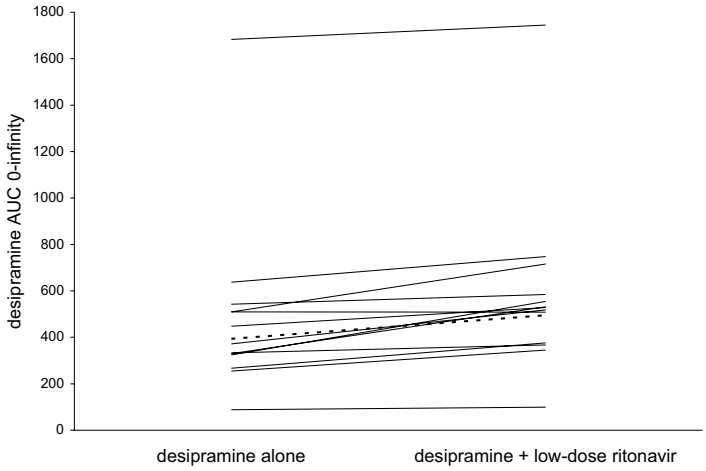

Figure 3. Individual desipramine AUC_{0}-∞ values when administered alone (first study period) and concurrently with low-dose ritonavir (100 mg BID, second study period). The dotted line joins the geometric mean desipramine AUC_{0}-∞ values in the first and second study period.

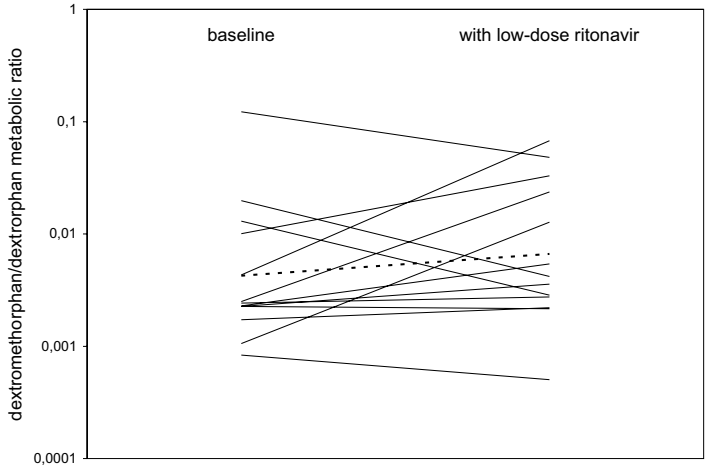

Figure 4. Individual dextromethorphan/dextrorphan urinary metabolic ratios after administration of dextromethorphan alone (first study period) and under steady-state conditions for low-dose ritonavir (100 mg BID, second study period). The dotted line joins the geometric mean metabolic ratios in the first and second study period.

dextromethorphan/dextrorphan metabolic ratios did not reveal a statistically significant difference (p=0.28), corresponding to a geometric mean ratio (metabolic ratio with/without ritonavir) plus 95% CI of 1.56 [0.66-3.71]. Examination of results for individual participants showed a variable change in metabolic ratio (figure 4). The metabolic ratio for all subjects remained at less than 0.3 after administration of low-dose ritonavir.

In the first study period, a significant linear association was found between the dextromethorphan/dextrorphan metabolic ratio and the desipramine $AUC_{0-\infty}$ (Spearman's rho=0.67, p=0.02). However, this association was not observed after co-administration of ritonavir in the second period (rho=0.36, p=0.25). In addition, there was no linear association between the change in desipramine $AUC_{0-\infty}$ ($AUC_{0-\infty}$ on day 24-27 / $AUC_{0-\infty}$ on day 3-6) and the change in dextromethorphan/dextrorphan metabolic ratio (ratio day 22-23 / ratio day 1-2) (rho=0.01, p=0.97).
The exposure to low-dose ritonavir (AUC0-12h) was not linearly associated with the change in desipramine $AUC_{0-\infty}$ or the change in dextromethorphan/dextrorphan metabolic ratio (rho=-0.16, p=0.60 and rho=-0.07, p=0.82, respectively).

Administration of desipramine and low-dose ritonavir was well-tolerated without any serious adverse reactions (WHO grade 2, 3 or 4). After administration of desipramine, mild fatigue and dry mouth were reported by three to five participants in each study period. The median fasting cholesterol increased from 4.4 mmol/L at baseline to 4.7 mmol/L at the end of the study (median increase was 0.6 mmol/L), and median triglyceride values increased from 0.9 mmol/L to 1.2 mmol/L (median increase was 0.3 mmol/L). No other laboratory abnormalities were observed.

Discussion

This study shows that co-administration of low-dose ritonavir (100 mg BID) results in a 26% increase in the geometric mean $AUC_{0-\infty}$ of a single dose of desipramine. Therefore bioequivalence could not be concluded between desipramine when administered alone and combined with low-dose ritonavir. These results suggest that low-dose ritonavir exerts an inhibitory effect on the activity of CYP2D6. However, this inhibitory effect is modest and was not apparent when the dextromethorphan/

dextrorphan metabolic ratio was used as an indicator for CYP2D6 activity in the same subjects.

The biotransformation of the antidepressant desipramine to its principal metabolite, 2-hydroxy-desipramine, is almost exclusively mediated by CYP2D6 [7]. Consequently the 2-hydroxylation of desipramine is used as an index reaction to assess CYP2D6 activity *in vitro* [14,15], and desipramine is applied as CYP2D6 index substrate in intensive pharmacokinetic studies that aim to assess the effect of other drugs on CYP2D6 activity *in vivo* [16,17]. The latter approach was used in this study and served as the primary measure to evaluate the effect of low-dose ritonavir on the activity of CYP2D6, considering that a pharmacokinetic evaluation directly shows the clinical consequences of CYP2D6 inhibition in terms of plasma concentrations that are unequivocal and easy to interpret. A previous pharmacokinetic study combined single oral doses of desipramine with a high dose of ritonavir (500 mg BID [3]). In that study, the mean AUC ratio for desipramine (AUC during ritonavir divided by AUC without ritonavir) was 2.45, corresponding to a 145% increase in the AUC of desipramine upon administration of ritonavir. Compared to these findings, the effect of low-dose ritonavir on CYP2D6, as assessed in the current study, is marginal. This is in accordance with the preliminary results of another study, that found no effect of low-dose ritonavir (100 mg BID) plus lopinavir (400 mg BID) on the activity of CYP2D6 [18].

Metabolic phenotyping with dextromethorphan was used as a secondary measure to assess the effect of low-dose ritonavir on CYP2D6 in this study. The main metabolic route for dextromethorphan is its O-demethylation to dextrorphan, a pathway that is largely mediated by CYP2D6 [8]. The transformation of dextromethorphan to dextrorphan is very rapid in extensive metabolizers for CYP2D6 (elimination half-life for dextromethorphan is 2-4 h), but not in poor metabolizers, and dextromethorphan and metabolites are readily excreted in urine. Thus the dextromethorphan/dextrorphan urinary metabolic ratio can be used to assess the CYP2D6 phenotype [8,19]. This approach is noninvasive and involves only one timed urine sample rather than intensive blood sampling. In this study, administration of low-dose ritonavir did not result in a significant change in the mean dextromethorphan/dextrorphan metabolic ratio. This finding contrasts with the clear but modest effect of low-dose ritonavir on the bio-availability of desipramine. However, it has been shown before that the

dextromethorphan/dextrorphan metabolic ratio is not a very sensitive marker for inhibition of CYP2D6 [16,20]. This may be explained by the large *intra*-individual variability in baseline dextromethorphan/dextrorphan metabolic ratios [21-24] that could mask small changes in CYP2D6 activity. Repeat baseline and treatment phenotyping assessments may be required if metabolic phenotyping for CYP2D6 is used as the primary measure to assess the effect of a drug on CYP2D6 activity [21]. As another explanation for the discrepancy between the effects of low-dose ritonavir on desipramine and dextromethorphan, it could be argued that assessment of the CYP2D6 phenotype with dextromethorphan is not sufficiently validated in the presence of a CYP3A4 inhibitor such as ritonavir, despite the fact that several studies have actually used the dextromethorphan/dextrorphan metabolic ratio to assess whether moderate to strong inhibitors of CYP3A (claritromycin [25], fluoxetine [26-28], fluvoxamine [29]) did also affect the activity of CYP2D6. Inhibition of CYP3A could be relevant to the dextromethorphan/dextrorphan metabolic ratio, considering that dextromethorphan is metabolized by CYP3A4 to a small extent and that dextrorphan is further metabolized by this iso-enzyme [30]. Although it has been demonstrated that grapefruit juice or erythromycin (inhibitors of CYP3A4) did not affect the dextromethorphan/dextrorphan metabolic ratio [30,31], it has not been shown that concurrent inhibition of CYP3A4 also permits the accurate assessment of CYP2D6 activity with dextromethorphan as probe.

The clinical consequences of the modest inhibitory effect of low-dose ritonavir on the activity of CYPD6 are dependent on the CYP2D6 substrate. This modest effect of low-dose ritonavir may be relevant for substrates that are both largely dependent on CYP2D6 for their metabolism and have a narrow therapeutic index (e.g. desipramine). However, based on the results of this study it is expected that standard, pre-emptive dose reductions are not warranted when such substrates are combined with low-dose ritonavir 100 mg BID. It seems rather sufficient to advise physicians to titrate the dose of these CYP2D6 substrates carefully, using plasma concentration measurements for the CYP2D6 substrate if a therapeutic range has been defined. In addition, these patients should be monitored for adverse reactions related to the CYP2D6 substrate. The effect of low-dose ritonavir on the activity of CYP2D6 is not relevant for CYP2D6 substrates that are only partly metabolized by CYP2D6, or for substrates that do not have a narrow therapeutic index. However, it should be recognized that such CYP2D6

substrates may still interact with low-dose ritonavir if they are metabolized by CYP3A4 to a large extent.

Some additional considerations are important for the extrapolation of the results of this study to patients. Firstly, it should be noticed that the participants in this study were all healthy and male subjects and extensive metabolizers for CYP2D6. It can not be excluded that the pharmacokinetics of ritonavir and CYP2D6 substrates are different in HIV-infected patients compared to healthy volunteers [32]. A slightly higher CYP2D6 activity in women versus men was found in some [23,33,34] but not all [21,24] studies. However, we do not expect that gender will have a large effect on the magnitude of the interaction between low-dose ritonavir and CYP2D6 substrates. The results of this study do not apply to poor metabolizers for CYP2D6, but it is worthwile to mention that the effects of CYP2D6 inhibition on the pharmacokinetics of CYP2D6 substrates are generally less pronounced in poor metabolizers [35-38].
Secondly, it should be considered that this study evaluated the effect of low-dose ritonavir on the activity of CYP2D6, irrespective of the PI that is administered with ritonavir. Other PIs do not affect CYP2D6 themselves, but they may have an effect on the exposure to low-dose ritonavir. Available data suggest that exposure to low-dose ritonavir is lower in the combination with lopinavir or amprenavir [39], and that indinavir slightly increases the exposure to low-dose ritonavir [4]. However, any such between-PI differences in the exposure to low-dose ritonavir will be small compared to the ritonavir exposure that strongly inhibits CYP2D6 (i.e. exposure to ritonavir 600 mg BID; AUC0-12h is 60.8 h*mg/L [40]). The relevance of these small differences will probably be limited, considering that the current study only found a modest effect of low-dose ritonavir on CYP2D6 activity and no association between the AUC0-12h of low-dose ritonavir and the change in desipramine pharmacokinetics. Administration of low-dose ritonavir with or without food may also have a slight effect on the exposure to low-dose ritonavir, and this warrants further study.
Finally, this study evaluated the pharmacokinetics of a single dose of desipramine administered under steady-state conditions for low-dose ritonavir. It would be valuable to substantiate the results of this study by evaluating steady-state concentrations of CYP2D6 substrates in HIV-infected patients who use a low-dose of ritonavir combined with regular doses of other antiretroviral drugs.

In conclusion, this study shows that low-dose ritonavir exerts a significant but modest inhibitory effect on the activity of CYP2D6 in extensive metabolizers. Based on these data, it is expected that no standard dose reductions are required if CYP2D6 substrates that are largely metabolized by CYP2D6 and have a narrow therapeutic index are combined with low-dose ritonavir. Patients who take such a combination should be closely monitored for adverse reactions to the CYP2D6 substrate. The effect of low-dose ritonavir on CYP2D6 appears to be clinically irrelevant for CYP2D6 substrates that are only partly metabolized by this iso-enzyme or that have a wide therapeutic index.

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Chapter 5.

Development of once-daily dosing regimens for protease inhibitors

Chapter 5.1

Pharmacokinetics, food intake requirements and tolerability of once-daily combinations of nelfinavir and low-dose ritonavir in healthy volunteers

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Abstract

Aims

This study was performed to evaluate the steady-state pharmacokinetics, food intake requirements and short term tolerability of once daily combinations of nelfinavir and low-dose ritonavir.

Methods

Twenty-seven healthy volunteers were randomized over three groups to receive a once-daily regimen of nelfinavir/ritonavir 2000/200 mg (group 1), 2000/400 mg (group 2) or 2500/200 mg (group 3) with food for 14 days. Pharmacokinetic parameters for nelfinavir and its active metabolite M8 were assessed on study days 15 and 16, after administration of the regimens with a full (610 kcal) or light (271 kcal) breakfast, respectively.

Results

Pharmacokinetic data were evaluable for eight volunteers in group 1, eight in group 2 and four in group 3. Administration of nelfinavir/ritonavir with a full breakfast resulted in geometric mean (GM) nelfinavir AUC24h values of 76.8, 51.3, and 61.9 h*mg/L in group 1, 2 and 3, respectively. GM 24-h Cmin concentrations of nelfinavir were 0.76 mg/L, 0.43 mg/L and 0.47 mg/L, respectively. Co-administration of ritonavir increased M8 concentrations more than nelfinavir concentrations, resulting in GM AUC24h and Cmin values for nelfinavir plus M8 that were higher than or comparable to reference values for the approved regimen of nelfinavir (1250 mg BID without ritonavir). In the 2000/200 mg group, seven out of eight subjects had a Cmin value of nelfinavir plus M8 above a threshold of 1.0 mg/L. Administration of the combinations with a light breakfast resulted in significant decreases in the AUC24h and Cmin of nelfinavir and nelfinavir plus M8, compared with intake with a full breakfast. For the Cmin of nelfinavir plus M8, the GM ratio (light/full breakfast) was 0.76 (90% confidence interval: 0.67-0.86, participants from all groups combined). Short-term tolerability was satisfactory, apart from a higher than expected incidence of mild rash (12%).

Conclusions

Administration of nelfinavir in a once daily regimen appears feasible. A nelfinavir/ritonavir 2000/200 mg combination appears appropriate for further evaluation. Once daily nelfinavir/ritonavir should be taken with a meal containing at least 600 kcal.

Introduction

The availability of highly active antiretroviral therapy (HAART) has dramatically decreased mortality and morbidity in HIV infection [1]. However, up to 50% of treatment-naive patients do not have sustained antiviral response after one year of therapy [2,3]. To a considerable extent this can be ascribed to difficulties in achieving adequate adherence to the complex HAART regimens [4,5]. Simpler dosing regimens are associated with better adherence [6-8], and there is a move to decrease the frequency of HAART dosing to a once-daily regimen.

Pharmacokinetic interactions between protease inhibitors (PIs) can be exploited as a means of decreasing the dosing frequency of these antiretroviral drugs. More specifically, the exposure to PIs can be raised, and their half-lives can be prolonged, by coadministration of low-dose ritonavir [9]. The latter impairs the metabolism of other PIs by potent inhibition of cytochrome (CYP) 3A4. Data from recent studies suggest that ritonavir can adequately boost concentrations of amprenavir, indinavir and saquinavir, to allow for once-daily dosing of these PIs [10-15].

Nelfinavir is another PI that is widely used for treatment of HIV infection. It is approved for twice-daily dosing (1250 mg BID), and should be taken with food [16]. In vitro studies revealed that nelfinavir is metabolized by at least five different pathways, catalysed by several CYP isoenzymes (CYP3A4, CYP2C19, CYP2D6 and CYP2C9 [17]). CYP3A4 and CYP2C19 are the predominant contributors to nelfinavir metabolism. CYP2C19 catalyses exclusively the conversion of nelfinavir to an active metabolite termed M8, which in turn is metabolized by CYP3A4 [18]. Plasma levels of M8 are about 30% of those of nelfinavir after BID dosing of the latter [19]. M8 has equipotent activity to nelfinavir *in vitro*, binds to plasma protein *in vivo* to a similar extent to nelfinavir (≥ 98%), and has an almost identical molar weight [20]. Assuming additive virological efficacy, this suggests that the sum nelfinavir and M8 plasma concentrations may represent all active drug after administration of the parent drug. Nelfinavir appears to be an appropriate PI for once-daily administration, because of its pharmacokinetic properties and its good tolerability. Nelfinavir shows slow oral absorption and an elimination half-life that is relatively long (3.5-5 h) compared with most other PIs [16]. In addition, previous pharmacokinetic studies have demonstrated

that exposure to nelfinavir can be increased by coadministration of low-dose ritonavir (100-400 mg BID) [21,22]. In these studies, ritonavir also increased M8 concentrations, as well as the M8:nelfinavir ratio, enhancing the relative contribution of M8 to the antiviral efficacy of nelfinavir. Thus, it appeared feasible that therapeutic plasma concentrations of nelfinavir plus M8 could be maintained over 24 h after once-daily administration of nelfinavir and ritonavir.
With respect to the tolerability of such a once-daily regimen, it appeared advantageous that no clear relationship has been demonstrated between the adverse effects of nelfinavir and its plasma concentration, particularly Cmax [23,24].
Therefore this study was performed to characterize the steady-state pharmacokinetics and short-term tolerability of possible once-daily nelfinavir/ritonavir combinations. We also evaluated whether ingestion with a light meal would permit adequate absorption of these combinations.

Methods

Subjects

Male or female healthy volunteers, aged 18 to 65 years, were eligible for enrolment. Subjects were excluded if they were hepatitis B- or C-seropositive, pregnant, hypersensitive to PIs or loperamide, if they had positive serology for HIV infection or prespecified abnormal laboratory parameters, and if they were taking any medication or illicit drugs.
All subjects gave written informed consent after full explanation of the study details. The study was approved by the Institutional Review Board of University Medical Centre Nijmegen, The Netherlands.

Study design and procedures

This study had an open-label, randomized, multiple-dose, parallel-group design. Twenty-seven volunteers were randomized (stratified by gender) over three dosage groups. Participants took once-daily doses of either 2000 mg nelfinavir plus 200 mg ritonavir (group 1), 2000 mg nelfinavir plus 400 mg ritonavir (group 2), or 2500 mg nelfinavir plus 200 mg ritonavir (group 3). Nelfinavir (Viracept®) was administered as

film-coated tablets, each containing 250 mg. Ritonavir (Norvir®) was given as capsules containing 100 mg.
Nelfinavir and ritonavir in all three combinations were ingested concomitantly with food (at least two slices of bread) at 24-h intervals and for 14 days.
Participants in the 2000/400 mg group (group 2) started with a 4-day lead-in period of 300 mg (instead of 400 mg) ritonavir combined with nelfinavir in order to attenuate possible ritonavir-associated adverse events in the initial period of the study [9].

Blood samples for two consecutive 24-h pharmacokinetic profiles were collected on study days 15 and 16. Participants attended in the morning after an overnight fast and a predose blood sample was drawn. On day 15 they ingested nelfinavir and ritonavir with a standardized, full breakfast, which consisted of 130 ml water and four slices of bread, filled with butter plus cheese, ham, paste or jam (610 kcal: 33% fat, 16% proteins and 51% carbohydrates). Blood samples were drawn at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 10.0, 12.0, 16.0 and 24.0 h post dose. Plasma was isolated by centrifugation within 12 h of sampling and was stored at -20^{0}C until analysis.
After an overnight fast, the same procedure was repeated on day 16. However, the drugs were ingested with a light instead of a full breakfast. This comprised one slice of bread with butter and cheese and 130 ml of semiskimmed milk (271 kcal: 37% fat, 24% proteins, 39% carbohydrates).

The study was conducted on an outpatient basis. Subjects received their study medication in a vial that contained sealed plastic sachets, each holding the appropriate number of tablets and capsules for one day. Drug administration was witnessed on days 1, 4, 11, 15 and 16. Compliance with study medication at home was verified at every study visit by inspection of drug-taking diaries, counting of sachets, measurement of plasma drug concentrations, and electronic monitoring of opening of vials, using the Medication Event Monitoring System (MEMS®) [25].

Drug analysis

Nelfinavir and ritonavir concentrations were assayed using a previously described validated reversed-phase HPLC method with u.v. detection [26]. M8 concentrations

were determined simultaneously using the same method without modifications. The retention time of M8 was 12.7 min. The accuracy of the method for nelfinavir ranged from 96% to 100%, depending on the concentration. Those for ritonavir and M8 were 102-108% and 93-108%, respectively. Intra-day precision and between-day precision were 2.1-7.5% and 0.4-3.5% for nelfinavir, 2.0-8.1% and 0-2.4% for ritonavir, and 2.8-4.3% and 2.0-3.0% for M8. The limit of determination was 0.04 mg/L for nelfinavir, ritonavir and M8.

Pharmacokinetic analysis

Pharmacokinetic parameters for nelfinavir, ritonavir and M8 were obtained by noncompartmental methods [27]. The highest observed plasma concentration was defined as Cmax, with the corresponding sampling time as tmax. Cmin was the concentration at 24 h after ingestion of the drugs. The terminal, log-linear period (log C versus t) was defined by visual inspection of the last data points ($n \geq 3$). The value of the slope (- ß/2.303) was calculated by least-squares linear regression analysis, where ß is the first-order elimination rate constant. The elimination half life (t1/2) was calculated from the expression 0.693/ß.
The area under the concentration vs time curve (AUC24h) was calculated using the trapezoidal rule from 0 to 24 h. The time of ingestion of nelfinavir/ritonavir on day 14 (the day preceding pharmacokinetic assessments) varied among participants, resulting in different contributions of this dose to the AUC24h on day 15. Therefore AUC24h values for study day 15 were corrected for the contribution of the previous dose by subtraction of C0/ß (where C0 is the concentration just before ingestion of nelfinavir/ritonavir at t=0), and area under the curve was extrapolated to infinity by adding Cmin/ß. Accordingly, the corrected AUC24h value ($AUC_{24h,corr}$) for day 15 was obtained from the equation:
$AUC_{24h,corr} = AUC24h - C0/ßday15 + Cmin/ßday15$.
The AUC24h for day 16 was corrected in the same way, but the contribution of the predose AUC was calculated using the elimination-rate constant ß determined on day 15: $AUC_{24h,corr} = AUC24h - C0/ßday15 + Cmin/ßday16$.
Apparent clearance (CL/F, where F is bioavailability) was calculated by dividing dose (D) by $AUC_{24h,corr}$, and apparent volume of distribution (Vd/F) was obtained by dividing CL/F by ß. Both were corrected for weight.

Safety and tolerability

Safety and tolerability were assessed by a questionnaire that described 15 possible adverse events that could occur during treatment with nelfinavir or ritonavir. The questionnaire was completed four times each, on study days 4, 8, 11 and 15. Participants were asked to grade every event as mild (symptoms do not interfere with daily activities), moderate (symptoms interfere with daily activities) or severe (symptoms markedly interrupt daily activities). An extensive blood chemistry and haematology screen and urinalysis were performed on the same four study days. If WHO grade 2 diarrhoea occurred, use of loperamide was allowed.

Data-analysis

The study was not powered to enable formal statistical comparisons of pharmacokinetic parameters between the study groups. Therefore pharmacokinetic parameters are presented descriptively for every study group.

To assess the effect of the composition of the concurrent meal on the pharmacokinetics of nelfinavir, M8, nelfinavir plus M8, and ritonavir, a two-way mixed analysis of variance (ANOVA) was performed on the logarhitmically transformed values of $AUC_{24h,corr}$, Cmax and Cmin, with the study group as between-subjects factor and the composition of the breakfast as within-subjects factor. Absence of an effect of the meal composition on $AUC_{24h,corr}$, Cmax or Cmin was concluded if the 90% confidence interval (CI) for a geometric mean ratio (light breakfast / full breakfast) was contained within 0.8 and 1.25 [28]. Tmax values were not log-transformed and were compared using the Wilcoxon signed-ranks test.

The incidence of adverse events was expressed as the percentage of participants who reported a particular event at least once during the four reporting moments. Consequently every reported mild, moderate or severe adverse event was ascribed a severity score of 1, 2 or 3 points respectively. Scores were added up for each participant and were divided by the number of reporting moments. In this way mean toxicity scores over the study period were obtained for all individual participants. All statistical evaluations were performed with SPSS for Windows, version 10.0 (SPSS Inc., Chicago, IL, USA).

Results

Subjects

Twenty-seven volunteers were enrolled into the study. Five male and four female subjects were included in each group. Median ages were 28, 23 and 25 years in groups 1, 2, and 3, respectively, and median weights were 71, 73 and 71 kg. All subjects were Caucasians.
Twenty-one volunteers completed the study and data for 20 volunteers were evaluated (eight volunteers in group 1, eight in group 2 and four in group 3).
One subject in group 1 and one in group 2 and three in group 3 were withdrawn because of toxicity concerns. An additional volunteer in group 3 withdrew his informed consent. One subject in group 3 did not convert nelfinavir to M8, probably because of CYP2C19 poor metabolizer genotype status, which occurs in 3-5% of Caucasian subjects [18]. Because of this, pharmacokinetic data for this volunteer were not analysed, which reduced the number of evaluable participants in group 3 to four.

Pharmacokinetics of nelfinavir and M8

Table 1 summarizes the pharmacokinetic parameters of nelfinavir, M8 and nelfinavir plus M8 after once daily administration of the combinations for 14 days. Figures 1 and 2 display the corresponding plasma concentration-time curves for nelfinavir and M8, showing that coadministration of nelfinavir and ritonavir in once-daily combinations resulted in detectable and appreciable concentrations of nelfinavir and M8 throughout the whole 24-h dosing interval. Concentration-time curves for nelfinavir and M8 after administration of nelfinavir alone are shown for reference.
M8 concentrations after once-daily administration of nelfinavir and ritonavir (table 1, figure 2) were relatively high compared with those of nelfinavir. Coadministration of nelfinavir and ritonavir appeared to raise M8 concentrations to a greater extent than nelfinavir concentrations, resulting in high M8:nelfinavir ratios for $AUC_{24h,corr}$ and especially for Cmin (table 1).

The geometric mean values for the $AUC_{24h,corr}$ and especially Cmin of nelfinavir were highest in group 1 (nelfinavir/ritonavir 2000/200 mg once daily). In contrast, the

Table 1. Pharmacokinetics of nelfinavir, M8, and nelfinavir plus M8 after once-daily administration of nelfinavir/ritonavir combinations for 14 days [a]

Parameter [b]	Values (geometric mean + range)		
	group 1: nelfinavir/ritonavir 2000/200mg (n=8)	Group 2: nelfinavir/ritonavir 2000/400mg (n=8)	group 3: nelfinavir/ritonavir 2500/200mg (n=4)
Nelfinavir			
$AUC_{24h,\ corr}$ (h.mg/L)	76.8 (32.4-121.3)	51.3 (23.5-114.8)	61.9 (48.1-89.3)
C_{max} (mg/L)	7.2 (3.4-9.3)	5.1 (2.9-9.4)	6.7 (6.4-7.6)
C_{min} (mg/L)	0.76 (0.28-2.1)	0.43 (0.16-1.8)	0.47 (0.18-1.8)
t_{max} (h) [c]	4.0 (2.5-5.0)	4.6 (2.6-6.0)	4.5 (3.0-5.0)
CL/F.kg (L/h.kg)	0.35 (0.16-0.84)	0.51 (0.24-0.95)	0.52 (0.27-0.74)
Vd/F.kg (L/kg)	3.2 (1.8-5.4)	4.5 (2.9-9.2)	4.3 (3.6-5.0)
$t_{1/2}$ (h)	6.5 (4.4-11.3)	6.1 (4.3-9.2)	5.8 (4.0-9.1)
M8 [d]			
$AUC_{24h,corr}$ (h.mg/L)	38.5 (21.5-57.7)	40.9 (30.1-58.8)	45.3 (30.0-63.4)
C_{max} (mg/L)	3.4 (2.3-5.6)	3.7 (2.8-4.9)	4.3 (2.9-5.9)
C_{min} (mg/L)	0.63 (0.46-1.1)	0.59 (0.32-1.1)	0.67 (0.35-1.7)
t_{max} (h) [c]	5.0 (4.0-5.0)	5.0 (4.0-6.1)	4.5 (4.0-5.0)
$t_{1/2}$ (h)	9.7 (7.5-13.6)	8.6 (6.0-11.8)	8.9 (7.4-13.3)
Nelfinavir + M8			
$AUC_{24h,corr}$ (h.mg/L)	116.4 (53.9-172.6)	94.6 (61.6-173.7)	108.3 (94.6-152.8)
C_{min} (mg/L)	1.4 (0.81-3.2)	1.1 (0.61-2.9)	1.2 (0.77-3.5)
M8-to-nelfinavir ratio			
Ratio for $AUC_{24h,\ corr}$	0.50 (0.32-0.75)	0.80 (0.42-1.6)	0.73 (0.46-0.98)
Ratio for C_{min}	0.83 (0.43-1.9)	1.36 (0.50-4.3)	1.43 (0.61-3.3)

[a] *Pharmacokinetic parameters were assessed after intake of drugs with a full breakfast: 610 kcal, 33% fat, 16% proteins, 51% carbohydrates and 130 ml water (study day 15).*
[b] *$AUC_{24h,corr}$, corrected 24 h-area under the concentration-time curve (see text); Cmin, trough concentration at 24 h; Cmax, highest observed plasma concentration; tmax, sampling time for Cmax; CL/F.kg, total clearance corrected for weight; Vd/F.kg, volume of distribution corrected for weight; t1/2, elimination half life; F, bio-availability.*
[c] *Median (and range).*
[d] *CL/F.kg and Vd/F.kg can not be calculated for M8, as dose is unknown.*

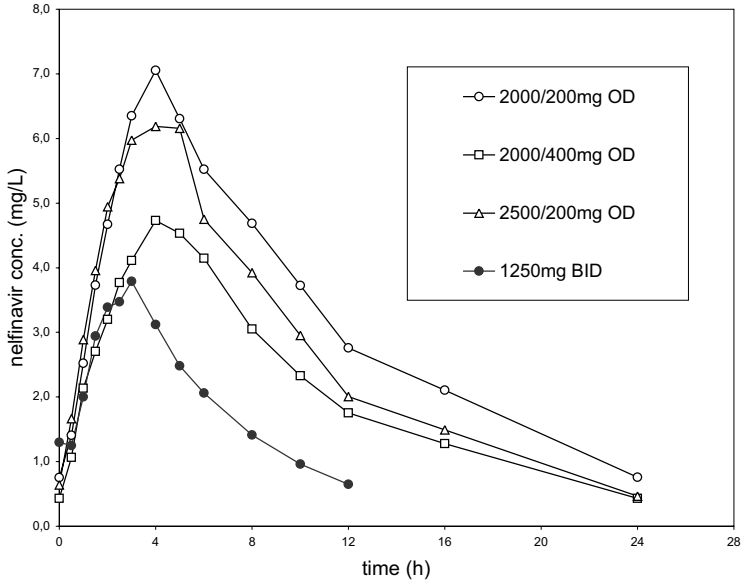

Figure 1. Nelfinavir steady state plasma concentrations on day 15 (geometric mean values) after administration of once-daily (OD) nelfinavir/ritonavir combinations[a]
[a] In-house reference data for nelfinavir 1250 mg BID are displayed for reference (see also table 5)

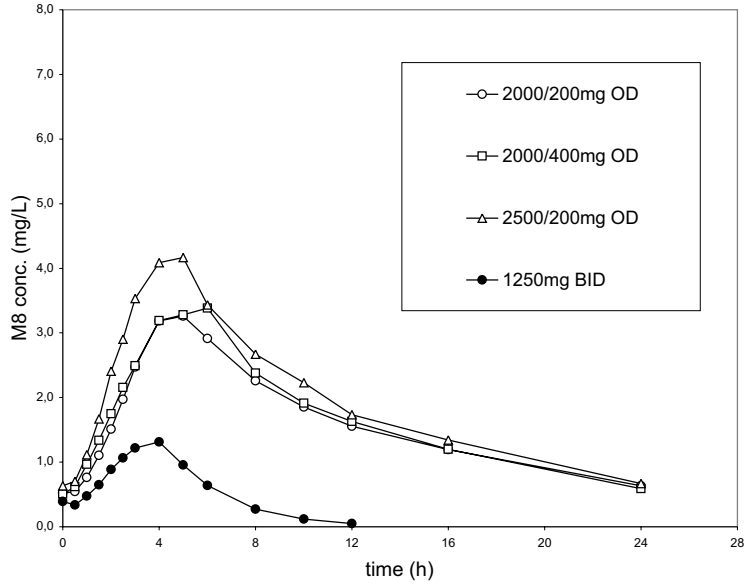

Figure 2. M8 steady-state plasma concentrations on day 15 (geometric mean values) after administration of once-daily (OD) nelfinavir/ritonavir combinations.[a]
[a] In-house reference data for M8 (after administration of nelfinavir 1250 mg) are displayed for reference (see also table 5)

geometric mean $AUC_{24h,corr}$ and Cmin values for M8 appeared remarkably similar across the study groups (table 1). As a result, the summed $AUC_{24h,corr}$ and Cmin values for nelfinavir plus M8 were also highest in group 1. Neither increasing the dosage of ritonavir to 400 mg (group 2), nor increasing the nelfinavir dose to 2500 mg (group 3) led to a proportional increase in nelfinavir (or nelfinavir plus M8) concentrations.
High inter-individual variability in pharmacokinetic parameters was noted in all three once-daily groups (table 1, figure 3). Figure 3 shows the variability in 24-h Cmin levels for nelfinavir and nelfinavir *plus* M8 in the three study groups, along with proposed therapeutic thresholds for nelfinavir and M8 [29-31]. It appeared that 7/8 participants in group 1 had a nelfinavir Cmin above a proposed therapeutic threshold of 0.45 mg/L for nelfinavir (M8 not included [29]), compared with 2/8 participants in group 2 and 2/4 participants in group 3 (figure 3, left panel). Comparison of individual's Cmin with a higher threshold concentration of 0.8 mg/L for nelfinavir [30,31] revealed that 4/8, 2/8 and 1/4 participants in groups 1, 2 and 3 had nelfinavir Cmin values above this concentration. The 0.8 mg/L threshold for nelfinavir corresponds roughly to a third threshold of 1.0 mg/L for nelfinavir and M8 together (0.8 + (30% * 0.8) = 1.0 mg/L). When nelfinavir and M8 Cmin concentrations were summed (figure 3, right panel), 7/8 volunteers in group 1 had a nelfinavir plus M8 Cmin above 1.0 mg/L, compared with 4/8 and 1/4 volunteers in groups 2 and 3 respectively.

In group 1, a linear association was found between the $AUC_{24h,corr}$ values for M8 and ritonavir (Spearman's rho = 0.786, p=0.021), but the $AUC_{24h,corr}$ values for nelfinavir and ritonavir, or nelfinavir and M8 were not related. No corresponding associations were found in group 2. Correlation analyses were not performed for group 3, because of the small number of participants remaining in this group.

Pharmacokinetics of ritonavir

The geometric mean $AUC_{24h,corr}$, Cmin and Cmax for ritonavir in groups 1 and 3 (200 mg of ritonavir once-daily) were comparable (table 2). Increasing the dose of ritonavir from 200 mg to 400 mg (group 2) resulted in a more than proportional increase in the $AUC_{24h,corr}$ for ritonavir. The $AUC_{24h,corr}$ of ritonavir in group 2 was significantly higher than that in groups 1 and 3 ($p < 0.001$).

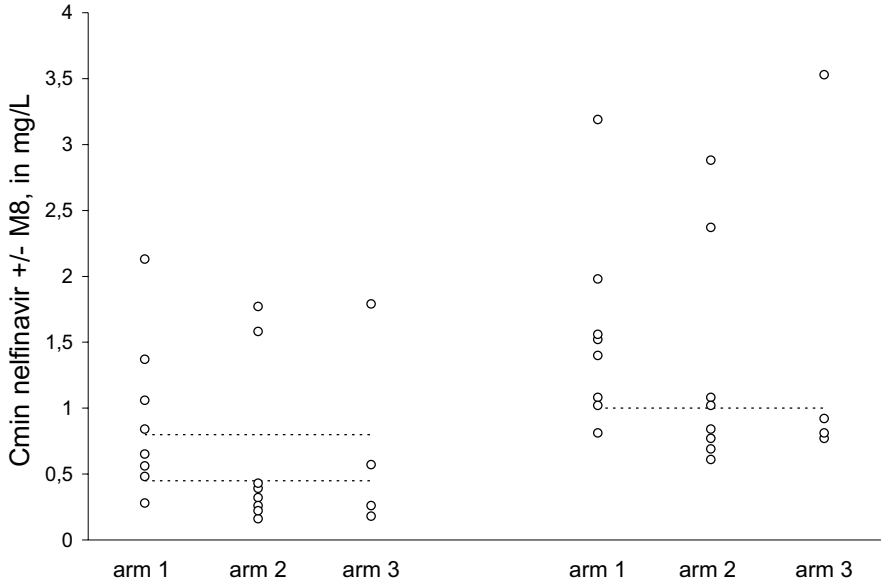

Figure 3. Individual 24-h trough concentrations (Cmin) for nelfinavir (left panel) or nelfinavir plus M8 (right panel) [a,b]
[a] *Group 1: nelfinavir/ritonavir 2000/200 mg once-daily*
Group 2: nelfinavir/ritonavir 2000/400 mg once-daily
Group 3: nelfinavir/ritonavir 2500/200 mg once-daily
[b] *Proposed threshold values for treatment-naïve patients are depicted as dotted lines (see text):*
Nelfinavir: 0.45 mg/L and 0.8 mg/L
Nelfinavir plus M8: 1.0 mg/L

Food effects

A significant effect of meal composition on the $AUC_{24h,corr}$ and Cmin values for nelfinavir and nelfinavir plus M8 was found. No significant differences between study groups could be demonstrated for these parameters and the interaction between study group and meal composition was never significant.
The geometric mean ratios (light/full breakfast) and 90% CI were 0.70 [0.62-0.78] and 0.76 [0.68-0.85] for nelfinavir $AUC_{24h,corr}$ and Cmin, respectively, and 0.71 [0.63-0.81] and 0.76 [0.67-0.86] for the $AUC_{24h,corr}$ and Cmin of nelfinavir plus M8 (data of all three groups combined). The geometric mean ratios fell below 0.8-1.25 limits and the 90% CI were not contained within these limits; thus, the absence of a food-effect could not be concluded.
As the lack of significant between-group differences and the lack of a group-by-meal interaction could be attributable to the small number of participants in each group,

Table 2. Pharmacokinetics of ritonavir after once-daily administration of nelfinavir/ritonavir combinations for 14 days [a]

Parameter [b]	Values (geometric mean + range)		
	group 1: nelfinavir/ritonavir 2000/200mg (n=8)	group 2: nelfinavir/ritonavir 2000/400mg (n=8)	group 3: nelfinavir/ritonavir 2500/200mg (n=4)
Ritonavir			
$AUC_{24h,corr}$ (h.mg/L)	12.2 (8.2-18.3)	32.4 (14.0-53.7)	11.8 (7.9-23.4)
C_{max} (mg/L)	1.7 (1.1-2.9)	4.0 (2.3-5.7)	1.8 (1.2-3.9)
C_{min} (mg/L)	0.04 (0.00-0.12)	0.15 (0.06-0.24)	0.04 (0.02-0.13)
t_{max} (h) [c]	4.0 (2.0-5.0)	4.1 (1.0-6.0)	5.0 (1.5-5.0)
CL/F.kg (L/h.kg)	0.22 (0.16-0.35)	0.15 (0.06-0.30)	0.22 (0.12-0.37)
Vd/F.kg (L/kg)	1.1 (0.58-1.5)	0.95 (0.32-2.2)	1.3 (0.62-2.6)
$t_{1/2}$ (h)	3.4 (2.4-5.7)	4.4 (3.7-7.0)	4.1 (2.7-5.4)

[a] *Pharmacokinetic parameters were assessed after intake of drugs with a full breakfast: 610 kcal, 33% fat, 16% proteins, 51% carbohydrates and 130 ml water (study day 15).*
[b] *Abbreviations of pharmacokinetic parameters: see table 1.*
[c] *Median (and range).*

table 3 shows geometric mean ratios and CIs for each of the study groups separately. The food-effect data in individual groups were in accordance with the data for all groups together. Confidence intervals for group 3 were broad due to the small number of participants in this group.
With respect to ritonavir, the data suggested that the $AUC_{24h,corr}$ in group 2 (400 mg of ritonavir) was less affected by the composition of the breakfast than $AUC_{24h,corr}$ values in groups 1 and 3 (200 mg of ritonavir).

Safety and tolerability

Five volunteers were withdrawn because of concerns over toxicity. Three of these (one in each study group) suffered a mild rash. A second subject in group 3 was withdrawn as a precautionary measure, as he had complaints (without any objective sign of toxicity) which reminded him of a hypersensitivity reaction that he experienced once before. A third subject in group 3 was withdrawn because of severe fatigue (WHO

Table 3. Effect of food on the steady-state pharmacokinetics of nelfinavir/ritonavir given once-daily

Parameter [a]	Geometric mean ratio (light/full breakfast) + 90% CI [b,c]		
	Group 1: nelfinavir/ritonavir 2000/200mg (n=8)	Group 2: Nelfinavir/ritonavir 2000/400mg (n=8)	Group 3: nelfinavir/ritonavir 2500/200mg (n=4)
Nelfinavir			
$AUC_{24h,corr}$ (h.mg/L)	0.75 [0.67-0.84]	0.75 [0.66-0.85]	0.53 [0.29-0.98]
C_{max} (mg/L)	0.88 [0.80-0.97]	0.84 [0.75-0.94]	0.57 [0.32-1.00]
C_{min} (mg/L)	0.78 [0.65-0.94]	0.77 [0.61-0.99]	0.69 [0.53-0.90]
t_{max} (h) [d]	+0.1 (-2.4 to +1.0)	-0.5 (-3.0 to +5.0)	-0.75 (-2.0 to 0.0)
M8			
$AUC_{24h,corr}$ (h.mg/L)	0.83 [0.68-1.01]	0.77 [0.65-0.91]	0.55 [0.28-1.06]
C_{max} (mg/L)	0.93 [0.90-0.96]	0.84 [0.71-0.98]	0.63 [0.40-0.99]
C_{min} (mg/L)	0.83 [0.69-0.99]	0.82 [0.63-1.07]	0.62 [0.36-1.05]
t_{max} (h) [d]	-1.0 (-1.0 to 0.0)	-1.0 (-2.0 to +5.0)	-0.75 (-2.0 to 0.0)
Nelfinavir + M8			
$AUC_{24h,corr}$ (h.mg/L)	0.78 [0.68-0.91]	0.75 [0.65-0.87]	0.53 [0.28-1.00]
C_{min} (mg/L)	0.80 [0.67-0.96]	0.80 [0.62-1.02]	0.63 [0.43-0.91]
Ritonavir			
$AUC_{24h,corr}$ (h.mg/L)	0.78 [0.66-0.92]	0.92 [0.78-1.08]	0.52 [0.29-0.95]
C_{max} (mg/L)	0.95 [0.78-1.15]	1.07 [0.89-1.27]	0.49 [0.24-0.98]
C_{min} (mg/L)	0.90 [0.74-1.09]	1.03 [0.59-1.80]	1.17 [0.91-1.50]
t_{max} (h) [d]	+0.5 (-1.0 to +2.0)	+0.5 (-1.0 to +5.1)	-0.5 (-2.0 to 0.0)

[a] *Abbreviations of pharmacokinetic parameters: see table 1.*
[b] *CI: confidence interval.*
[c] *A ratio refers to the fraction of a pharmacokinetic parameter after administration of nelfinavir/ritonavir with a light breakfast (day 16) to the same parameter after administration with a full breakfast (day 15).*
Full breakfast: 610 kcal, 33% fat, 16% proteins, 51% carbohydrates, 130 ml water.
Light breakfast: 271 kcal, 37% fat, 24% proteins, 39% carbohydrates, 130 ml fluid.
[d] *Median difference in tmax after administration of nelfinavir/ritonavir with a light breakfast compared to a full breakfast (tmax day 16 - tmax day 15), and range.*
No significant differences in tmax values (light breakfast versus full breakfast) were found for nelfinavir, M8, or ritonavir, except for M8 in group 1 (p = 0.03, Wilcoxon-signed ranks test).

Table 4. Adverse events: incidence and toxicity scores [a]

Adverse event	Study group			
	group 1: nelfinavir/ ritonavir 2000/200mg	group 2: nelfinavir/ ritonavir 2000/400mg	group 3: nelfinavir/ ritonavir 2500/200mg	All groups:
	(n=9)	(n=9)	(n=8) [a]	(n=26)
Incidence (any severity) [b]				
Diarrhoea	100	78	50	77
Flatulence	56	78	38	58
Nausea	11	56	25	31
Vomiting	0	11	25	12
Abdominal pain	22	44	50	38
Asthenia	11	22	38	23
Fatigue/somnolence	22	56	75	50
Fever	0	0	13	4
Headache	56	33	25	38
Skin reaction or rash	22	33	63	38
Taste perversion	11	11	25	15
Peroral paraesthesia	33	11	13	19
Peripheral paraesthesia	22	22	13	19
Arthralgia	22	22	25	23
Myalgia	11	22	13	15
Median severity score [c]	1.5	2.5	3.1	2.4

[a] Data are from all participants, including those who withdrew. One participant in group 3 withdrew informed consent before taking the drugs and before the first evaluation of adverse events.
[b] Incidence expressed as the percentage of participants who reported a particular adverse event at least once.
[c] A severity score of 3.0 represents three mild adverse events, or one moderate adverse event (2 points) plus a mild one, or one severe adverse event (3 points) at an average reporting moment.

grade 3 toxicity) after 11 days of nelfinavir/ritonavir. One day after stopping the medication, increased liver transaminases were found in this subject (AST grade 2, ALT grade 3 toxicities), but these readings normalized in the next three weeks.
Apart from these adverse events, the nelfinavir/ritonavir regimens were tolerated reasonably well. The most common adverse reactions are shown in table 4.

Median toxicity scores were 1.5, 2.5 and 3.1 in study groups 1, 2 and 3 respectively. This means that subjects in group 1 had an average of 1.5 mild adverse events (or almost one event of moderate intensity, which would count for 2.0) at an average moment of questioning. Diarrhoea was mild, as expressed in a median score of less than 1.0 for this adverse event in all three study groups. No significant correlations were found between the toxicity scores and the $AUC_{24h,corr}$, Cmax or Cmin of nelfinavir, nelfinavir plus M8, or ritonavir.

Small increases in fasting cholesterol were observed in the majority of participants who completed the study (6/8, 6/8 and 2/4 participants in group 1, 2 and 3, respectively). Median changes in cholesterol were +0.50, +0.25 and 0.0 mmol/L in groups 1, 2 and 3, whereas median changes in triglycerides were negligible (+0.04, +0.04 and +0.16 mmol/L in groups 1, 2 and 3). The study medication had no material effect on other laboratory parameters.

Discussion

The results of this study suggest it is possible to achieve effective exposure to nelfinavir and M8 after once-daily dosing of nelfinavir in combination with low-dose ritonavir. These data are in agreement with results from a similar study, presented in abstract [32]. A nelfinavir-based HAART regimen with once-daily dosing for all drugs is simple and also facilitates witnessed therapy of HAART. Both these advantages may result in improved long-term adherence [6-8], which is associated with improved efficacy of HAART [4,5]. Once-daily administration of HAART may be particularly useful for a subgroup of patients who cannot adhere to more complex drug regimens, due to unstable lifestyles, imprisonment, or injectable drug misuse.
Whereas once-daily administration of nelfinavir may prove more convenient, the number of tablets that need to be taken and food restrictions still make a once-daily

regimen quite complex. The proposed development of 625-mg tablets of nelfinavir will enable further simplification of the regimen. With respect to food restrictions, once-daily administration offers patients the flexibility to adapt the dosing time to their dietary habits, assuming that the pharmacokinetics of nelfinavir/ritonavir do not change with the time of dosing.

It is important not to over-interpret the apparent pharmacokinetic differences or similarities between the three nelfinavir/ritonavir regimens in this exploratory study. Each study group comprised a relatively small number of participants, and considerable interindividual variability was observed for all pharmacokinetic parameters. The number of participants who completed the study in group 3 was particularly small (n = 4). Therefore, no firm conclusions can be drawn with regard to the nelfinavir/ritonavir 2500/200mg combination.
With these considerations taken into account, two approaches could be applied to evaluate the pharmacokinetics of nelfinavir and nelfinavir plus M8 after once-daily administration. According to a first approach, geometric *mean* pharmacokinetic parameters for nelfinavir and nelfinavir plus M8 (table 1) are compared to reference values for the approved regimen of nelfinavir (1250 mg BID without ritonavir). The $AUC_{24h,corr}$ and Cmin values should be regarded as the most important parameters for this comparison, since both AUC and Cmin values of PIs have been related to efficacy of these drugs [33]. Reference pharmacokinetic data for the approved BID regimen of nelfinavir are presented in table 5 [16,19,22,31,34]. Comparison of table 1 and table 5 reveals that the geometric mean $AUC_{24h,corr}$ values for once-daily nelfinavir are at least comparable to similar values reported for the approved BID regimen of nelfinavir, and are considerably higher when the contribution of M8 is included. Therefore, $AUC_{24h,corr}$ values predict efficacy for all three once daily regimens. In contrast, only group 1 (nelfinavir/ritonavir 2000/200 mg once daily) yielded a geometric mean 24-h Cmin value for nelfinavir that was comparable to the lowest 12-h Cmin values reported for the BID regimen. When M8 levels are taken into consideration, the mean 24-h Cmin values in all groups are comparable to reference data, with group 1 showing the most favourable results again.
Comparing experimental data to reference data in this way could be confounded by differences across studies. Firstly, the current study was performed in healthy volunteers, whereas most reference data (table 5) are from HIV-infected patients. No

substantial differences in nelfinavir pharmacokinetics have been observed between these two groups [16], but they can not be excluded. Secondly, differences in the composition of meals taken with nelfinavir as well as different analytical methods could also confound comparison of pharmacokinetic parameters. Our in-house reference data (table 5) were obtained using the same breakfast content and the same bio-analytical method as in the current study. Thirdly, some reference AUC12h values reported for the BID regimen were doubled to enable comparison with once-daily $AUC_{24h,corr}$ values. However, this neglects circadian variations that appear to occur in the pharmacokinetics of nelfinavir.
According to a second approach for evaluation of the pharmacokinetics of once-daily nelfinavir, *individual* (instead of mean) 24-h Cmin values for nelfinavir and nelfinavir plus M8 could be compared to therapeutic threshold values (see figure 3). The 0.45 mg/L (800 nM) threshold for nelfinavir is based upon *in vitro* 95% effective concentrations (IC95) against HIV, with adjustment for *in vivo* protein binding and the elevated concentrations of α1-acid glycoprotein in HIV infected patients [29]. Thresholds derived from *in vivo* patient response data may be more relevant, and the 0.8 mg/L nelfinavir threshold for wild-type HIV-1 (i.e. in treatment-naive patients) has been independently assessed in two patient cohorts [30,31]. This threshold corresponds to about 1.0 mg/L when the Cmin values of nelfinavir and M8 are added. Regardless of the threshold chosen and the inclusion or exclusion of M8 concentrations, the 2000/200 mg regimen (group 1) appeared to be most favourable again (figure 3). As this combination was also associated with the lowest toxicity score (table 4), it appears to be an appropriate regimen for further evaluation.

The boosting effect of ritonavir on nelfinavir $AUC_{24h,corr}$ values appears less pronounced than those seen after addition of ritonavir to indinavir and especially saquinavir therapy. This could be explained by the relatively small contribution of CYP3A to nelfinavir metabolism, compared with the other PIs. The increase in M8 levels may reflect the possible inducing effect of ritonavir on CYP2C19 (leading to enhanced formation of M8) and inhibition of CYP3A4 (which limits the clearance of M8) [9]. Exposures to M8 seemed similar among study groups, which may suggest saturation of M8 formation, or a lack of additional effects of ritonavir (above a dose of 200 mg once-daily) on the subsequent metabolism of M8.
The apparent lack of increase in nelfinavir concentrations with further dose escalations

Table 5. Reference pharmacokinetic data for nelfinavir, M8 and nelfinavir plus M8 after twice-daily administration of nelfinavir (1250 mg BID) [a]

Parameter [b]	Reference [16,34] [c] n = 10 intens. PK [h]	[31] [c] n = 84 pop. PK	[19] n = 355 pop. PK	[22] [d,f] n = 12 / 12 intens. PK	[in house data] [e,g] n = 5 intens. PK
Nelfinavir					
AUC_{24h} (h.mg/L)	51.0	52.0 [i]	48.0 [i]	52.5 / 55.5 [f]	41.8 [i]
C_{max} (mg/L)	4	8.33	3.4	3.39 / 4.25 [f]	3.9
C_{min}, morning (mg/L)	2.2	1.02	1.60	1.16 / 1.24	1.30
C_{min}, evening (mg/L)	0.7		0.85	1.76 / 1.35	0.65
M8					
AUC_{24h} (h.mg/L)			15.2 [i]	24.6 / 26.7 [f]	12.9 [i]
C_{max} (mg/L)			1.1	1.76 / 1.95 [f]	1.4
C_{min}, morning (mg/L)			0.41	0.44 / 0.48	0.39
C_{min}, evening (mg/L)			0.28	0.71 / 0.65	0.05
Nelfinavir + M8 [i]					
AUC24h (h.mg/L)			63.2	77.1 / 82.2	54.7
Cmin, morning (mg/L)			2.01	1.60 / 1.72	1.69
Cmin, evening (mg/L)			1.13	2.47 / 2.00	0.70
M8-to-nelfinavir ratio [i]					
ratio for AUC_{24h}			0.32	0.47 / 0.48	0.31
ratio for C_{min},morning			0.26	0.38 / 0.39	0.30
ratio for C_{min}, evening			0.33	0.40 / 0.48	0.08

[a] Reference data were assessed in HIV-infected patients, except for [22] (healthy volunteers).
[b] AUC24h, 24 h-area under the concentration-time curve; Cmax , highest observed plasma concentration; Cmin, trough concentration, either in the morning (before the morning dose) or in the evening (before the evening dose).
[c] Median values.
[d] Mean values.
[e] Geometric means.
[f] Reference 22 was a two-group study; data for both groups are shown. Mean values for AUC24h were estimated by summing mean AUC12h values reported for the morning dose and evening dose. Cmax values refer to the morning dose.
[g] In-house reference data were retrieved from our own dataset of intensive pharmacokinetic assessments. These data were assessed after intake of nelfinavir with a full breakfast that was identical to the breakfast used in the study, and the same bioanalytical method was used for measurement of drug concentrations.
[h] Reference data were assessed by intensive (intens.) pharmacokinetic (PK) measurements in a limited number of individuals, or by population (pop.) pharmacokinetic approaches.
[i] AUC24h values were obtained by doubling AUC12h values that were reported in the references.
[i] Values for Nelfinavir + M8 and for M8:nelfinavir ratios were derived from the mean values reported for nelfinavir and M8 separately (this is an approximation).

of ritonavir (from 200 mg in group 1 to 400 mg in group 2) or nelfinavir (from 2000 mg in group 1 to 2500 mg in group 3) seems counterintuitive and may reflect random variability in this exploratory study. However, the effect of ritonavir on the pharmacokinetics of nelfinavir (comparison of groups 1 and 2) is hard to predict, due to the complex metabolism of nelfinavir, the influence of ritonavir as both an inhibitor or inducer of the metabolic enzymes involved, and the presence of such enzymes in both the gut wall and liver. For example, it could be argued that higher doses of ritonavir (group 2) increase the first-pass metabolism of nelfinavir in the gut wall, resulting in lower nelfinavir Cmax and $AUC_{24h,corr}$ values in group 2 (see table 1). Any differences between groups 1 and 3 (increase in the dose of nelfinavir) should be interpreted even more cautiously, considering the small number of participants in group 3 of the study. Nevertheless, the apparent lack of increase in nelfinavir concentrations after a higher dose of nelfinavir corresponds to other literature data [19,21] and may be explained by saturation of absorption from the gut.

As regards to the effect of food, it was hypothesized that food restrictions for nelfinavir might possibly change with co-administration of ritonavir, as has been demonstrated for indinavir in combination with ritonavir (400/400 mg BID) [9]. However, $AUC_{24h,corr}$ and Cmin values for nelfinavir and M8 significantly decreased after administration with a light compared with a full breakfast. Accordingly, it is recommended that once-daily regimens of nelfinavir/ritonavir are administered with a meal comparable in calories (circa 600 kcal) and fat content (circa 33%) to the full breakfast used in this study.

The spectrum of adverse events was as expected. Mild diarrhoea is a common adverse effect of both nelfinavir and ritonavir. The incidence of rash in this study (3 out of 26 participants who took at least one dose of nelfinavir/ritonavir, i.e. 12%) was higher than the 3% value reported for phase III studies in which nelfinavir was administered without ritonavir [29]. This adverse event requires close study when once-daily nelfinavir/ritonavir regimens are tested in HIV-infected patients. Similarly, the observed increases in cholesterol values warrant close monitoring of blood lipids when the nelfinavir/ritonavir regimen is used in HIV-infected patients, especially if combined with other antiretroviral agents with known lipid-elevating effects.

In conclusion, data from this study demonstrate that coadministration of nelfinavir and low-dose ritonavir offers the potential for once-daily administration of nelfinavir. A once-daily regimen of 2000 mg nelfinavir and 200 mg ritonavir seems appropriate for further evaluation. The once daily combination of nelfinavir and ritonavir should be taken with a meal containing at least 600 kcal. Short-term tolerability was satisfactory, apart from a higher than expected incidence of rash. Follow-up pharmacokinetic and tolerability assessments in HIV-infected patients are warranted to confirm the findings of this study in healthy volunteers.

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Chapter 5.2

Combination of nelfinavir/ritonavir and nevirapine in a once-daily antiretroviral regimen in healthy volunteers

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In preparation

Abstract

Background

We have previously reported that administration of nelfinavir (NFV) with low-dose ritonavir (RTV) offers the potential for OD administration of NFV. This study was an extension of the previous study and evaluated the influence of OD nevirapine (NVP) on the pharmacokinetics (PK) of OD NFV/RTV combinations.

Methods

The study had a three-arm, one-sequence, two-period design. Twenty healthy volunteers who had been taking one of 3 possible NFV/RTV OD regimens (A. 2000/200 mg, n=8; B. 2000/400 mg, n=8; or C. 2500/200 mg, n=4) with food during 15 days were included in the extension study. OD nevirapine was added to the NFV/RTV combinations from day 16-38, starting with a dose of 200 mg OD (2 weeks) followed by 400 mg OD (1 week). PK parameters for NFV, its active metabolite M8, and RTV were assessed on days 15 and 38, and NVP parameters were assessed on day 38. Since RTV increases the M8/NFV-ratio, NFV and M8 concentrations were also summed. Geometric mean (GM) ratios plus 90% confidence intervals (CIs) were calculated for the PK parameters on day 38 (with nevirapine) relative to the parameters on day 15 (without nevirapine).

Results

All participants completed the study. The GM ratio and 90% CI for the $AUC_{0\text{-}24h}$ and the 24-h trough level (Cmin) of nelfinavir were 1.13 [0.94-1.37] and 1.42 [1.06-1.90] for regimen A; 1.83 [1.52-2.19] and 3.04 [2.17-4.27] for regimen B; and 1.30 [0.80-2.11] and 1.80 [0.74-4.36] for regimen C. The GM ratio and 90% CI for the sum $AUC_{0\text{-}24h}$ and Cmin for nelfinavir *plus* M8 were 0.95 [0.79-1.14] and 1.01 [0.83-1.25] for regimen A; 1.44 [1.23-1.68] and 2.01 [1.52-2.66] for regimen B, and 0.96 [0.62-1.48] and 0.99 [0.54-1.81] for regimen C. These results indicate that the exposure to NFV and M8 in the presence of OD NVP was equivalent to, or higher than exposures without NVP. The GM $AUC_{0\text{-}24h}$ values for OD NVP (155, 171, and 146 h*mg/L for regimen A, B and C, respectively) appeared to be higher than reported before. In the period from day 15 to 38, two participants developed grade I and one participant grade II hepatotoxicity. Another participant developed grade II-III hepatotoxicity after the study. Rash was not observed.

Conclusions
It is feasible to add a OD dose of NVP to a OD combination of NFV/RTV to obtain a once-daily dual PI and NNRTI antiretroviral regimen. A combination of NFV/RTV 2000/200 mg plus NVP 400 mg OD seems preferable, since this combination results in adequate plasma concentrations of NFV and M8, whereas exposure to RTV is limited.

Introduction

Adherence to highly active antiretroviral treatment (HAART) has become one of the most important challenges for HIV-infected patients who have access to these potent drug regimens. Patients have to maintain near perfect adherence to their prescribed regimen on a long-term basis to prevent the emergence of viral resistance and an increase in viral replication [1], which is usually followed by immunologic deterioration. Unfortunately, many patients do not achieve sustained optimal adherence [1,2]. This has been associated with a wide array of determinants, among which the complexity of HAART regimens appears to have a weighty impact [3]. Consequently, current efforts to maximize adherence focus on development of less complex HAART regimens that can be dosed once-daily [4]. Several protease-inhibitors (PIs), nucleoside reverse transcriptase inhibitors (NRTIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs) have been approved for once-daily administration, or are being studied in a once-daily dosing scheme.

Once-daily dosing of PIs can be achieved by exploiting the pharmacokinetic interaction of these drugs with ritonavir, a strong inhibitor of cytochrome (CYP) 3A4. Previously we reported that administration of the PI nelfinavir with low-dose ritonavir offers the potential for once-daily administration of nelfinavir [5]. Three once-daily combinations of nelfinavir and ritonavir were studied in healthy volunteers; nelfinavir/ritonavir 2000/200 mg, 2000/400 mg, and 2500/200 mg. Co-administration of nelfinavir and ritonavir resulted in an increase in the formation of the active metabolite of nelfinavir, M8. The 2000/200 mg combination yielded the most favorable concentrations of nelfinavir and M8 at 24 h post dose. Furthermore it was found that nelfinavir/ritonavir combinations have to be taken with a sufficient amount of food to assure adequate absorption of nelfinavir.

Since entirely once-daily HAART regimens comprise several antiretroviral drugs, further evaluation of once-daily nelfinavir/ritonavir combinations requires an assessment of possible pharmacokinetic interactions between these combinations and other once-daily antiretrovirals. We were specifically interested in combinations of nelfinavir/ritonavir and the NNRTIs nevirapine and efavirenz. Such combinations of PIs and NNRTIs could be applied as first-line therapy to spare the class of NRTIs and to prevent toxicity related to NRTIs [6]. In addition, PI/NNRTI combinations are increasingly used as second-line therapy (with or without NRTIs) in patients who are resistant or intolerant to NRTIs [6]. The pharmacokinetics of nevirapine in a once-daily dose have been characterized before [7,8], and this NNRTI has demonstrated good antiviral response in once-daily regimens [9-12]. Therefore we evaluated the influence of once-daily nevirapine on the pharmacokinetics of once-daily nelfinavir/ritonavir combinations in healthy volunteers.

Methods

Subjects

This study was performed as an extension of the previously reported pharmacokinetic study that explored the feasibility of once-daily administration of nelfinavir plus ritonavir in healthy volunteers. All volunteers who completed this study were subsequently included in the extension study. The participants were healthy males or females, aged 18 to 65 years, who were not using any medication or illicit drugs prior to these studies. The subjects had negative serology for HIV infection, were hepatitis B- or C-seronegative, were not pregnant or hypersensitive to PIs, and had normal laboratory parameters according to prespecified criteria.
All subjects gave written informed consent after full explanation of the study details. The study was approved by the Institutional Review Board of University Medical Centre Nijmegen, The Netherlands.

Study design and procedures

This was an open-label, randomized, three-arm, one-sequence (fixed-order), two-period pharmacokinetic interaction study. In the preceding study (period 1), twenty-seven volunteers were randomized (stratified by gender) over three groups of nine participants

[5]. The participants took once-daily doses of either nelfinavir/ritonavir 2000/200 mg (group 1), 2000/400 mg (group 2), or 2500/200 mg (group 3) with food during 15 days (period 1). Nelfinavir (Viracept®) was administered as film-coated tablets, each containing 250 mg. Ritonavir (Norvir®) was applied as capsules containing 100 mg. The drugs were taken in the morning. Blood samples for two consecutive 24-h pharmacokinetic profiles were collected on study day 15 (administration with food) and day 16 (administration without food) [5].
On day 17, the extension of this previous study was started (period 2). The remaining participants continued their nelfinavir/ritonavir combination, and nevirapine was added to these combinations, starting with a dose of 200 mg once-daily during two weeks (nevirapine lead-in period) followed by 400 mg once-daily during one week, until study day 38. The lower dose of nevirapine in the lead-in period accounts for autoinduction of metabolic enzymes by nevirapine, and this dose escalating scheme also reduces the incidence of rash [13]. Nevirapine was administered as 200 mg tablets of Viramune® and was taken together with nelfinavir/ritonavir and with food. On the morning of day 38, the participants reattended the pharmacokinetic unit for a repeat pharmacokinetic assessment.

Pharmacokinetic assessments on day 15 (nelfinavir/ritonavir) and day 38 (nelfinavir/ritonavir plus nevirapine) were performed after an overnight fast. A predose blood sample was drawn and participants ingested nelfinavir and ritonavir (plus nevirapine on day 38) with a standardized, full breakfast, which consisted of 4 filled slices of bread and 130 ml of water (610 kcal in total: 33% fat, 16% proteins and 51% carbohydrates). Blood samples were drawn at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 10.0, 12.0, 16.0 en 24.0 h post dose. Plasma was isolated by centrifugation within 12 hours and was stored at -20^{0}C until analysis.

The study was conducted on an outpatient basis. Subjects received their study medication in a vial that contained sealed plastic sachets, each holding the appropriate amount of tablets and capsules for one day. Drug administration was directly observed on 12 study days. Compliance at home was verified at every study visit by inspection of drug taking diaries, counting of sachets, measurement of plasma drug concentrations, and electronic monitoring of opening of vials, using the Medication Event Monitoring System (MEMS®).

Bio-analysis

Nelfinavir and ritonavir concentrations were assayed with a previously described validated high-performance liquid chromatographic (HPLC) method with UV detection [14]. Concentrations of the active metabolite of nelfinavir (M8) were determined simultaneously using the same method without modifications. Retention time for M8 was 12.7 min. The accuracy of the method for nelfinavir ranged from 96% to 100%, depending on the concentration level. Those for ritonavir and M8 were 102-108% and 93-108%, respectively. Intra-day precision and between-day precision were 2.1-7.5 % and 0.4-3.5 % for nelfinavir, 2.0-8.1% and 0-2.4% for ritonavir, and 2.8-4.3% and 2.0-3.0% for M8. The limit of quantitation was 0.04 mg/L for nelfinavir, ritonavir and M8. Nevirapine concentrations were measured with another method that was described previously [15]. The accuracy of this method varied from 97 to 103%, depending on the concentration level. Intra-day precision and between-day precision were 1.3-3.9% and 1.9-3.0 %, and the limit of quantitation was 0.1 mg/L.

Pharmacokinetic analysis

Pharmacokinetic parameters for nelfinavir, M8, ritonavir, and nevirapine were obtained by non-compartmental methods. The highest observed plasma concentration was defined as Cmax, with the corresponding sampling time as tmax. Cmin was the concentration at 24 h after ingestion of the drugs. The terminal, log-linear period (log C versus t) was defined by visual inspection of the last data points ($n \geq 3$). The value of the slope (- ß/2.303) was calculated by least-squares linear regression analysis, where ß is the first-order elimination rate constant. The elimination half life (t1/2) was calculated by the equation 0.693/ß. The area under the concentration versus time curve ($AUC_{0\text{-}24h}$) was calculated using the trapezoidal rule from 0 to 24 h. $AUC_{0\text{-}24h}$ values for nelfinavir, M8 and ritonavir were corrected for contribution of the predose AUC by subtraction of C0/ß (where C0 is the concentration just before ingestion of nelfinavir/ritonavir at t=0), and area under the curve was added at the end of the curve (extrapolation to infinity by adding Cmin/ß). $AUC_{0\text{-}24h}$ values for nevirapine were not corrected. The AUC values for nelfinavir and M8 and the Cmin values for nelfinavir and M8 were also summed, considering that M8 has equipotent activity to nelfinavir *in vitro*, similar *in vivo* protein binding [16], and an almost identical molar

weight. Cmax values were not summed, since these do not necessarily refer to the same time post dose.

Safety and tolerability

Safety and tolerability were assessed by a questionnaire, that presented 14 possible adverse events that could occur during treatment with nelfinavir, ritonavir or nevirapine. The questionnaire was presented 11 times during the study, 4 times during period 1 (day 1-15) and 7 times during period 2 (day 16-38). An extensive blood chemistry and hematology screen were performed on the same study days.

Statistical analysis

The effect of nevirapine on the pharmacokinetics of nelfinavir/ritonavir was evaluated with the confidence interval approach for bioequivalence studies that is recommended for drug interaction studies [17,18]. Ratios of geometric means (day 38 / day 15, i.e. with nevirapine / without nevirapine) and accompanying 90% confidence intervals (CI) were calculated for the AUC, Cmax, Cmin and t1/2 of nelfinavir, M8, nelfinavir plus M8, and ritonavir. Equivalence between the test condition (administration of nelfinavir/ritonavir with nevirapine) and the reference condition (administration of nelfinavir/ritonavir without nevirapine) was concluded for a specific pharmacokinetic parameter if the 90% CI for the geometric mean ratio of that parameter was entirely contained within 80-125% limits. *In*equivalence was concluded if a 90% CI fell entirely outside 80-125% limits. Equivalence was suggested if a geometric mean ratio was enclosed in the 80-125% interval, but one limit of its 90% CI fell outside the 80-125% interval. Likewise, inequivalence was suggested if a geometric mean ratio fell outside the 80-125% interval, whereas one limit of its 90% CI was enclosed in the 80-125% interval [18]. Tmax values were not log-transformed and were compared with Wilcoxon signed-ranks test.
Individual Cmin values in each of the study groups were compared to a 0.8 mg/L efficacy threshold for nelfinavir in treatment-naive patients [19,20], and to a corresponding threshold of 1.0 mg/L for the sum of nelfinavir and M8.
All statistical evaluations were performed with SPSS for Windows, version 10.0 (SPSS Inc., Chicago, IL, USA).

Results

Subjects

Twenty-one participants from the previous study were enrolled into the extension study, eight participants in group 1 (nelfinavir/ritonavir 2000/200 mg), eight in group 2 (nelfinavir/ritonavir 2000/400 mg) and five in group 3 (nelfinavir/ritonavir 2500/200 mg). All participants completed the extension study. After analysis of plasma samples, it appeared that one volunteer in group 3 did not convert nelfinavir to M8. This volunteer was probably a poor metabolizer for CYP2C19, the iso-enzyme that exclusively catalyzes the conversion of nelfinavir to M8. Because of this different metabolic pattern, pharmacokinetic data for this volunteer were excluded. The remaining participants were 5 men and 3 women (median age and weight: 30 years, 71 kg) in group 1; 4 men and 4 women (median age and weight: 24 years, 76 kg) in group 2; and 2 men and 2 women (median age and weight: 33 years, 73 kg) in group 3. All subjects were Caucasians.

Pharmacokinetics of nelfinavir, M8 and ritonavir

Table 1 shows the steady-state pharmacokinetic data for nelfinavir, M8, nelfinavir plus M8, and ritonavir after once-daily administration of nelfinavir/ritonavir without nevirapine (period 1) and with nevirapine (period 2). The accompanying geometric mean ratios (day 38 / day 15, with nevirapine / without nevirapine) and 90% CIs in table 2 illustrate the effect of nevirapine in the three groups. The pharmacokinetic data for period 1 have been presented before [5].

Considering the results for the nelfinavir/ritonavir 2000/200 mg combination (group 1), equivalence was suggested when comparing the $AUC_{0\text{-}24h}$ and Cmax values of nelfinavir after administration of the 2000/200 mg combination with or without nevirapine (table 2 and figure 1). Inequivalence was suggested for nelfinavir Cmin values, but this resulted from an increase (rather than a decrease) in the geometric mean nelfinavir Cmin upon addition of nevirapine. M8 concentrations strongly decreased in seven of eight participants after administration of nevirapine in group 1. The decrease in M8 concentrations paralleled with a uniform decrease in exposure to

the pharmacokinetic enhancer ritonavir. Based on the $AUC_{0\text{-}24h}$ and Cmin values of nelfinavir *plus* M8, equivalence between the test and reference regimen was suggested.

The results in group 2 (nelfinavir/ritonavir 2000/400 mg) differed from those in group 1 (table 2 and figure 2). Uniform increases in nelfinavir concentrations were observed upon addition of nevirapine to this combination, and inequivalence between the test and reference regimen was concluded for the AUC, Cmax and Cmin of nelfinavir. A variable change in M8 concentrations was observed upon addition of nevirapine. The sum of nelfinavir and M8 revealed an increase in $AUC_{0\text{-}24h}$ and Cmin values in seven of eight participants after addition of nevirapine. As a consequence, inequivalence between the test and reference condition was suggested or confirmed, respectively, after comparison of $AUC_{0\text{-}24h}$ and Cmin values of nelfinavir *plus* M8. The exposure to ritonavir (400 mg once-daily) was not affected by nevirapine (equivalence documented), contrary to the results in group 1 (200 mg of ritonavir).

Group 3 (nelfinavir/ritonavir 2500/200 mg) comprised only four participants, hence the broad 90% CI in table 2. In view of the small number of participants, no conclusions can be drawn for the effect of nevirapine on this combination. However, the effects of nevirapine seemed to correspond to those in group 1.

As reported before, M8-to-nelfinavir ratios were elevated after co-administration of nelfinavir and ritonavir (period 1, see table 1) compared to a mean M8-to-nelfinavir ratio of circa 0.3 after administration of nelfinavir (1250 mg BID) without ritonavir [5]. Addition of nevirapine (period 2) lead to a decrease in M8-to-nelfinavir ratios in all three groups (table 1), caused by decreasing M8 concentrations and increasing nelfinavir concentrations (groups 1 and 3) or by increasing nelfinavir concentrations (group 2).
Tmax values of nelfinavir, M8 or ritonavir were not affected by the addition of nevirapine in all three groups.

Table 1. Pharmacokinetics of once-daily nelfinavir/ritonavir combinations administered without nevirapine (period 1) and with nevirapine (period 2) [a,b]

Parameter	Geometric means					
	Group 1 (n=8): NFV/RTV 2000/200mg		Group 2 (n=8): NFV/RTV 2000/400mg		Group 3 (n=4): NFV/RTV 2500/200mg	
	Period 1	*Period 2*	*Period 1*	*Period 2*	*Period 1*	*Period 2*
NFV						
$AUC_{0\text{-}24h}$ (h.mg/L)	76.8	87.1	51.3	93.8	61.9	80.5
C_{max} (mg/L)	7.2	7.2	5.1	8.1	6.7	7.1
C_{min} (mg/L)	0.76	1.1	0.43	1.3	0.47	0.84
t_{max} (h)[c]	4.0	4.5	4.6	4.5	4.5	3.3
$t_{1/2}$ (h)	6.5	7.8	6.1	8.3	5.8	8.5
M8						
$AUC_{0\text{-}24h}$ (h.mg/L)	38.5	22.1	40.9	37.3	45.3	21.8
C_{max} (mg/L)	3.4	1.7	3.7	2.5	4.3	1.8
C_{min} (mg/L)	0.63	0.36	0.59	0.73	0.67	0.30
t_{max} (h)[c]	5.0	5.0	5.1	5.0	4.5	5.0
$t_{1/2}$ (h)	9.7	10.4	8.6	10.9	8.9	9.8
NFV + M8						
$AUC_{0\text{-}24h}$ (h.mg/L)	116.4	110.2	94.6	136.0	108.3	103.8
C_{min} (mg/L)	1.4	1.5	1.1	2.2	1.2	1.2
M8:nelfinavir ratio						
$AUC_{0\text{-}24h}$ (h.mg/L)	0.50	0.25	0.80	0.40	0.73	0.27
C_{min} (mg/L)	0.83	0.33	1.36	0.55	1.43	0.35
RTV						
$AUC_{0\text{-}24h}$ (h.mg/L)	12.2	7.3	32.4	33.6	11.8	6.7

[a] *Pharmacokinetic parameters were assessed after intake of drugs with a full breakfast: 610 kcal, 33% fat, 16% proteins, 51% carbohydrates and 130 ml water*
[b] *Abbreviations: NFV: nelfinavir, RTV: ritonavir, $AUC_{0\text{-}24h}$: 24 h-area under the concentration-time curve, Cmax : highest observed plasma concentration, Cmin : trough concentration at 24h, tmax : sampling time for Cmax, t1/2: elimination half life*
[c] *Median*

Comparison of nelfinavir and M8 Cmin values to thresholds

In group 1, the number of participants with a nelfinavir Cmin above 0.80 mg/L increased from 4/8 on day 15 (end of period 1) to 7/8 participants on day 38 (end of period 2). In group 2, the number of Cmin values above this threshold increased

Table 2. Effect of nevirapine on the pharmacokinetics of nelfinavir/ritonavir combinations[a]

Parameter	Geometric mean ratio (with NVP, period 2 / without NVP, period 1)[b] + 90% confidence interval		
	Group 1 (n=8): NFV/RTV 2000/200mg	Group 2 (n= 8): NFV/RTV 2000/400mg	Group 3 (n=4): NFV/RTV 2500/200mg
NFV			
$AUC_{0\text{-}24h}$ (h.mg/L)	1.13 [0.94-1.37]	1.83 [1.52-2.19]	1.30 [0.80-2.11]
C_{max} (mg/L)	0.99 [0.85-1.17]	1.59 [1.31-1.91]	1.06 [0.69-1.61]
C_{min} (mg/L)	1.42 [1.06-1.90]	3.04 [2.17-4.27]	1.80 [0.74-4.36]
t_{max} (h)[c]	+0.5	0.0	-0.75
$t_{1/2}$ (h)	1.21 [1.03-1.41]	1.35 [1.15-1.59]	1.47 [0.93-2.32]
M8			
$AUC_{0\text{-}24h}$ (h.mg/L)	0.57 [0.45-0.72]	0.91 [0.68-1.22]	0.48 [0.28-0.82]
C_{max} (mg/L)	0.50 [0.45-0.57]	0.68 [0.55-0.83]	0.41 [0.28-0.61]
C_{min} (mg/L)	0.56 [0.45-0.71]	1.24 [0.87-1.78]	0.44 [0.20-0.99]
t_{max} (h)[c]	0.0	-0.1	0.0
$t_{1/2}$ (h)	1.07 [0.93-1.22]	1.28 [1.09-1.50]	1.10 [0.88-1.38]
NFV + M8			
$AUC_{0\text{-}24h}$ (h.mg/L)	0.95 [0.79-1.14]	1.44 [1.23-1.68]	0.96 [0.62-1.48]
C_{min} (mg/L)	1.01 [0.83-1.25]	2.01 [1.52-2.66]	0.99 [0.54-1.81]
RTV			
$AUC_{0\text{-}24h}$ (h.mg/L)	0.59 [0.53-0.67]	1.03 [0.91-1.18]	0.57 [0.44-0.74]

[a] *Pharmacokinetic parameters were assessed after intake of drugs with a full breakfast: 610 kcal, 33% fat, 16% proteins, 51% carbohydrates and 130 ml water*
[b] *NVP: nevirapine. Other abbreviations: see table 1*
[c] *Median change in tmax after administration of nelfinavir/ritonavir with nevirapine (period 2) compared to administration without nevirapine (period 1). No significant differences in tmax values were found for nelfinavir, M8 or ritonavir (Wilcoxon-signed ranks test).*

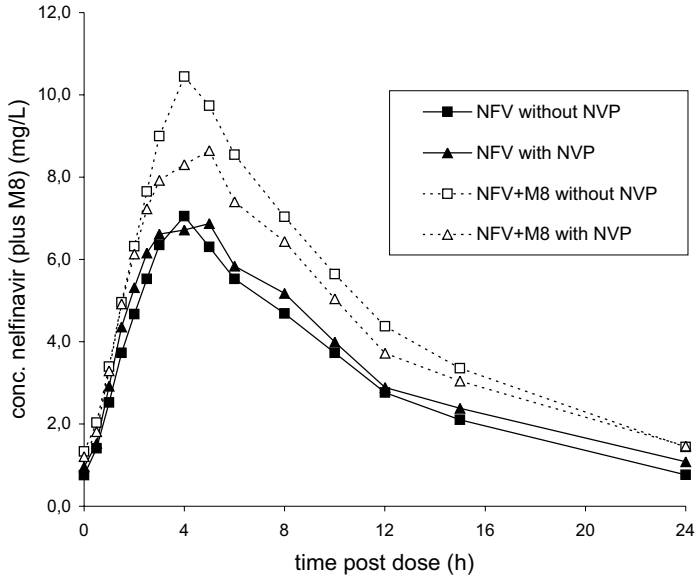

Figure 1. Steady-state plasma concentrations of nelfinavir, and nelfinavir plus M8, after administration of nelfinavir/ritonavir 2000/200 mg once-daily (group 1) without and with nevirapine 400 mg once-daily (geometric mean values).

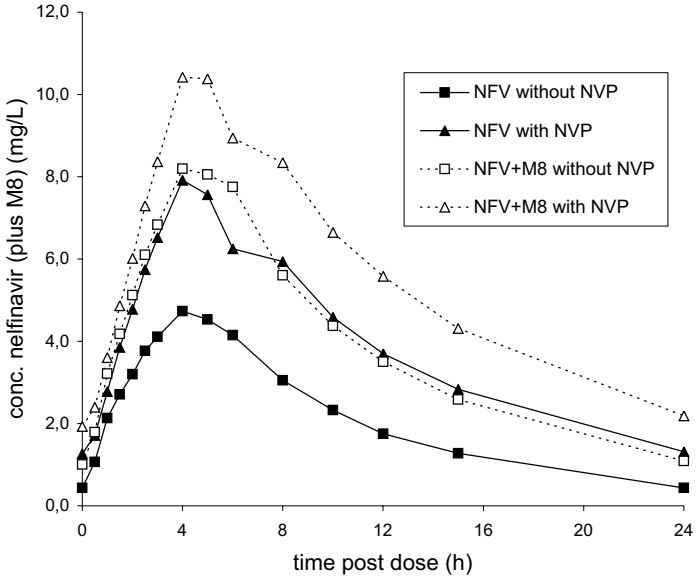

Figure 2. Steady-state plasma concentrations of nelfinavir, and nelfinavir plus M8, after administration of nelfinavir/ritonavir 2000/400 mg once-daily (group 2) without and with nevirapine 400 mg once-daily (geometric mean values).

from 2/8 to 8/8 participants after co-administration of nevirapine, and in group 3 these figures were 2/4 and 3/4 respectively.
After summing nelfinavir and M8 Cmin concentrations, 7/8 volunteers in group 1 had a combined Cmin above a threshold of 1.0 mg/L, both on day 15 and on day 38. In group 2, 4/8 participants had a combined Cmin above 1.0 mg/L and this increased to 8/8 participants after addition of nevirapine. In group 3, 1 /4 and 2/4 volunteers had nelfinavir plus M8 Cmin values above 1.0 mg/L on day 15 and 38 respectively. Thus, addition of nevirapine to nelfinavir/ritonavir caused an increase in the number of participants with nelfinavir Cmin values above thresholds, especially in group 2.

Pharmacokinetics of nevirapine

Table 3 presents the pharmacokinetic data for nevirapine after co-administration with nelfinavir/ritonavir in an entirely once-daily combination. The results do not suggest relevant differences in the exposure to nevirapine between the three study groups. The exposure to nevirapine was not related to any pharmacokinetic parameter (or change in parameter) of nelfinavir, M8 or ritonavir.

Table 3. Pharmacokinetics of nevirapine (400 mg once-daily) after co-administration with nelfinavir/ritonavir combinations [a]

Parameter[b]	*Geometric mean*		
	Group 1 (n=8): NFV/RTV 2000/200mg	*Group 2 (n=8): NFV/RTV 2000/400mg*	*Group 3 (n=4): NFV/RTV 2500/200mg*
Nevirapine			
$AUC_{0\text{-}24h}$ (h.mg/L)	155.1	170.5	146.0
Cmax (mg/L)	8.0	9.2	8.1
Cmin (mg/L)	5.2	6.0	4.7
tmax (h)[c]	4.0	4.0	2.0
t1/2 (h)	34.0	41.6	34.8

[a] Pharmacokinetic parameters were assessed after intake of drugs with a full breakfast: 610 kcal, 33% fat, 16% proteins, 51% carbohydrates and 130 ml water
[b] Abbreviations: see table 1
[c] Median

Safety and tolerability

As reported before, five participants were withdrawn during period 1 [5]. No participants withdrew during the extension phase (period 2). The most prevalent adverse events in period 2 (all groups combined) were mild diarrhoea (reported at least once by 85% of the participants), fatigue (80%), flatulence (65%) and abdominal pain (60%). The severity of adverse events was generally mild. As result, the combinations of nelfinavir/ritonavir and nevirapine were reasonably well tolerated. Of note, no participant developed rash after administration of nevirapine from day 15 to day 38. No significant correlations were found between incidences or severities of adverse reactions on the one hand and pharmacokinetic parameters for nelfinavir, M8, ritonavir or nevirapine on the other.

The study medication appeared to have an effect on fasting cholesterol concentrations (median values at baseline, day 15, day 38: 4.7, 4.8, 5.3 mmol/L; median change from baseline to day 38: +0.6 mmol/L) and fasting triglyceride values (median values at baseline, day 15, day 38: 1.3, 1.5, 1.7 mmol/L, median change from baseline to day 38: +0.2 mmol/L). No general effect was discernible on other laboratory parameters, especially the liver enzymes aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT) and alkaline phosphatase. At the end of the study three participants had elevated gamma-glutamyltransferase (GGT) values, corresponding to grade I toxicity (two participants) and grade II toxicity (one participant), according to the criteria of the AIDS Clinical Trial Group. The grade II toxicity was probably due to intercurrent illness during the study. A fourth participant showed grade II-III toxicity for ASAT, ALAT and GGT at the post-study visit. These parameters normalized in the subsequent weeks.

Discussion

The results of this study show that is feasible to add a once-daily dose of nevirapine to a once-daily combination of nelfinavir and low-dose ritonavir to obtain a once-daily dual PI and NNRTI antiretroviral regimen. This follows from the observed effects of nevirapine on the $AUC_{0\text{-}24h}$ and Cmin values of nelfinavir (and nelfinavir *plus* M8) in participants who used nelfinavir/ritonavir 2000/200 mg or 2000/400 mg once-daily.

The effect of nevirapine on Cmin values of nelfinavir (plus M8) may be considered most important, since there is accumulating evidence that PIs require certain minimum concentrations throughout the whole dosing interval (i.e. time-dependent viral inhibition). It was reported before that the nelfinavir/ritonavir 2000/200 mg combination yields the most favorable nelfinavir (and nelfinavir *plus* M8) Cmin concentrations without co-administration of nevirapine [5]. The results of this study suggest that addition of nevirapine to this combination caused a modest increase (rather than a decrease) in nelfinavir Cmin, and the results suggested equivalence between the sum nelfinavir plus M8 Cmin values in the presence or absence of nevirapine. The mean 24-h Cmin values for nelfinavir (and nelfinavir plus M8) in the nelfinavir/ritonavir 2000/200 mg plus nevirapine regimen were comparable to 12-h Cmin values after twice daily administration of nelfinavir (1250 mg BID) without ritonavir [19,21,22]. Individual nelfinavir Cmin values of this combination also compared well to therapeutic thresholds that have been derived for twice-daily administration of nelfinavir. As to the 2000/400 mg combination, an unexpected, uniform increase in nelfinavir concentrations was observed after addition of nevirapine to this combination. This raised the Cmin values for nelfinavir (and nelfinavir plus M8) of this combination to adequate levels as well. No conclusions can be drawn for the 2500/200 mg combination, considering the small number of participants in group 3.

This study was not designed to evaluate the influence of nelfinavir/ritonavir on the pharmacokinetics of nevirapine in a once-daily dose. Based on available data, no such influence was expected. Nevertheless, an interaction cannot be excluded, considering that the geometric mean $AUC_{0\text{-}24h}$, Cmax and Cmin values of once-daily nevirapine in this study (table 3) appear to be higher than reported previously. More specifically, Havlir et al reported an $AUC_{0\text{-}24h}$ of 130 h.mg/L, a Cmax of 7.2 mg/L and a Cmin of 4.0 mg/L for once-daily administration of nevirapine [7], whereas van Heeswijk et al found $AUC_{0\text{-}24}$h, Cmax and Cmin values of 106 h.mg/L, 6.8 mg/L and 2.9 mg/L [8], respectively; these data were all assessed in HIV-infected patients. If these cross-study differences are real, it is unknown what their clinical relevance would be, as there is no clear upper (toxicity) threshold for nevirapine at this moment. Some studies did not show any, or only a weak, association between nevirapine plasma concentrations and hepatotoxicity [23,24], but one study did find such a relationship [25]. A possible relationship between nevirapine plasma concentrations and

hepatotoxicity could be explained as high nevirapine concentrations causing hepatotoxicity, but it is also possible that liver damage itself caused reduced clearance of nevirapine and increased concentrations of this drug.

The results of this study indicate that the interaction between nelfinavir, ritonavir and nevirapine is complex. This is not surprising, considering the complicated metabolism of nelfinavir and the inhibiting and inducing effects of ritonavir and nevirapine on the hepatic enzymes involved.
In vitro studies revealed that nelfinavir is metabolized by at least five different pathways, catalysed by several CYP isoenzymes (CYP3A4, CYP2C19, CYP2D6 and CYP2C9) [26]. CYP3A4 and CYP2C19 are the predominant enzymes in the metabolism of nelfinavir. CYP2C19 exclusively catalyzes the conversion of nelfinavir to M8, which in turn is metabolized by CYP3A4 [27].
The effect of ritonavir on nelfinavir and M8 concentrations can be explained by inhibition of the metabolism of nelfinavir and M8, since ritonavir is a strong inhibitor of CYP3A. In addition, ritonavir may be an inducer of CYP2C19, thereby increasing the formation of M8 [28].
The effect of nevirapine on nelfinavir concentrations has raised some controversy in the past [29-31], but it is now assumed that this effect is clinically insignificant [13]. Furthermore, it is known that nevirapine only slightly affects ritonavir concentrations, at least when ritonavir is used in high doses [13]. In the current study it appeared that nevirapine, a moderate inducer of CYP3A, was able to decrease the exposure to a low-dose of ritonavir (200 mg, groups 1 and 3). In contrast, nevirapine was not able to reverse the enzyme inhibition by a larger dose of ritonavir (400 mg, group 2). The effect of nevirapine on the pharmacokinetic enhancer ritonavir may drive the effects on M8, since decreases in ritonavir concentrations paralleled with decreases in M8 concentrations. Whereas the effect of nevirapine on M8 concentrations can be explained in this way, no obvious explanation seems to be available at this time for the uniform and strong increase in nelfinavir concentrations that occurred following addition of nevirapine to the nelfinavir/ritonavir 2000/400 mg combination.

All participants in this extension study completed the 23 study days (from day 15 to 38) in which they took nelfinavir/ritonavir plus nevirapine. The absence of any rash after administration of nevirapine was not anticipated, as the incidence of rash

attributable to nevirapine (dosed according to the escalation scheme) among HIV-infected patients was 16% in phase II/III trials, and the majority of rashes occur within the first 6 weeks of treatment [13]. An explanation for the absence of rash may be that several participants with predisposition for rash were withdrawn in the study that preceded this extension study. Hepatic reactions are an another frequent adverse reaction to nevirapine in the first 6 weeks of treatment [13]. These reactions are of special concern, since one large study showed that once-daily administration of nevirapine is associated with a higher incidence of liver associated laboratory abnormalities than twice-daily administration of nevirapine [12]. In the current study, two of 20 participants had grade I hepatotoxicity (based on isolated elevations of GGT) that was probably related to the study medication, and one participant developed grade II-III toxicity. Hepatotoxic reactions, as well as increases in cholesterol values, warrant close monitoring when the nelfinavir/ritonavir plus nevirapine combination is used in HIV-infected patients.

Based on the results of this study, both the nelfinavir/ritonavir 2000/200 mg and the nelfinavir/ritonavir 2000/400 mg combinations could be combined with nevirapine. The combination of nelfinavir/ritonavir 2000/200 mg plus nevirapine seems to be preferable. This combination results in adequate plasma concentrations of nelfinavir and M8 that are not unnecessarely high, whereas the exposure to ritonavir is much lower than observed with the nelfinavir/ritonavir 2000/400 mg plus nevirapine combination. Higher plasma concentrations of ritonavir will probably infer more toxicity in the long-term [32]. In addition, nelfinavir/ritonavir 2000/200 mg comprises less pills than nelfinavir/ritonavir 2000/400 mg. A once-daily combination of nelfinavir/ritonavir 2000/200 mg plus nevirapine still represents a large number of tablets and capsules, but the announced introduction of 625-mg tablets of nelfinavir will enable further simplification.

In conclusion, this study shows that once-daily combinations of nelfinavir and low-dose ritonavir can be combined with once-daily nevirapine to obtain an entirely once-daily dual PI plus NNRTI regimen. A combination of nelfinavir/ritonavir 2000/200 mg plus nevirapine 400 mg appears to be preferable. The combinations of nelfinavir/ritonavir plus nevirapine were reasonably well-tolerated, but require close monitoring of liver enzymes and cholesterol values. Follow-up pharmacokinetic and tolerability

assessments in HIV-infected patients are warranted to confirm the results of this study in healthy volunteers.

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Chapter 5.3

A once-daily HAART regimen containing indinavir + ritonavir plus one or two nucleoside reverse transcriptase inhibitors (PIPO study)

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Abstract

Introduction

There is an increased interest in developing once-daily regimens for the treatment of HIV-infected patients. A phase II study was conducted to investigate the pharmacokinetics and short-term safety and efficacy of an indinavir/ritonavir combination as part of a once-daily regimen.

Methods

HIV-infected patients with either proven poor compliance to HAART regimens in the past or an anticipated poor compliance to such a regimen in the future were eligible for this study. They received a once-daily regimen consisting of indinavir 1200 mg, ritonavir 400 mg, and one or two nucleoside reverse transcriptase inhibitors, also administered once-daily with food. A 24h pharmacokinetic profile was constructed in a subset of patients. Short-term safety and efficacy were evaluated at 4, 12, and 24 weeks after initiation of treatment.

Results

A total of 64 patients were included in this study, of whom 27 (42.2%) were treatment-naive. The geometric mean (+ 95% CI) of indinavir $AUC_{0\text{-}24h}$, Cmax and Cmin as determined in an unselected group of 16 patients were 84.9 (69.7-103.5) mg/L.h, 12.0 (10.2-14.1) mg/L, and 0.15 (0.09-0.26) mg/L, respectively. A large interpatient variability was observed, with 5 out of the 16 subjects having a Cmin value below the minimum effective concentration of 0.10 mg/L. During the 24 weeks of follow-up 9 patients (14.1%) discontinued study medication, two due to medication-related toxicity. Gastrointestinal adverse events were reported most frequently (50.0%), followed by skin effects (45.3%), joint pain (9.4%) and urological complaints (7.8%). No patient developed nephrolithiasis. The median (+ interquartile range) serum creatinine level in the 64 patients increased slightly from 74 (63-88) micromol/L to 79 (66-92) micromol/L during the 24 weeks of follow-up. One patient reached a grade 1 elevation in serum creatinine which normalized during the follow-up; 5 other patients with elevated serum creatinine at baseline remained stable. During the 24 weeks of follow-up, the proportion of patients with a viral load < 500 copies/mL increased from 35.1% at baseline to 71.4% (ITT NC=F analysis) or 83.3% (OT analysis), and from 0% at baseline to 76.2% (ITT NC=F analysis) or 100.0% (OT analysis) in treatment-experienced and -naive patients, respectively. This was accompanied by a mean

increase in CD4 cell count of 52 and 220 cells/mm^3 in these two subgroups, respectively.

Conclusion

The 24-weeks follow-up data of this study indicate favorable pharmacokinetics of an indinavir/ritonavir 1200/400 mg combination as part of a once-daily regimen consisting also of one or two NRTIs. Short-term safety and efficacy were also satisfactory. Long-term follow up is planned to evaluate the durability of these results.

Introduction

Highly active antiretroviral therapy (HAART) with two nucleoside reverse transcriptase inhibitors (NRTIs) and one non-nucleoside reverse transcriptase inhibitor (NNRTI) or a (boosted) protease inhibitor (PI) has become standard treatment of HIV-1-infected patients [1]. Although the convenience of taking these medications has improved by the introduction of newer formulations and the use of ritonavir as a booster of other PIs, many patients still find it hard to adhere to these complex regimens [2,3]. Nearly-perfect adherence is required for optimal response [4], so HAART regimens should be as simple as possible. One approach to simplify therapy is to develop drugs or combinations of drugs that can be taken once-daily. This is attractive for the general patient population, and especially in settings in which observed therapy is given, e.g. ambulant methadone clinics, prisons, nursing homes.

So far, only a few antiretroviral agents have been approved for once-daily use (didanosine, lamivudine, efavirenz, and tenofovir) and the approvals for a number of others is expected soon (abacavir, stavudine extended-release, and atazanavir). The relatively long elimination half-life of these compounds or their intracellular metabolites has made once-daily use possible. All currently available PIs have relatively short elimination half-lives, but this is considerably changed when their metabolism is inhibited by a low-dose of ritonavir [5,6]. Several studies have demonstrated that a once-daily PI containing regimen can be constructed by increasing the dose of the PI in combination with low-dose ritonavir [7-11]. With regard to indinavir, dose-finding studies in healthy volunteers [12,13] have indicated that a 1200 mg dose of indinavir in combination with 200 or 400 mg of ritonavir once-daily should provide adequate exposure to indinavir. A number of pilot studies in HIV-infected patients were

conducted to translate this experience with once-daily use of indinavir into clinical practice [14-16]. In most of these studies, however, other components of the combination were given twice-daily. So, the benefit of administering indinavir/ritonavir once-daily was partly lost by the twice-daily schedule of the other components.

We have conducted a phase II study of a regimen containing indinavir + ritonavir and one or two NRTIs, all given once-daily, to explore further the pharmacokinetics and short-term safety and efficacy of an indinavir-based once-daily combination.

Methods

Patients and regimens

The PIPO study was a phase II study to investigate the 24 weeks pharmacokinetics, safety, and efficacy of a once-daily regimen containing indinavir 1200 mg, ritonavir 400 mg, and one or two NRTIs. Patients could be either treatment-naive or -experienced. Patients were eligible for participation in the study if the physician judged a once-daily regimen to be the most appropriate regimen for a specific patient. Poor compliance to other, more complex regimens was either proven to be a problem in the past, or it was anticipated to become a problem in the near future. Concomitant use of NNRTIs or other agents known to interfere with the metabolism of indinavir and/or ritonavir was not allowed. Other exclusion criteria were pregnancy or breastfeeding, a change in the antiretroviral regimen within 4 weeks prior to start of the study, abnormal liver or renal function, an active opportunistic infection, and previous hypersensitivity to one of the drugs in the regimen.
The use of two NRTIs in combination with indinavir would make a triple drug regimen. The use of a double drug regimen (one NRTI + indinavir/ritonavir combination) was also considered acceptable based on the favorable results of the Prometheus trial [17] in which a ritonavir/saquinavir + one NRTI combination demonstrated excellent antiretroviral activity. It was specifically recommended to add two NRTIs in patients with high viral load at baseline (i.e.: > 500,000 copies/mL). Allowed NRTIs administered once-daily were didanosine (400 mg as chewing tablets, later replaced by the enteric coated formulation), lamivudine (300 mg) and stavudine (60-80 mg immediate-release capsules, doses based on body weight).

The study was approved by the local Ethics Committees and all patients gave written informed consent.

Pharmacokinetics

An unselected cohort of study participants was sequentially enrolled in a pharmacokinetic substudy. Two weeks after the start of treatment, a 24h pharmacokinetic curve was recorded. Patients were admitted to the hospital and took their medication with a standardized, low-fat meal (approximately 350 kcal). Blood was sampled just prior to dosing, and at 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 10, 12, 16, and 24 hours post ingestion. Blood was centrifuged immediately and plasma was frozen at -20°C until analysis. Indinavir and ritonavir plasma concentrations were determined by a validated reversed-phase high-performance liquid chromatographic assay as previously described [18]. Pharmacokinetic parameters were calculated by noncompartmental methods [19].

Safety and efficacy measurements

Patients were seen at the outpatient clinic at week 0, 4, 8, 12, 16 and 24 after start of treatment. At each study visit, patients were questioned about adverse events. Laboratory abnormalities were evaluated at the same time points. Serum creatinine

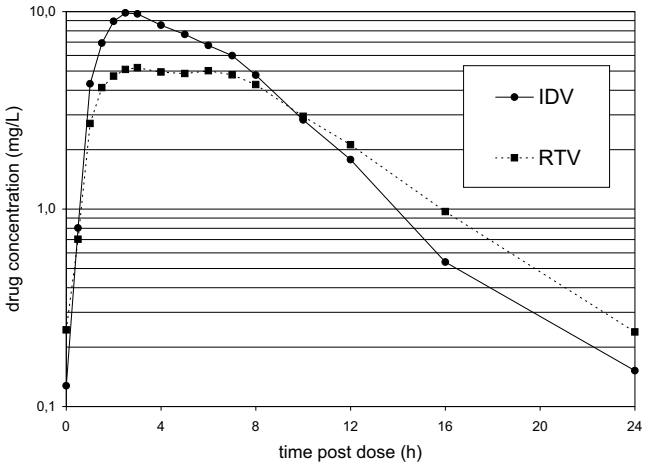

Figure 1. Geometric mean indinavir (IDV) and ritonavir (RTV) plasma concentrations vs. time curves of the 16 patients who participated in the pharmacokinetic substudy.

levels were evaluated for toxicity according to AIDS Clinical Trials Group (ACTG) guidelines, i.e. grade 1 = 1.1 – 1.6 times the upper limit of normal (ULN); grade 2 = 1.6 – 3.0 times ULN; grade 3 = 3.1 – 6.0 times ULN; grade 4: > 6.0 times ULN. Also, blood was sampled for measurement of HIV-1 RNA (Roche Amplicor®, detection limit 500 copies/mL) and CD4 cell counts at week 0, 4, 12, and 24. During the study, a more sensitive assay became available with a detection limit of 50 copies/mL, but for a uniform analysis of all HIV-1 RNA data points, the results below 500 were considered < 500 copies/mL in all analyses.

Data analysis

Geometric means, 95% confidence intervals and min-max range were calculated for all pharmacokinetic parameters and compared to literature data of indinavir three times daily and twice-daily indinavir/ritonavir combinations. Viral load responses were evaluated by intent-to-treat (ITT, non-completer equals failure) and on-treatment (OT) analysis.

Results

Patients

A total of 64 patients (49 males, 15 females) have been included between May 1999 and May 2002. The initial sample size was 20 patients, but due to the encouraging initial safety and virological response data as reported previously [20] it was decided to extend this study to 60-70 patients to assess these items in a larger study population. The majority of the population (59.4%) was Caucasian, the remainder was African (35.9%) or Hispanic (4.7%). Median (+ interquartile range) age was 39 years (35-46). This cohort contained 52 (former) intravenous drug users. Twenty-seven patients were treatment-naive vs. 37 pretreated patients; 13 of these pretreated patients had received indinavir previously. Concomitant medications (n; %) were lamivudine (48; 75.0%), didanosine (1; 1.6%), stavudine (5, 7.8%), stavudine+lamivudine (8; 12.5%), stavudine+didanosine (1; 1.6%), or didanosine+lamivudine (1; 1.6%). During the 24 weeks of follow-up, 9 patients (14.1%) discontinued the study medication: one patient died due to a cocaine

Table 1. Summary of pharmacokinetic data for indinavir 1200 mg and ritonavir 400 mg once-daily; steady state pharmacokinetic parameters (n=16)

Parameter	Geometric mean	95% CI[a]	Range
Indinavir			
$AUC_{0\text{-}24h}$ (h.mg/L)	84.9	69.7-103.5	36.2-156.0
C_{max} (mg/L)	12.0	10.2-14.1	5.1-18.5
C_{min} (mg/L)	0.15	0.09-0.26	0.03-0.94
t_{max} (h)	2.2 [b]	-	0.5-6.0
CL/F (L/h)	14.1	11.6-17.2	7.7-33.1
Vd/F (L)	55.3	36.7-83.3	5.7-145.2
$t_{1/2}$ (h)	2.7	2.0-3.8	0.51-5.2
Ritonavir			
$AUC_{0\text{-}24h}$ (h.mg/L)	64.0	49.3-83.0	22.7-129.6
C_{max} (mg/L)	6.4	5.1-8.1	2.1-12.4
C_{min} (mg/L)	0.24	0.13-0.45	0.05-1.8
t_{max} (h)	3 [b]	-	1.0-7.0
CL/F (L/h)	6.3	4.8-8.1	3.1-17.6
Vd/F (L)	36.8	30.8-44.0	23.9-92.2
$t_{1/2}$ (h)	4.1	3.3-5.1	2.6-8.5

[a]. *CI: confidence interval*
[b]. *Median value*

overdose, one patient developed severe anemia, one patient suffered from severe vomiting, 5 patients withdrew consent for personal reasons, and one patient discontinued for unknown reasons.

Pharmacokinetics

An unselected cohort of 16 patients participated in the 24h pharmacokinetic substudy. Median (+ IQR) age and body weight were 38 years (32-55) and 67 kg (52-81), respectively. The pharmacokinetic curve of the geometric mean values of these 16 patients (1 female) are presented in figure 1. The pharmacokinetic parameters of indinavir and ritonavir are listed in table 1. A large interpatient variability was observed, in particular for the indinavir Cmin, leading to 5 out of the 16 subjects

having a value below 0.10 mg/L, the proposed minimum effective concentration for indinavir in treatment-naive patients. However, virological failure was not detected during the 24 weeks of follow-up in any of these 5 patients with low indinavir Cmin values.

Safety

The treatment was generally well-tolerated during the 24 weeks of follow-up. Only two subjects discontinued medication because of adverse events. No serious adverse event occurred with the exception of one patient who died of myocardial infarction following a cocaine overdose, which was considered to be unrelated to the study medication (see above). Gastrointestinal (GI) adverse events were reported most frequently (50.0%), followed by skin effects (45.3%), joint pain (9.4%) and urological complaints (7.8%). No patient developed nephrolithiasis. At baseline, 5 patients (of whom 3 indinavir-pretreated) had elevated serum creatinine (4 patients grade 1 and one patient grade 2). One new patient reached a grade 1 elevation in serum creatinine which normalized during the follow-up; those with elevated serum creatinine at baseline remained stable. The median (+ interquartile range) serum creatinine level in these 64 patients increased from 74 (63-88) micromol/L to 79 (66-92) micromol/L during the 24 weeks of treatment (figure 2).

Efficacy

In the subgroup of 37 treatment-experienced patients, 13 had a viral load < 500 copies/mL at the start of the trial (35.1%). This proportion of patients with a viral load < 500 copies/mL increased to 71.4% (ITT NC=F analysis) or 83.3% (OT analysis), respectively (figure 3). For the 27 treatment-naïve patients in this study, the percentage of patients with an undetectable viral load increased from 0% at baseline to 76.2% (ITT NC=F analysis) or 100.0% (OT analysis), respectively, during the 24 weeks of follow-up. Parallel to this virological response, the geometric mean CD4 cell count rose from 112 at baseline to 164 cells/mm^3 at week 24 in the treatment-experienced patients (OT analysis); for the treatment-naive patients, the change in CD4 cell count was from 64 at baseline to 284 cells/mm^3 at week 24 (figure 4).

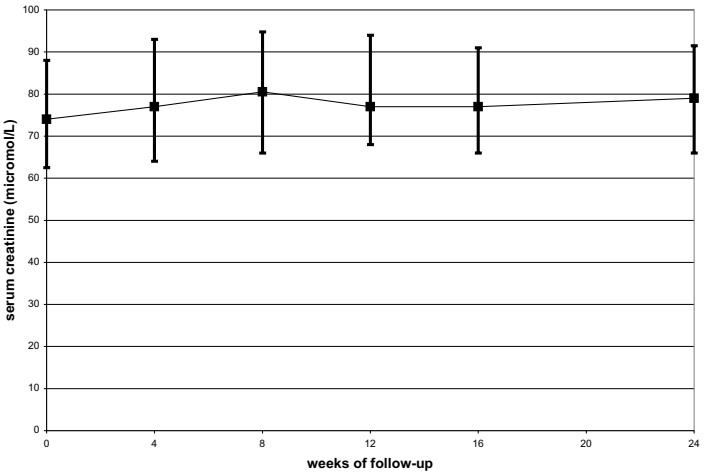

Figure 2. Evolution of serum creatinine levels (median ± IQR) during 24 weeks of follow-up.

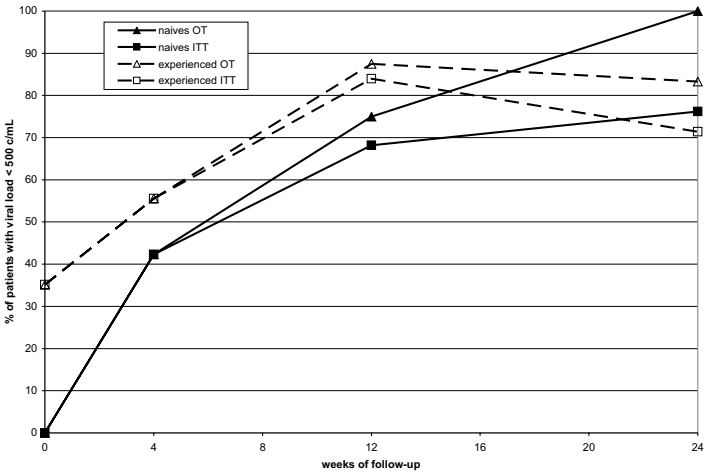

Figure 3. Viral load response separated for treatment-naive (n=27) and -experienced (n=37) patients. ITT = intention-to-treat non-completer equals failure. OT = on treatment.

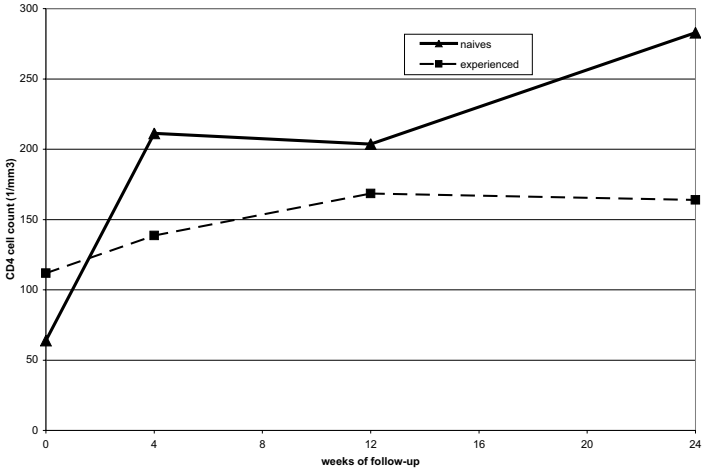

Figure 4. CD4 cell response (geometric means) separated for treatment-naive (n=27) and -experienced (n=37) patients

Discussion

This phase II study demonstrated that a once-daily regimen containing indinavir/ritonavir plus one or two NRTIs results in adequate pharmacokinetic parameters of indinavir as compared to the licensed use of indinavir three times daily without ritonavir [21]. Average Cmin levels of indinavir were similar (0.15 mg/L), and interpatient variability (5/16 subjects had a Cmin below 0.10 mg/L) was also comparable to previous observations [22-26]. In addition, $AUC_{0\text{-}24h}$ of this once-daily indinavir regimen was on average 1.5 times higher than usually reported for indinavir three times daily (corrected for 24h exposure) [22-26].
Given the fact that both AUC and Cmin concentrations of indinavir have been related to antiviral response (for review see [27]), it can be expected that this once-daily regimen of indinavir would have adequate antiviral activity. In line with the adequate pharmacokinetic parameters for indinavir, the short-term efficacy of this regimen was satisfactory. This may be somewhat surprising given the fact that mainly patients were selected based on a proven or predicted poor adherence with antiretroviral regimens. In this study, adherence was not independently assessed, although some of the drop

outs actually did not meet their appointments and can be considered non-adherent. We have no information on adherence of patients who remained in the study.

Indinavir was originally licensed in a three times daily dose of 800 mg. Several studies have shown that addition of ritonavir makes twice-daily dosing possible [28-30]. A subsequent step to once-daily use was investigated in two separate healthy volunteer studies [12,13]. These studies evaluated three different indinavir doses (400, 800 and 1200 mg) and three ritonavir doses (100, 200 and 400 mg). Optimal exposure was predicted in the 1200/400 mg dose group and this dose was selected for the phase II study as described here. The observation that 5 out of the 16 patients had a Cmin level below the minimum effective level of 0.10 mg/L, and the absence of published data on other doses, suggests that this dose can be considered the minimum effective dose and that Therapeutic Drug Monitoring may be needed to optimize treatment in selected cases.

The question remains, however, whether this threshold of 0.10 mg/L as derived from three times daily use of indinavir without ritonavir can be extrapolated to boosted indinavir regimens taken twice or once-daily. In this study, none of the 5 patients with a Cmin below 0.10 mg/L had virological failure during the short-term follow-up. One explanation for this apparent discrepancy may be that a suboptimal trough level of indinavir may occur in some patients three times a day in three times daily regimens vs. only once a day in a once-daily regimen as described here. As a result, it can be postulated that patients with suboptimal exposure to indinavir are at a higher risk for virological failure when they have a trough below 0.10 mg/L in a three times daily regimen compared to a once-daily regimen. So far, no studies have demonstrated a minimum effective concentration of indinavir in these boosted regimens. Use of the 0.10 mg/L threshold for all indinavir containing regimens can be considered a conservative approach while awaiting more data for the boosted indinavir regimens. Another explanation for a difference in minimum effective concentrations for indinavir in three times daily vs. once daily regimens may be a difference in the relationship between indinavir Cmin and AUC in the different schedules. Usually indinavir Cmin and AUC show co-linearity, but this may change when the dose and dose interval are changed. For example, the average indinavir trough of 0.15 mg/L in the unboosted three times daily regimen corresponds to an indinavir $AUC_{0\text{-}24h}$ of approximately 60

mg/L.h [21-23]. In contrast, the $AUC_{0\text{-}24h}$ as observed in this once-daily regimen is approximately 50% higher (GM 84.9 mg/L.h) in the absence of a change in the indinavir trough. Therefore, if we consider a similar $AUC_{0\text{-}24h}$ value as the target for unboosted three times daily indinavir and boosted once-daily indinavir, the target C24h in the latter regimen may be considerably lower than the proposed minimum value for C8h of 0.10 mg/L in the unboosted regimen [27]. Long-term follow up of patients using once-daily indinavir regimens is required to evaluate the relationships between indinavir Cmin or AUC and virological response for this regimen.

When the once-daily use of indinavir was initially considered, there was a considerable fear of increased nephrotoxicity due to the elevated indinavir peak levels that were observed in the healthy volunteer studies of the once-daily dose. Indeed, Cmax levels of indinavir as observed in this study are substantially higher than those in other indinavir regimens: 12.0 mg/L for once-daily (+ ritonavir) vs. 8-11 mg/L for twice-daily (800 mg + 100 mg of ritonavir) [29,30] vs. 7-9 mg/L for three times daily (800 mg without ritonavir) [21-23,25,26]. The higher Cmax levels of indinavir in this study, however, do not appear to result in increased renal toxicity as compared to other indinavir regimens. No patient developed nephrolithiasis. Only 5 out of the 64 patients had urological complaints (i.e. hematuria and dysuria) during the 24 weeks of follow up, and none discontinued indinavir for this reason. In addition, there was a slight increase of 5 micromol/L in serum creatinine during the 24 weeks of treatment. A possible explanation for the absence of an increased incidence in renal toxicity, despite the high Cmax levels of indinavir in this once-daily regimen, may be the fact that this peak occurs only once-a-day. Subjects were instructed to drink at least 1.5 liter of fluid around the intake of indinavir, and this advice may be better adhered to than when a similar recommendation needs to be followed at three different time points during the day. The incidence of renal toxicity in patients using other indinavir regimens has been presented in largely varying incidences, probably due to differences in definitions and duration of follow-up. Long-term follow up of this cohort is planned and may indicate any long-term renal complications of this regimen.
In contrast to the low incidence of renal toxicity, a much higher frequency of GI and skin toxicity was observed. GI toxicity may be partly related to ritonavir plasma levels as a relatively high dose for boosting was used here. Skin toxicity (esp. dry skin) is probably most related to indinavir exposure; a higher incidence of skin effect was

observed in the boosted twice daily 800/100 mg regimen vs. the unboosted three times daily 800 mg regimen in the BEST study [31].

The initial antiviral response is encouraging, especially considering the proven poor compliance in the past or an anticipated poor compliance for new patients and the high proportion of treatment-experienced patients in this cohort (57.8%). The duration of follow-up of this phase II study, however, was too short to draw any firm conclusions with regard to antiviral response. Furthermore, comparisons with other once-daily regimens are needed to assess the place of this regimen in current treatment options.

In conclusion, the 24-weeks follow-up data of this study indicate favorable pharmacokinetics of an indinavir/ritonavir 1200/400 mg combination as part of a once-daily regimen consisting also of one or two NRTIs. Short-term safety and efficacy were also satisfactory. Long-term follow up is planned to evaluate the durability of these results.

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Chapter 6.

General discussion

Introduction

The overall objective of this thesis was to contribute to the optimization of dosage regimens for antiretroviral protease inhibitors (PIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs) by the application of pharmacokinetics. After a review of the literature, an interlaboratory quality control program was introduced as a means to enable laboratories to assess and improve their ability to analyze PIs and NNRTIs in plasma. Pharmacokinetic studies were performed that aimed to provide pharmacokinetically-based dosing recommendations for (combinations of) antiretroviral drugs. Finally, the feasibility of once-daily administration of two PIs was evaluated in pharmacokinetic studies. This chapter discusses the main findings of these studies and presents perspectives for future studies.

Bioanalysis and interlaboratory quality control

A prerequisite for any pharmacokinetic study and for Therapeutic Drug Monitoring (TDM) is the availability of bioanalytical methods for the drugs in question. **Chapter 2.1** presented a review of HPLC methods for analysis of PIs in human biological matrices. This review summarized analytical techniques that have been applied for sample pretreatment, chromatographic separation and detection of PIs, and discussed the requirements for HPLC measurement of these drugs in terms of accuracy, sensitivity and selectivity of the method. The review suggested variability between laboratories with respect to the rigour of intralaboratory method validation. This prompted the development of an international interlaboratory quality control (QC) program for measurement of antiretroviral drugs in plasma (**chapters 3.1 and 3.2**). The first rounds of this newly established QC program defined the state-of-the-art in terms of analytical methodology for and performance of laboratories in measurement of PIs and NNRTIs, and evaluated sources of error in analytical performance. Most importantly, the program alerted laboratories to unknown deficiences in their methods, and this enabled them to undertake corrective actions.

The results of the first rounds of the interlaboratory QC program revealed large variability in the ability of laboratories to measure PIs and NNRTIs accurately. In the third round of the program (chapter 3.2), 82% of all measurements performed by 30

laboratories were considered satisfactory, i.e. within 80-120% limits for accuracy. Nineteen of 30 laboratories (63%) reported at least 80% of their results within the acceptance range. These results can be considered as indicative for the performance of laboratories, considering that every round of the program represented a comprehensive set of measurements for multiple drugs at three concentration levels. Moreover, some general limitations to the validity of a QC program as an indicator for laboratory performance were not applicable [1,2]. That is, the risk of disturbing matrix effects was limited since all samples were prepared in human plasma and were not lyophilized, and criteria for a satisfactory measurement were well-founded and accepted by all participants. However, it can not be excluded that laboratories focused an inordinate amount of effort to the measurement of the QC samples, compared to real samples [1,2]. If this is taken into consideration, it can only be concluded that too many laboratories had an unsatisfactory performance in the first rounds of the program. In pharmacokinetic studies, this could lead to assessment of incorrect pharmacokinetic parameters. In TDM, inaccurate measurements could result in inappropriate dose adjustments or the advice not to adjust doses where it might actually be desirable.

The reasons for unsatisfactory measurements were inquired by the participating laboratories themselves as part of the third round of the program (chapter 3.2). A common reason for inaccurate measurements appeared to be an insufficient validation of analytical methods. Several methods for particular PIs and/or NNRTIs were not tested for interference by other antiretrovirals. Since the interlaboratory QC samples contained more than one drug, the drugs affected eachother's assay. In this way the design of the program may have caused an overestimation of the number of unsatisfactory measurements, but these findings also highlighted that the specificity and selectivity of these methods were not (appropriately) validated. Another deficiency in method validation was reflected in the significantly worse performance for measurement of low plasma concentrations versus medium and high concentrations. This indicates that several methods were not suitable for measurement of low (trough) plasma concentrations, or lower limits of quantitation were not well validated. Interestingly, review of the literature with respect to HPLC analysis of PIs (chapter 2.1) already suggested that some methods had deficiencies with respect to the validation parameters specificity/selectivity and limit of quantitation. Apparently, there is some

scope for individual interpretation with respect to the conduct and acceptance criteria for a validation procedure in the bioanalysis of drugs, despite (or maybe due to) the availability of many guidelines for validation of bioanalytical methods.
Apart from inadequate method validation, laboratories reported other sources of error, such as dilution and pipetting errors, acceptance of runs even though controls were out of range, and use of ageing stock solutions. These errors reflect deficiencies in regular intralaboratory quality control.

The results of the first rounds of the QC program raise the question how the overall performance of laboratories in measuring antiretroviral drugs could be improved. Clearly, laboratory directors have the prime responsibility to ensure that critical validation parameters are evaluated appropriately before a method is put into use. It seems that scientific journals could verify this more carefully before a method is accepted for publication. A continuing role remains for this (and other) programs as a tool to alert laboratory directors to unrecognized deficiencies in their quality assurance systems. Whereas participation in the program is voluntary at this moment, it could be questioned whether this is desirable in the long-term, considering the importance of bioanalysis for pharmacokinetic studies and TDM. In the interest of patients, it seems justified (and useful, based on data from the USA [3]) to use interlaboratory QC not only as an educational tool, but also as a regulatory tool. As for the current program, subsequent steps in this direction would be the definition of specific criteria for an acceptable performance, the issue of certificates after an adequate performance over time, the mandated (rather than recommended) participation in the program (or similar programs) and, finally, the accreditation of laboratories for measurement of antiretroviral drugs, based upon performance in QC programs and other criteria. Some of these steps are in the hands of regulatory authorities. The combined use of interlaboratory QC both as an educational and regulatory tool requires courage from laboratories to allow interlaboratory QC to detect errors in the short-term, in order to profit from it in the long run, and a judicious response by regulatory authorities when failure occurs [1]. In the short term, the (educational) role of the current program could be improved by an increase in the frequency of send-arounds, an extension of the program to measurement of NRTIs, and incorporation of the post-analytical stage of antiretroviral drug measurements. The latter issue means that the program includes QC samples that are accompanied by a real-life TDM casus that should be interpreted by the laboratory.

Drug-drug and drug-food interactions

The studies in chapters 4 and 5 aimed to provide dosing recommendations for (combinations of) antiretroviral drugs. All these studies evaluated pharmacokinetic interactions of PIs and NNRTIs. The studies in chapter 4 focused on potentially undesirable interactions, whereas the studies in chapter 5 actually exploited the interactions between PIs to achieve once-daily administration of these drugs. All studies involved PIs that were combined with low-dose ritonavir (boosted PIs). The interactions that were studied can be classified as interactions between an individual drug (combination) and food (chapters 4.1 and 5.1), interactions between antiretroviral drugs (chapters 4.2-4.4 and 5.1-5.3), and interactions between antiretroviral drugs and concomitantly administered drugs (chapter 4.5). The interactions involved alterations in absorption (chapters 4.1 and 5.1) or metabolism (chapters 4.2-4.5, 5.1-5.3) of the agents that were co-administered. The relevance of the interactions was evaluated by comparison of the obtained pharmacokinetic parameters (especially the area under the curve, AUC; the peak plasma concentration, Cmax; and the trough concentration, Cmin) to reference values. These reference values were either the mean pharmacokinetic parameters for a drug (combination) in an approved dosing scheme that is known to confer adequate efficacy and limited toxicity, or they were threshold values that have been derived from pharmacokinetic-pharmacodynamic relationships.

Interactions between antiretroviral drugs and food

The studies described in chapters 4.1 and 5.1 assessed the effect of food (or the amount of food) on the bioavailability of indinavir/ritonavir 800/100 mg BID or once-daily nelfinavir/ritonavir combinations. In the study in **chapter 4.1** it was found that intake of indinavir/ritonavir with a light meal decreased the rate, but not the extent, of absorption of indinavir, compared to intake on an empty stomach. A decrease in the absorption rate of drugs is the most commonly observed effect of food [4]. It can be explained by a food-induced delay in gastric emptying [4-6]. Although this effect is generally of minor clinical significance, it could be relevant for indinavir, since available data suggest an association between indinavir Cmax values and nephrotoxicity [7-16]. Thus, based on this study, it was recommended that indinavir/ritonavir 800/100 mg should preferably be taken with food. A heavy, high

caloric meal is expected to delay gastric emptying more strongly than the light meal that was used in this study [5,6] and could therefore induce a further reduction in indinavir Cmax.
In the study in **chapter 5.1**, it was assessed that intake of once-daily combinations of nelfinavir and ritonavir with a light meal resulted in a decrease in the extent of absorption of nelfinavir, compared to administration with a full meal. Since plasma concentrations of nelfinavir are only slightly higher than proposed therapeutic thresholds [17,18], it was recommended that once-daily combinations of nelfinavir plus ritonavir should be taken with a full meal. The mechanism of the effect of food on the bioavailability of nelfinavir/ritonavir remains unknown. In theory, this effect may have been exerted in each step of the bioavailability pathways of nelfinavir and ritonavir, from tablet disintegration, dissolution of the drugs, transit through the gastro-intestinal tract, to metabolic transformation in the gastro-intestinal wall and liver [4-6]. The lack of data on food-effects of PIs continues to raise questions in everyday clinical practice. In fact, the minimum requirements for a meal to guarantee adequate absorption are still unknown for all available PIs. There is a need for comprehensive food-effect studies that evaluate such minimum requirements and the effect of determinants such as fat content, caloric content, volume of food and amount of fluids on the bioavailability of PIs.

Interactions between antiretroviral drugs

The studies in chapters 4.2-4.4 included both healthy volunteers and HIV-infected patients to evaluate the pharmacokinetics of the same dual PI plus NNRTI combination of indinavir/ritonavir 800/100 mg BID and efavirenz. Such combinations of PIs and NNRTIs could be applied as first-line therapy to spare the class of NRTIs and to prevent toxicity related to NRTIs [19]. In addition, PI/NNRTI combinations are increasingly used as second-line therapy (with or without NRTIs) in patients who are resistant or intolerant to NRTIs [19]. Blood sampling schemes and bioanalytical and pharmacokinetic methodology were similar across the studies in chapters 4.2-4.4.

The combination of indinavir/ritonavir plus efavirenz was first studied in healthy volunteers who were all Caucasian and male (**chapter 4.2**). It was concluded that efavirenz decreases the plasma concentrations of indinavir in the indinavir/ritonavir

combination, but not to an extent that dose modifications are required for treatment-naive patients. However, it could not be excluded that such pharmacokinetic data in healthy volunteers would differ from those in HIV-infected patients. For example, it is known that the activity of CYP P450 enzymes can be altered by certain proinflammatory cytokines that are released during inflammation and infection [20]. As to the specific effects of HIV infection, some studies have found differences in zidovudine phosphorylation between healthy volunteers and HIV-infected patients [21,22], and other studies suggested that patients with AIDS might have alterations in specific patterns of drug metabolism [23]. Results from one study indicated that the magnitude of pharmacokinetic interactions could differ between healthy volunteers and HIV-infected patients [24]. Therefore it was desirable to obtain follow-up pharmacokinetic data of the indinavir/ritonavir plus efavirenz combination in HIV-infected patients, and these data were assessed in Caucasian and Asian (Thai) HIV-infected patients (**chapters 4.3 and 4.4**). Most importantly, these assessments confirmed that indinavir Cmin values and efavirenz concentrations were adequate in HIV-infected patients, as predicted by the study in healthy volunteers. Cross-study comparisons suggested that indinavir Cmax and AUC values were higher in Thai patients who used the combination of indinavir/ritonavir plus efavirenz; efavirenz concentrations seemed to be somewhat higher in HIV-infected patients compared to healthy volunteers. An explanation for these apparent differences is complicated by the simultaneous occurrence of several determinants of pharmacokinetic variability in the study participants, i.e. disease, race or ethnicity, gender and body weight. For example, the seemingly higher indinavir Cmax in Thai patients could possibly be explained by genetic (racial) differences in absorption of indinavir (e.g. due to differences in gut or hepatic first-pass effects [25]), but could also be ascribed to interethnic differences in meals that may have affected the rate of absorption of indinavir [25]. Moreover, it could be argued that the high indinavir Cmax values in Thai patients were attributable to their relatively low body weights, associated with lower volumes of distribution. Similarly, the apparently higher efavirenz levels in HIV-infected patients could be explained as an effect of disease, but could also be ascribed to the presence of women (50%) among the included HIV-infected patients. Women generally have higher efavirenz plasma concentrations [26]. Clearly, more research is warranted to elucidate the relative importance of several determinants of pharmacokinetic variability for PIs and NNRTIs. Although the effects of race/ethnicity,

gender, body weight and other determinants could be subtle, they may be relevant for drugs with a narrow therapeutic index such as PIs and NNRTIs. It is not expected that these determinants could change the direction of pharmacokinetic interactions, but the magnitude of interactions could be affected.

The study in **chapter 5.2** also evaluated the effect of an NNRTI (nevirapine) on the pharmacokinetics of a boosted PI (nelfinavir/ritonavir in a once daily dosing scheme). This interaction appeared to be very complex and probably involved multiple hepatic enzymes. The results showed that once-daily nevirapine can be added to once-daily nelfinavir/ritonavir to obtain an entirely once-daily regimen.

The studies in **chapters 5.1 and 5.3** evaluated interactions between PIs and low-dose ritonavir. Ritonavir inhibited the metabolism of these PIs to an extent that once-daily administration was made possible (see next paragraph).

Interactions between antiretroviral drugs and concomitantly administered drugs

There is a large potential for interactions between antiretroviral drugs and concomitantly administered drugs. Especially low-dose ritonavir can be expected to raise levels of many drugs, not just PIs. These drugs include many substrates for CYP2D6. Unfortunately, there is a limit to the number of in vivo interaction studies that can reasonably be performed, and in vitro methods for predicting drug interactions have their limitations [27]. The study in **chapter 4.5** used an in vivo *model drug* approach to investigate the effect of low-dose ritonavir on the activity of CYP2D6. According to such a model drug approach, the elimination of one (or a few) model drugs is studied as a means to predict the elimination of many other compounds [28]. In this case, the effect of low-dose ritonavir on CYP2D6 was evaluated in a pharmacokinetic interaction study with desipramine, a model substrate for CYP2D6, and by assessment of urinary ratios of dextromethorphan and its CYP2D6 mediated metabolite dextrorphan (metabolic phenotyping of CYP2D6) in the presence or absence of ritonavir. The results showed that low-dose ritonavir caused a modest increase in the exposure to desipramine. Based on this finding, it is expected that many CYP2D6 substrates can be combined with low-dose ritonavir without dose-

adjustments. Interestingly, the modest inhibitory effect of low-dose ritonavir on CYP2D6 was not detected by changes in metabolic ratios as assessed by metabolic phenotyping. Thus metabolic phenotyping with dextromethorphan appeared to be less sensitive (i.e. would have required more participants or phenotyping sessions) for the assessment of the effect of ritonavir on CYP2D6 than an intensive pharmacokinetic study with a model substrate. Another (more general) disadvantage of metabolic phenotyping is the difficulty of interpreting the relevance of changes in metabolic ratios of model drugs (probes). In this respect, changes in plasma drug levels, as determined in an intensive pharmacokinetic study with a model substrate, are easier to understand. In any case, each of these two model drug approaches requires (nontoxic) model drugs that are specifically metabolized by a certain metabolic route and have documented predictive value to other drugs. Whereas such model drugs are available for CYP2D6, they are not available for all hepatic enzymes at this time. In addition, many drugs do affect multiple enzymes whereas other drugs are biotransformed by multiple pathways. This means that the model drug approach often requires several concomitantly administered model drugs. In the end, it will probably be difficult to predict many interactions. This means that direct pharmacokinetic evaluations of interacting drugs will remain essential to yield information that is clinically applicable.

Once-daily administration of protease inhibitors

The studies in chapter 5 evaluated the feasibility of once-daily administration of the PIs nelfinavir and indinavir by combining these drugs with low-dose ritonavir. In this way, pharmacokinetic interactions were exploited as a means to modulate adherence, an important determinant of treatment response to HAART.

Each of the studies in chapter 5 represents a stage that needs to be passed through before a once-daily combination can be applied in clinical practice. Firstly, explorative studies should reveal whether once-daily administration of an individual antiretroviral drug is feasible at all, based on pharmacokinetic data and a short-term evaluation of safety and tolerability in healthy volunteers. **Chapter 5.1** described such an explorative study for once-daily combinations of nelfinavir plus low-dose ritonavir. As a next step in the development of a once daily HAART regimen, it should be considered that potential once-daily agents can not always be assembled in an entirely

once-daily regimen, due to pharmacokinetic interactions or different food restrictions. **Chapter 5.2** described the pharmacokinetic interaction between once-daily nelfinavir/ritonavir combinations and once-daily nevirapine. Based on these studies in healthy volunteers, a once-daily nelfinavir/ritonavir regimen could now be tested in HIV-infected patients. **Chapter 5.3** described such a study, but for the once-daily combination of indinavir plus low-dose ritonavir. The favourable results of this study pave the way for comparative (e.g. once-daily versus twice-daily) long-term trials that would constitute the final step in the development of this once-daily indinavir/ritonavir combination. Such trials should incorporate adequate assessments of adherence, as it remains unknown whether once-daily administration of HAART really improves adherence. Data for other chronic diseases suggest advantages for once-daily versus twice-daily administration of drugs, both in terms of doses taken and timing-accuracy, but these differences did not reach statistical significance [29].

Future trials should also include pharmacokinetic substudies, since there are several pharmacokinetic issues concerning once-daily administration that need to be resolved. Firstly, there is a lack of data about pharmacokinetic-pharmacodynamic relationships after once-daily administration of antiretroviral drugs. For PIs, it is assumed that the 24-h Cmin value is the most important pharmacokinetic parameter associated with therapeutic response, consistent with pharmacokinetic-pharmacodynamic relationships obtained for twice- and thrice-daily administration of these drugs. However, it is unknown whether the same Cmin threshold would apply to once-daily and twice- or thrice-daily dosing regimens of a PI. More specifically, thresholds for Cmin could possibly be lower after once-daily administration, considering that Cmin levels occur only once every 24 h in a once-daily dosing scheme. In the absence of data, the studies in chapters 5.1 and 5.2 used conservative target values for the 24-h Cmin of nelfinavir and M8, i.e. target values that were similar to mean Cmin values after twice daily-administration of nelfinavir without ritonavir. Similar to therapeutic response, there is uncertainty about the relationship between pharmacokinetics and toxicity in once-daily combinations. For example, the once-daily indinavir/ritonavir combination (chapter 5.3) was associated with a low incidence of indinavir-related nephrolithiasis, despite high indinavir Cmax and AUC values. This could possibly be explained by the occurrence of just one Cmax every 24 h.

A second pharmacokinetic issue relates to the consequences of missing a dose in a once-daily dosing scheme. Some argue that this results in a prolonged period of

suboptimal drug exposure, compared to missing a dose in a twice-daily scheme [30,31]. However, the forgiveness of a regimen is dependent on the pharmacokinetic properties of the drugs involved, not on the number of times a drug is dosed. In this respect, a long elimination half-life could be advantageous, as long as plasma levels remain above concentration windows that favor resistance development [32]. On the other hand, a long half-life could actually be undesirable if plasma levels are only slightly higher than concentration windows that are associated with the emergence of resistance. In this case it would be desirable if drug concentrations declined rapidly through these windows [33]. Pharmacokinetic studies should reveal whether individual PIs and NNRTIs differ in this respect. In the end, only clinical trials can demonstrate whether improved adherence afforded by once-daily dosing outweighs the consequences of missing a dose.

As a third issue, there is concern that interindividual variability in key pharmacokinetic parameters may be increased after once-daily administration of antiretroviral drugs. Lopinavir Cmin values showed substantially greater interpatient variability among patients receiving the once-daily regimen versus the twice-daily combination [34]. In contrast, variability in indinavir Cmin after once-daily administration of indinavir (chapter 5.3) appeared to be similar to variability observed after thrice-daily administration of this drug.

Therapeutic Drug Monitoring

The concept of TDM goes beyond the application of pharmacokinetics in the design of fixed dosing regimens for the average patient. It seeks to individualize drug dose, guided by measurement of plasma drug concentrations. **Chapter 2.2** provided a review of the prospects, limitations, clinical trial results and recent developments with respect to this ultimate application of pharmacokinetics in HIV-infection. Data from two clinical trials support the use of TDM in treatment-naive patients who start with an indinavir- or nelfinavir-based regimen. Application of TDM in other patient groups (treatment-experienced patients) or for other drugs (other PIs, NNRTIs) is speculative at this moment and warrants further study. Trials in treatment-naive patients can probably no longer be performed in some European countries such as the Netherlands and France, where TDM for antiretroviral drugs is widely available. Studies in these countries could focus on the combined application of TDM and resistance testing as a

means to optimize response in treatment-experienced patients. Large TDM-studies in treatment-naive and experienced patients will likely provide more insight in pharmacokinetic-pharmacodynamic relationships (especially the target values to be used) for antiretroviral drugs. In turn, this information could be applied in the design of fixed dosing regimens, demonstrating the close association between these applications of pharmacokinetics to optimize dosing regimens.

Methodological issues

Study design

Several studies in chapters 4 and 5 can be categorized as experimental studies. Some experiments were designed to test a hypothesis (chapters 4.1, 4.2, 4.5, 5.2), whereas one experimental study was explorative in nature (chapter 5.1). The studies in chapters 4.3, 4.4 and 5.3 are descriptive pharmacokinetic studies that were performed in the context of a clinical experiment (trial).

The studies that were designed to test a hypothesis were typical drug interaction studies. In drug interaction studies, drugs that cause a change in the pharmacokinetics or pharmacodynamics of another drug are denoted as "precipitant drugs", whereas drugs affected by the precipitant drug are designated as "object drugs" [35]. Just like other experiments, drug interaction studies can either have a parallel design, with one group of subjects receiving the object drug and another group receiving the object drug *plus* the suspected precipitant drug, or a self-controlled design in which participants receive both treatments. The latter self-controlled (or within-subject, or paired) design was chosen for the studies in chapters 4.1, 4.2, 4.5 and 5.2. This was motivated by the large interindividual variability in pharmacokinetic parameters of antiretroviral drugs and the efforts that are required to characterize the pharmacokinetics of just one patient. A self-controlled design controls for variability among patients, and therefore requires much less patients and less pharmacokinetic assessments to obtain the same statistical power [35,36]. The self-controlled studies in chapters 4.1, 4.2, 4.5 and 5.2 were all two-period (nonreplicated) studies; the number of treatments was equal to the number of study periods.

A two-period self-controlled interaction study can be performed in two ways [35,36]. The studies in chapters 4.2, 4.5 and 5.2 had a fixed-order design in which the participants received the object drug in the first study period (A) and the object plus precipitant drug in the second period (A+B). The alternative design is a crossover study in which the participants are randomized over two sequences, sequence A - A+B, or sequence A+B - A. This design was used in chapter 4.1 with food as precipitant agent. Each of these two designs have advantages and disadvantages [35,36]. The advantage of a two-period fixed-order design is that the precipitant drug is never administered in the first period. This means that the effect of this drug (e.g. inhibition or induction of CYP P450 enzymes) will never persist in the second period (no carry-over effect – i.e. treatment-by-period interaction). This is advantageous when the occurrence of carry-over or the duration of an adequate washout period is unknown a priori; in fact, this was the main reason to use a fixed-order design in the study described in chapter 4.5. As a second advantage, a fixed-order design is very efficient when the object and/or precipitant drug are to be studied at steady-state. Steady-state achieved in the first study period can be maintained in the second period, as no wash-out period (to exclude carry-over) is usually required. This was the main reason to use a fixed-order design in the studies described in chapters 4.2 and 5.2. An important disadvantage of a fixed-order design is that any apparent treatment effect could have been caused by the precipitant drug, but also by some other intercurrent difference between the study periods (period effect).
The advantage of a crossover design is that it allows for detection of (and correction for) period effects [37]. The disadvantage of a crossover design is that differential carry-over could occur [35-37]. A carry-over effect can be tested for, but the statistical power of this test is low with the sample size used in crossover studies [37]. Therefore the crossover design can only be used if the assumption of no carry-over is absolutely valid on theoretical grounds, and this condition was met in the study in chapter 4.1.

Pharmacokinetic analysis and statistical analysis

The studies in this thesis were individual-based pharmacokinetic studies, i.e. studies in which pharmacokinetic estimates for each individual subject were assessed after sampling of the subject at a series of prespecified times. The pharmacokinetic analysis

was performed using noncompartmental methods; these methods can be applied irrespective of the distribution characteristics of the drugs involved [38].

Several studies in this thesis were designed to test hypotheses based on the pharmacokinetic data. Two statistical approaches were used, a traditional analysis (chapters 4.1 and 4.2) and a bio-equivalence approach (chapters 4.5 and 5.2). According to the traditional analysis, the objective is to reject a null hypothesis of no difference (pharmacokinetic parameters for the object drug(s) are equivalent in the presence or absence of the precipitant drug, i.e. lack of interaction) versus an alternative hypothesis (there is a pharmacokinetic interaction). The disadvantages of this approach are that a clinically important difference may not be statistically significant if the sample size is small and within- and between-subject pharmacokinetic variability is large, whereas a clinically irrelevant difference could become statistically significant. Therefore it is said that the traditional approach does not adequately control the consumer risk of an incorrect conclusion of "lack of interaction", nor the producer risk of an incorrect conclusion of "interaction" [35,39]. In the studies in chapters 4.1 and 4.2 significant differences were found and these were either large and clinically relevant (chapter 4.2) or interpreted cautiously (chapter 4.1).
For the studies in chapters 4.5 and 5.2 it was decided to use the bioequivalence approach that better controls the consumer and producer risks. According to this approach, the null hypothesis is inequivalence and the alternative hypothesis of equivalence is defined by a prespecified equivalence criterion and limits [40]. In the studies in chapters 4.5 and 5.2, equivalence (lack of interaction) was concluded when the 90% confidence interval of the geometric mean ratio for a specific pharmacokinetic parameter was contained within 80-125% limits, or a similar statement.
The bioequivalence approach has been elaborated further in the past years. New approaches for bioequivalence testing, the population approach and the individual approach, have been included in recent guidelines for bioequivalence testing [40]. These developments will likely influence the execution and evaluation of interaction studies in the near future.

Final remarks

The studies in this thesis lead to the introduction of an interlaboratory QC program for measurement of PIs and NNRTIs in plasma. This program highlighted large variability in the performance of laboratories in measuring antiretroviral drugs, revealed common sources of error, and appeared to be useful in alerting laboratories to unknown deficiencies in their analytical methods. In addition, studies in this thesis have provided pharmacokinetically-based dosing recommendations for the mode of administration of indinavir/ritonavir 800/100 mg, for the combined use of indinavir/ritonavir 800/100 mg plus efavirenz, and for combination of low-dose ritonavir with CYP2D6 substrates. Studies in healthy volunteers provided a basis for once-daily administration of nelfinavir/ritonavir and nelfinavir/ritonavir plus nevirapine, and once-daily administration of indinavir/ritonavir was supported by pharmacokinetic, safety and efficacy data in HIV-infected patients.

In the near future, the interlaboratory QC program should be continued as an educational tool, but its additional value as regulatory tool should be explored. The frequency of send-arounds could be increased, and the program could be extended to measurement of NRTIs and to interpretation of drug levels in the context of TDM. More food-effect studies should be performed to assess the minimum requirements for a meal to achieve adequate absorption of PIs. The introduction of new antiretroviral drugs, the combined use of antiretroviral drugs, and the wide use of low-dose ritonavir will continuously require drug interaction studies. Results from drug interaction studies in healthy volunteers should be confirmed in HIV-infected patients, and dosing recommendations based upon such studies should be validated. More research is warranted to elucidate the relevance of other determinants of pharmacokinetic variability besides the occurrence of drug interactions. Clinical trials that evaluate once-daily dosing regimens should incorporate pharmacokinetic assessments to obtain more data about pharmacokinetic-pharmacodynamic relationships after once-daily administration of antiretroviral drugs. The consequences of missing a dose in a once-daily regimen should be evaluated and interindividual variability in the pharmacokinetics of once-daily dosing schemes should be assessed. Finally, more studies are warranted to assess the value of TDM for antiretroviral drugs other than indinavir and nelfinavir, and for treatment-experienced patients. Ultimately it is hoped

that pharmacokinetic optimization of fixed dosing regimens and individualization of doses by plasma concentration measurements will help to improve the response to HAART.

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Summary

The introduction of protease inhibitors (PIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs) has allowed for treatment of HIV-infected patients with "highly active antiretroviral treatment" (HAART). Unfortunately, response to HAART is suboptimal. There is convincing evidence for PIs and accumulating evidence for NNRTIs to conclude that the plasma concentration of these drugs is a better correlate of response than the dose. This provides a rationale to evaluate the pharmacokinetic characteristics of these agents and to incorporate this information into the design of dosing regimens. The overall objective of the studies in this thesis was to contribute to the optimization of dosage regimens for antiretroviral drugs by the assessment and interpretation of pharmacokinetic characteristics of these agents, i.e. by the application of pharmacokinetics. Optimized dosage regimens are expected to improve the response to antiretroviral drugs.

Chapter 2 presented a review of the literature on bioanalytical methods for PIs and the practice of Therapeutic Drug Monitoring (TDM) for antiretroviral drugs.
The availability of bioanalytical methods is a prerequisite for any pharmacokinetic study and for TDM. **Chapter 2.1** reviewed the available high-performance liquid chromatographic (HPLC) methods for analysis of PIs in human biological matrices. It summarized analytical techniques that have been applied for sample pretreatment, chromatographic separation and detection of PIs, and discussed the requirements for a reliable HPLC method.
Whereas adequate bioanalytical methods provide the foundation for pharmacokinetic studies, TDM may be considered the ultimate application of pharmacokinetics in therapeutics. TDM is the individualization of the dose of a drug guided by measurement of plasma drug concentrations. **Chapter 2.2** presented a review on the practice of TDM for antiretroviral drugs. Clinical trials support the use of TDM for treatment-naive patients who start with an indinavir- or nelfinavir based regimen. In addition, TDM appears to be worthwile in certain selected patient groups. More clinical trials are needed to assess the value of TDM for patients who take other PIs or NNRTIs, and for patients who are treatment-experienced.

Since plasma concentration measurements are essential for any pharmacokinetic study and for TDM, an international interlaboratory quality control (QC) program was developed to enable laboratories to assess and improve their performance with respect to these measurements. The studies in chapter 3 described the design and first results of this QC program. **Chapter 3.1** described the results of the first round, that was confined to nine laboratories and to measurements of PIs. The results of the mature program, as reflected in the third round, are described in **chapter 3.2.** The program revealed large variability in the ability of laboratories to measure PIs and NNRTIs accurately. Measurement of these drugs needs to be improved in about one third of the laboratories that participated in the program. The performance in measurement of low plasma concentrations was worse than for medium or high concentrations. Sources of error were inquired and appeared to be inadequate method validation and common deficiencies in intralaboratory quality assurance. By participating in the program, laboratories were alerted to previously unknown errors in their methods, and this may enable and incite them to optimize their analytical methods or intralaboratory quality assurance system.

The studies in chapter 4 aimed to provide pharmacokinetically-based dosing recommendations for some existing antiretroviral drugs (or drug combinations) when used alone, in combination with other antiretroviral drugs, or when administered with co-medicated agents. These studies evaluated pharmacokinetic interactions that were potentially undesirable.
The study in **chapter 4.1** assessed the effect of food on the peak plasma concentration (Cmax) of indinavir when administered in an indinavir/ritonavir (800/100 mg twice-daily) combination in HIV-infected patients. High indinavir Cmax values have been associated with indinavir-related nephrotoxicity. Administration of indinavir/ritonavir on an empty stomach resulted in a higher indinavir Cmax and a trend to a shorter time to Cmax (tmax) compared to administration with a light meal. Stated the other way round, intake with a light meal reduced indinavir Cmax. This probably reflected a food-induced delay in the absorption of indinavir. The mode of administration of indinavir/ritonavir did not affect the indinavir area under the concentration versus time curve (AUC) or Cmin, nor the urinary excretion of indinavir. Based on this study it is recommended to administer indinavir/ritonavir 800/100 mg with food, as a possible means to prevent indinavir-related nephrotoxicity in patients

who start or continue with this regimen.
Chapters 4.2, 4.3 and 4.4 were devoted to the pharmacokinetics of a combination of indinavir/ritonavir (800/100 mg twice-daily) combined with efavirenz 600 mg once-daily. The study in **chapter 4.2** assessed the effect of multiple-dose efavirenz on the steady-state pharmacokinetics of indinavir/ritonavir in healthy volunteers. The addition of efavirenz resulted in significant decreases in the AUC, Cmax, and especially Cmin of indinavir. However, all indinavir Cmin levels remained equivalent to or above the mean Cmin value described for the approved regimen of 800 mg indinavir three times a day, without ritonavir. Based on these findings, it was recommended that the dose of indinavir or ritonavir should be increased to maintain similar indinavir concentrations after addition of efavirenz to the indinavir/ritonavir combination. It was argued that dose modifications might not be needed in antiretroviral-naive HIV-infected patients, since the indinavir Cmin appeared to remain sufficiently high for these patients.
Follow-up pharmacokinetic assessments to the study in chapter 4.2 were performed in HIV-infected patients, considering that the pharmacokinetics of healthy volunteers and HIV-infected patients may differ. The study in **chapter 4.3** assessed the steady-state pharmacokinetic parameters for the combination of indinavir/ritonavir plus efavirenz (without dose modifications) in a small group of predominantly treatment-naive patients who were included in the EASIER study. Indinavir plasma concentrations were similar to values previously observed in the healthy volunteers, but efavirenz concentrations appeared to be higher. The treatment response of the patients to the antiretroviral combination was satisfactory. This supports the conclusion that co-administration of indinavir/ritonavir and efavirenz results in concentrations of both indinavir and efavirenz that are adequate for treatment-naive patients.
The study described in **chapter 4.4** also evaluated the steady-state pharmacokinetics of indinavir/ritonavir plus efavirenz, but in a larger group of Thai HIV-infected patients. Again, all participants in this study recorded an indinavir Cmin that was at least comparable to the reported mean population Cmin for indinavir 800 mg thrice daily without ritonavir. The Thai patients had an elevated indinavir Cmax compared to the Caucasian healthy volunteers who were studied in the study in chapter 4.2. This may be related to their lower body mass, but could also be ascribed to interethnic differences in meals.
Taken together, the studies in chapters 4.3 and 4.4 confirm the pharmacokinetic data for the indinavir/ritonavir plus efavirenz combination as obtained in healthy volunteers

(chapter 4.2). The three studies together provide a strong pharmacokinetic basis for the application of this combination regimen in HIV-infected patients.
The study in **chapter 4.5** was performed to assess the effect of low-dose ritonavir on the activity of cytochrome P450 isoenzyme CYP2D6 in vivo. Many drugs are metabolised by this isoenzyme and could possibly interact with low-dose ritonavir. The results of this study showed that low-dose ritonavir (100 mg twice-daily) exerts a modest inhibitory effect on the activity of CYP2D6 in extensive metabolizers for CYP2D6. Therefore it is expected that no standard dose reductions are required if CYP2D6 substrates that are largely metabolized by CYP2D6 and have a narrow therapeutic index are combined with low-dose ritonavir. Patients who take such a combination should be closely monitored for adverse reactions to the CYP2D6 substrate. The effect of low-dose ritonavir on CYP2D6 appears to be clinically irrelevant for CYP2D6 substrates that are only partly metabolized by this iso-enzyme or that have a wide therapeutic index.

The studies in chapter 5 aimed to provide dosing recommendations for new PI-based dosing regimens that can be administered once-daily. The interactions that were studied in this chapter were desirable rather than undesirable.
The study in **chapter 5.1** evaluated the steady-state pharmacokinetics, food intake requirements and short-term tolerability of once-daily combinations of nelfinavir and low-dose ritonavir in healthy volunteers. Co-administration of nelfinavir and ritonavir resulted in appreciable concentrations of nelfinavir and M8 (the active metabolite of nelfinavir) throughout the 24-h dosing interval. Mean AUC and Cmin values of nelfinavir plus M8 were at least comparable to reference values for the approved regimen of nelfinavir 1250 mg twice daily without ritonavir. Administration of the nelfinavir/ritonavir combinations with a light meal (300 kcal) reduced the bioavailability of nelfinavir compared to administration with a full meal (600 kcal). Short-term tolerability of the nelfinavir/ritonavir combinations was satisfactory, apart from a higher than expected incidence of mild rash. It was concluded that once-daily administration of nelfinavir is feasible. A once-daily nelfinavir/ritonavir 2000/200 mg combination appeared most appropriate for further evaluation, and this combination should be taken with a full meal.
The study in **chapter 5.2** was an extension of the study in chapter 5.1 and assessed the influence of once-daily nevirapine on the pharmacokinetics of the once-daily

nelfinavir/ritonavir combinations. The AUC and Cmin values of nelfinavir and nelfinavir plus M8 combined with nevirapine were at least comparable to plasma concentrations without nevirapine. This means that once-daily nelfinavir/ritonavir and nevirapine can be combined to obtain an entirely once-daily dual PI plus NNRTI regimen. A combination of nelfinavir/ritonavir 2000/200 mg plus nevirapine 400 mg once-daily seemed preferable, since this combination results in adequate plasma concentrations of nelfinavir and M8, whereas exposure to ritonavir is limited compared to a combination of nelfinavir/ritonavir 2000/400 mg plus nevirapine. The study in **chapter 5.3** investigated the pharmacokinetics and short-term (24 week) safety and efficacy of a once-daily indinavir/ritonavir combination in treatment-naive and treatment-experienced HIV-infected patients. The participants in this study received a regimen consisting of indinavir 1200 mg, ritonavir 400 mg, and one or two nucleoside reverse transcriptase inhibitors, all to be administered once-daily with food. A pharmacokinetic evaluation in this study revealed that the mean indinavir Cmax value of the once-daily combination was high, and the mean Cmin was comparable to the Cmin reported for the approved thrice-daily regimen of indinavir. The high indinavir Cmax value did not result in nephrolithiasis. The short-term treatment response was adequate, both in treatment-naive and -experienced patients. Long-term follow-up is planned to evaluate the durability of the favorable response to this entirely once-daily antiretroviral combination.

Chapter 6 discussed the main findings of the studies in this thesis and presented perspectives for future studies.

Samenvatting

De ontwikkeling van proteaseremmers (PIs) en non-nucleoside reverse transcriptase remmers (NNRTIs) heeft het mogelijk gemaakt om HIV-geïnfecteerde patiënten te behandelen met krachtige combinatietherapie ("highly active antiretroviral treatment", HAART). Helaas blijkt de therapeutische respons op HAART suboptimaal te zijn. Voor de PIs is er overtuigend bewijs dat de respons op deze geneesmiddelen beter wordt voorspeld door de bereikte plasmaconcentraties dan door de gegeven dosis. Ook voor de NNRTIs zijn er aanwijzingen voor een relatie tussen plasmaconcentratie en effect. Dergelijke concentratie-effect relaties vormen de rationale voor onderzoek naar de farmacokinetische eigenschappen van PIs en NNRTIs en voor het gebruik van farmacokinetische gegevens bij het vaststellen van doseerregimes voor deze geneesmiddelen. De doelstelling van de onderzoeken in dit proefschrift was om bij te dragen aan de optimalisatie van doseerregimes van PIs en NNRTIs door het vaststellen en interpreteren van de farmacokinetische eigenschappen van deze geneesmiddelen en hun combinaties. Geoptimaliseerde doseerregimes worden geacht de respons op PIs en NNRTIs te verbeteren.

Hoofdstuk 2 geeft een literatuuroverzicht van analysemethoden voor PIs in biologische matrices en van het gebruik van Therapeutic Drug Monitoring (TDM) van antiretrovirale geneesmiddelen.
De beschikbaarheid van adequate analysemethoden is een voorwaarde voor elk farmacokinetisch onderzoek en voor TDM. **Hoofdstuk 2.1** beschrijft de beschikbare HPLC methoden voor bioanalyse van PIs. Dit hoofdstuk beschouwt diverse analytische technieken voor de voorbehandeling van monsters, voor chromatografische scheiding en voor detectie van PIs, en bespreekt de kenmerken van een betrouwbare HPLC methode voor PIs.
Terwijl geschikte analysemethoden het fundament vormen voor elke farmacokinetisch onderzoek, kan TDM worden beschouwd als de meest geavanceerde toepassing van farmacokinetiek in de behandeling van patiënten. TDM is het individualiseren van de dosis van geneesmiddelen aan de hand van metingen van plasmaconcentraties.
Hoofdstuk 2.2 geeft een literatuuroverzicht van TDM voor antiretrovirale geneesmiddelen. Uit klinische trials is gebleken dat TDM zinvol is voor de PIs indinavir

en nelfinavir als deze middelen worden toegepast bij patiënten die niet eerder werden behandeld met antiretrovirale geneesmiddelen. Bovendien lijkt TDM waardevol voor bepaalde geselecteerde patiëntengroepen. Meer klinische trials zijn gewenst om vast te stellen of TDM ook zinvol is bij NNRTIs of bij andere PIs dan indinavir of nelfinavir, alsook bij patiënten die wél zijn voorbehandeld met antiretrovirale geneesmiddelen.

Omdat meting van plasmaconcentraties de basis vormt voor zowel farmacokinetisch onderzoek als voor TDM werd een internationaal kwaliteitscontroleprogramma opgezet voor analyse van antiretrovirale geneesmiddelen in plasma (Hoofdstuk 3). Het hoofddoel van dit programma was om laboratoria in staat te stellen de kwaliteit van hun analyses te toetsen en waar mogelijk te verbeteren. **Hoofdstuk 3.1** beschrijft de resultaten van de eerste ronde van het kwaliteitscontroleprogramma. Deze ronde beperkte zich tot 9 laboratoria en tot meting van PIs. De resultaten van het volgroeide programma (derde ronde) worden beschreven in **hoofdstuk 3.2**. Deze resultaten gaven aan dat er grote verschillen tussen laboratoria bestaan in hun vermogen om plasmaspiegels van PIs en NNRTIs accuraat te meten. De analyse van deze geneesmiddelen diende te worden verbeterd in ongeveer één derde van de deelnemende laboratoria. Lage plasmaconcentraties bleken slechter te worden gemeten dan middelhoge of hoge plasmaconcentraties. De oorzaken van onjuiste metingen werden geïnventariseerd; onvoldoende validatie van analysemethoden en tekortkomingen in reguliere, dagelijkse kwaliteitsborging verklaarden de meeste onjuiste metingen. Door de deelname aan het kwaliteitscontroleprogramma werden laboratoria gewezen op foutenbronnen die tevoren onvoldoende waren onderkend. Deze kennis zou laboratoria in staat moeten stellen (en moeten aansporen) om verbeteringen aan te brengen in hun analysemethoden of kwaliteitsborgingssysteem.

In de onderzoeken in hoofdstuk 4 werden aan de hand van farmacokinetisch onderzoek doseringen (of wijzen van toediening) vastgesteld voor antiretrovirale geneesmiddelen, voor combinaties van antiretrovirale geneesmiddelen, en voor combinaties van antiretrovirale geneesmiddelen en andere geneesmiddelen. De studies in hoofdstuk 4 hadden alle betrekking op ongewenste farmacokinetische interacties. Het onderzoek in **hoofdstuk 4.1** bestudeerde het effect van voedsel op de piek plasma spiegel (Cmax) van indinavir bij gebruik van deze PI in combinatie met een lage dosis ritonavir (indinavir/ritonavir 800/100 mg twee maal daags). Hoge

indinavir Cmax waarden worden in verband gebracht met nefrotoxiciteit op indinavir. De toediening van indinavir/ritonavir op een nuchtere maag resulteerde in een hogere Cmax van indinavir en een trend tot een kortere tijd tot Cmax (tmax) vergeleken met toediening van indinavir/ritonavir met een lichte maaltijd. Andersom geformuleerd leidde inname van indinavir/ritonavir met een lichte maaltijd tot een lagere Cmax van indinavir. Dit kan waarschijnlijk worden verklaard als een vertraging in de absorptie van indinavir ten gevolge van de inname met voedsel. De wijze van toediening van indinavir had geen invloed op de "area under the concentration versus time curve" (AUC), de plasma dalspiegel (Cmin) of de excretie van indinavir in de urine. Op basis van deze resultaten werd geadviseerd om indinavir/ritonavir 800/100 mg mét voedsel in te nemen, als een mogelijke manier om nefrotoxiciteit op indinavir te voorkomen.

De hoofdstukken 4.2, 4.3 en 4.4 waren alle gewijd aan de farmacokinetiek van een combinatie van indinavir/ritonavir 800/100 mg twee maal daags in combinatie met efavirenz 600 mg één maal daags. In het onderzoek in **hoofdstuk 4.2** werd onder gezonde vrijwilligers het effect vastgesteld van herhaalde toediening van efavirenz op de steady-state farmacokinetische parameters van indinavir/ritonavir. Toevoeging van efavirenz aan indinavir/ritonavir leidde tot significante afnames in de AUC, Cmax en met name Cmin van indinavir. Individuele indinavir Cmin waarden bleven echter tenminste gelijkwaardig aan de gemiddelde Cmin die is beschreven voor het geregistreerde doseerregime van indinavir (zonder ritonavir), 800 mg drie maal daags. Naar aanleiding van de resultaten van deze studie werd geconcludeerd dat de dosering van indinavir of ritonavir in de indinavir/ritonavir combinatie verhoogd zou moeten worden om bij gelijktijdig gebruik van efavirenz dezelfde indinavir plasmaspiegels te handhaven. Voor patiënten die niet zijn voorbehandeld met indinavir is een dosisaanpassing wellicht niet nodig, aangezien de indinavir Cmin voor deze patiënten voldoende hoog blijft.

Het onderzoek onder gezonde vrijwilligers beschreven in hoofdstuk 4.2 werd gevolgd door farmacokinetisch onderzoek onder HIV-geïnfecteerde patiënten die dezelfde combinatie van indinavir/ritonavir plus efavirenz (zonder dosisaanpassingen) gebruikten. Er kan namelijk niet worden uitgesloten dat er verschillen bestaan tussen gezonde vrijwilligers en HIV-geïnfecteerden voor wat betreft de farmacokinetiek van antiretrovirale geneesmiddelen. De studie in **hoofdstuk 4.3** onderzocht de steady-state farmacokinetische parameters van de indinavir/ritonavir plus efavirenz

combinatie in een kleine groep van voornamelijk niet-voorbehandelde patiënten die waren geïncludeerd in de EASIER-studie. Indinavir plasma concentraties waren vergelijkbaar met de waarden zoals vastgesteld in gezonde vrijwilligers, maar de concentraties efavirenz leken hoger onder HIV-geïnfecteerde patiënten. De therapeutische respons van de patiënten op de combinatie van indinavir/ritonavir en efavirenz was adequaat. Dit bevestigt dat gelijktijdig gebruik van indinavir/ritonavir plus efavirenz resulteert in plasmaconcentraties van zowel indinavir als efavirenz die voldoende hoog zijn voor niet-voorbehandelde patiënten.
In het onderzoek in **hoofdstuk 4.4** werd opnieuw de steady-state farmacokinetiek van de indinavir/ritonavir plus efavirenz combinatie onderzocht, maar nu in een grotere groep van Thaise HIV-geïnfecteerde patiënten. In dit onderzoek bleken alle deelnemers een indinavir Cmin te hebben die tenminste gelijk was aan de gemiddelde populatie-Cmin voor het doseerschema van indinavir 800 mg drie maal daags zonder ritonavir. Vergeleken met Caucasische gezonde vrijwilligers (hoofdstuk 4.2) hadden de Thaise patiënten een hogere indinavir Cmax. Dit kan worden toegeschreven aan hun lagere lichaamsgewicht of aan interethnische verschillen in de samenstelling van de maaltijd die met indinavir/ritonavir werd ingenomen. De onderzoeken in de hoofdstukken 4.3 en 4.4 bevestigen de farmacokinetische gegevens voor de combinatie van indinavir/ritonavir plus efavirenz in gezonde vrijwilligers (hoofdstuk 4.2). De studies vormen samen een solide farmacokinetische basis voor toepassing van dit combinatieschema in HIV-geïnfecteerde patiënten.
De studie in **hoofdstuk 4.5** onderzocht het effect van lage dosis ritonavir (100 mg twee maal daags) op de activiteit van het cytochroom P450 iso-enzym CYP2D6. CYP2D6 medieert de metabole omzetting van een heterogene groep aan geneesmiddelen. Uit het onderzoek bleek dat lage dosis ritonavir een bescheiden remmend effect heeft op de activiteit van CYP2D6 in "extensive metabolizers" voor CYP2D6. Op grond van dit onderzoek wordt verwacht dat er géén dosisaanpassingen vereist zijn bij gebruik van lage dosis ritonavir (100 mg twee maal daags) samen met CYP2D6 substraten die grotendeels door CYP2D6 worden gemetaboliseerd en ook een nauwe therapeutische breedte hebben. Patiënten die een dergelijke combinatie gebruiken zouden wel moeten worden gemonitord op het optreden van bijwerkingen van het CYP2D6 substraat. Het effect van lage dosis ritonavir op CYP2D6 lijkt in het geheel niet relevant voor CYP2D6 substraten die slechts gedeeltelijk door CYP2D6 worden gemetaboliseerd of CYP2D6 substraten met een breed therapeutisch gebied.

De onderzoeken in hoofdstuk 5 waren erop gericht doseerrichtlijnen vast te stellen voor één maal daagse toepassing van PIs. De interacties die in dit hoofdstuk werden bestudeerd waren niet ongewenst, maar werden juist geëxploiteerd.
De studie in **hoofdstuk 5.1** onderzocht de steady-state farmacokinetische parameters, de voedselvoorschriften voor inname, en de korte termijns-verdraagbaarheid van één maal daagse combinaties van nelfinavir en ritonavir in gezonde vrijwilligers. Gezamenlijke toediening van nelfinavir en ritonavir resulteerde in detecteerbare concentraties van nelfinavir en M8 (de actieve metaboliet van nelfinavir) gedurende het gehele 24-uurs doseerinterval. De gemiddelde AUC en Cmin van nelfinavir *plus* M8 in de één maal daagse combinaties waren tenminste vergelijkbaar met dezelfde waarden voor het geregistreerde doseerregime van nelfinavir zonder ritonavir (1250 mg twee maal daags). Toediening van nelfinavir/ritonavir met een lichte maaltijd (300 kcal) leidde tot een lagere biologische beschikbaarheid aan nelfinavir vergeleken met toediening met een volledige maaltijd (600 kcal). Op grond van dit onderzoek werd geconcludeerd dat één maal daagse toepassing van nelfinavir mogelijk is. Een één maal daagse combinatie van 2000 mg nelfinavir en 200 mg ritonavir lijkt het meest geschikt voor vervolgonderzoek; deze combinatie moet daarbij met een volledige maaltijd worden ingenomen.
Het onderzoek beschreven in **hoofdstuk 5.2** was een vervolg op de studie van hoofdstuk 5.1. Dit onderzoek bestudeerde het effect van één maal daagse toepassing van nevirapine op de farmacokinetiek van één maal daagse nelfinavir/ritonavir combinaties. De AUC en Cmin waarden van nelfinavir en nelfinavir plus M8 mét nevirapine bleken tenminste vergelijkbaar met dezelfde waarden zonder nevirapine. Dit betekent dat één maal daags nelfinavir/ritonavir en één maal daags nevirapine kunnen worden gecombineerd tot een volledig één maal daags combinatieschema van een PI plus een NNRTI. Een combinatie van nelfinavir/ritonavir 2000/200 mg plus nevirapine 400 mg lijkt daarbij te verkiezen, daar deze combinatie resulteert in adequate spiegels van nelfinavir en M8, terwijl de blootstelling aan ritonavir wordt beperkt in vergelijking tot een nelfinavir/ritonavir 2000/400 mg combinatie.
In het onderzoek in **hoofdstuk 5.3** werden de steady-state farmacokinetische parameters en korte-termijns (24-weeks) effectiviteit en veiligheid bestudeerd van een één maal daagse indinavir/ritonavir combinatie in HIV-geïnfecteerde patiënten. De deelnemers aan dit onderzoek gebruikten een volledig één maal daags combinatieschema van indinavir 1200 mg, ritonavir 400 mg en één of twee

nucleoside reverse transcriptase inhibitors, alle in te nemen met voedsel. De gemiddelde Cmax van indinavir in dit één maal daagse regime bleek hoog, en de gemiddelde Cmin voor indinavir was vergelijkbaar met de gemiddelde Cmin bij drie maal daagse toepassing van indinavir zonder ritonavir. Ondanks de hoge indinavir Cmax waarden werd in deze studie geen nefrolithiasis waargenomen. De korte-termijns-effectiviteit van het één maal daagse regime bleek adequaat, zowel in voorbehandelde als niet-voorbehandelde patiënten. De deelnemers aan dit onderzoek zullen verder worden vervolgd om de duurzaamheid van de respons op dit volledig één maal daagse regime vast te stellen.

In **hoofdstuk 6** werden de belangrijkste resultaten van het proefschrift bediscussieerd en mogelijkheden voor toekomstig onderzoek beschreven.

Dankwoord

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Die Studie beschrieben in Kapitel 4.1 war das erste Resultat der Zusammenarbeit (unter dem Namen BONUS) der Forschungsgruppe in Nijmegen mit den Abteilungen für Innere Medizin der Universitäten Bonn und Köln. Im besonderen möchte ich mich bei Dr Jürgen Rockstroh, Dr Jan-Christian Wasmuth (Bonn), Dr Gert Fätkenheuer und Dr Karina Schmitz (Köln) für ihre Zusammenarbeit und Gastfreundschaft bedanken.
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Rob Aarnoutse
september 2003

Curriculum vitae

Rob Aarnoutse werd op 11 oktober 1968 te Nijmegen geboren. In 1987 behaalde hij het diploma Gymnasium ß aan het R.K. Lyceum Dominicus College te Nijmegen. Daarna begon hij met de studie Farmacie aan de Universiteit Utrecht. Zijn afstudeerproject had betrekking op bio-analyse van geneesmiddelen en werd uitgevoerd bij Bio-Pharmacie Servier te Orléans, Frankrijk. In 1992 werd het doctoraalexamen afgelegd en in 1994 het apothekersdiploma behaald. Daarna werkte hij als apotheker in verschillende ziekenhuizen en werd hij opgeleid tot ziekenhuisapotheker in het Albert Schweitzer ziekenhuis te Dordrecht (1996-1999). In 1999 trad hij als ziekenhuisapotheker in dienst bij de Afdeling Apotheek/Klinische Farmacie van het UMC St Radboud te Nijmegen, waar hij nu hoofd van de sectie Laboratorium is. Vanaf 1999 heeft hij gewerkt aan het onderzoek dat heeft geleid tot het voorliggende proefschrift. Hij woont samen met Margreet Filius en is de trotse vader van hun zoon Joep.

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