Clinical pharmacology of the HIV integrase inhibitor raltegravir: drug-drug interactions and pharmacokinetics

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Financial support for the printing of this thesis was kindly provided by
the Department of Pharmacy of the Radboud university medical center.

**Clinical pharmacology of the HIV integrase inhibitor raltegravir:
drug-drug interactions and pharmacokinetics.**
Thesis, Radboud University, Nijmegen, The Netherlands.

ISBN
978-94-028-0023-4

Cover & chapter design
studio ilse van klei, Rotterdam

Lay-out design
Promotie In Zicht, Arnhem

Print
Ipskamp Printing, Enschede

Clinical pharmacology of the HIV integrase inhibitor raltegravir: drug-drug interactions and pharmacokinetics

Proefschrift

ter verkrijging van de graad van doctor
aan de Radboud Universiteit Nijmegen
op gezag van de rector magnificus,
volgens besluit van het college van decanen
in het openbaar te verdedigen
op vrijdag 22 april 2016
om 12.30 uur precies

door

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geboren op 10 augustus 1977
te Groningen

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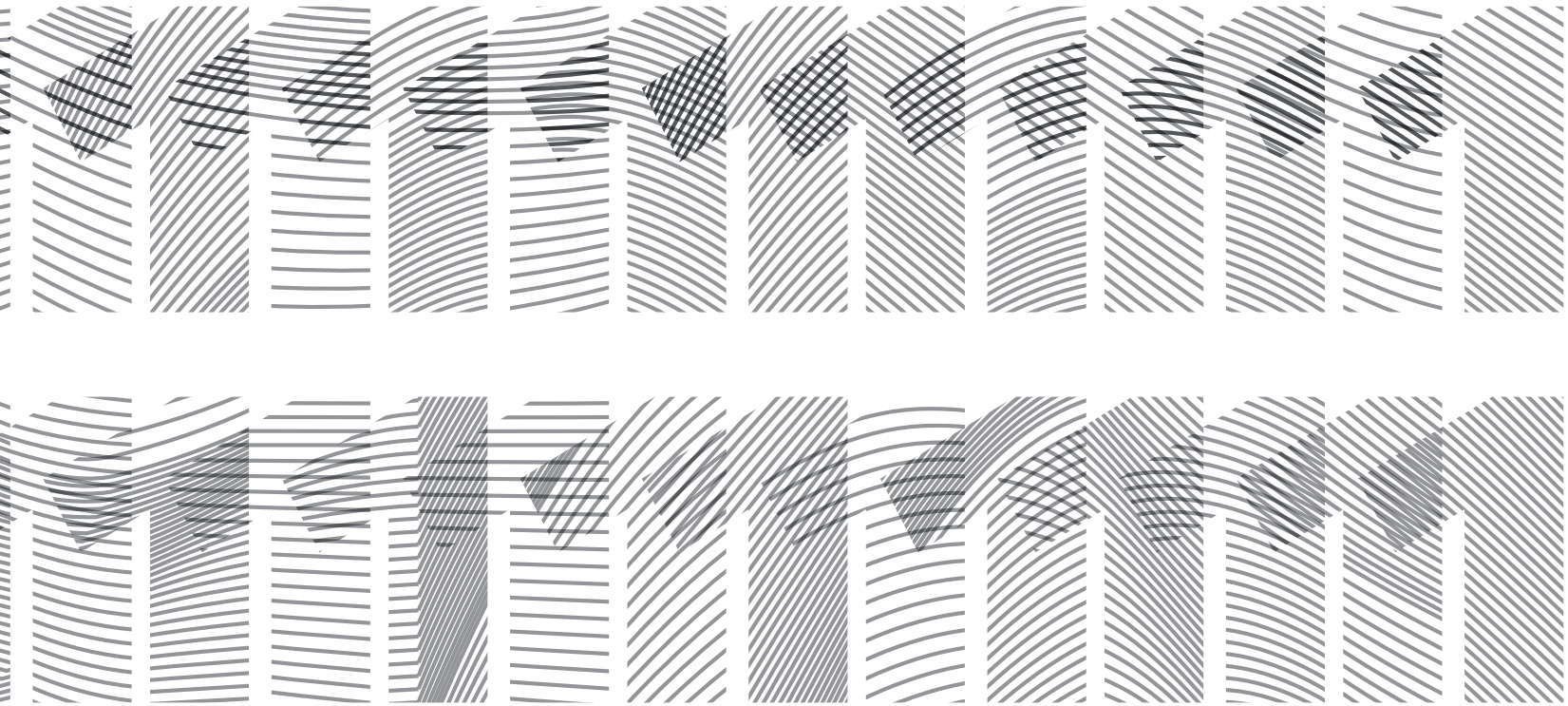
Dr. M. van Luin, Rijnstate

"Every experience is a form of exploration"
- Ansel Adams

Voor mijn vader

Contents



Introduction

The global impact of HIV

Currently, 35 million people are living with human immunodeficiency virus (HIV) worldwide and the HIV population is still growing. Globally, the majority (70%) of the HIV population lives in sub-Saharan Africa. Western and central Europe and North America accounts for approximately 2.3 million HIV-infected patients.

Although the rate of new HIV infections is continuing to decline in most parts of the world, the number of new HIV infections is still very high with more than 2 million new HIV infections in 2013.

Mother-to-child HIV transmission (MTCT) is the most common route of HIV- infection among infants and children. An estimated 1.4 million pregnant women infected with HIV give birth annually worldwide. Each day, approximately 1000 infants acquire HIV due to MTCT during pregnancy, delivery or breastfeeding.[1] Providing access to antiretroviral treatment for pregnant women living with HIV has averted more than 900 000 new HIV infections among children since 2009.[2] Its implementation together with other effective interventions has led to dramatic declines in the number of perinatally HIV-infected children from 15-40% to <2%.[3]

Nowadays, fewer people are dying of acquired immunodeficiency syndrome (AIDS)-related illnesses. In the past three years alone, the number of AIDS-related deaths have fallen by approximately one-fifth which represents the largest decline in the past decade. This is a direct result of the progress that has been made on the global access to antiretroviral treatment. Despite this success AIDS remains one of the top causes of infectious disease-related mortality worldwide, responsible for nearly 1.5 million AIDS-related deaths in 2013.[2]

Antiretroviral treatment

During the past 30 years there has been a remarkable progress in the treatment of HIV infection. The development of combination antiretroviral therapy against HIV is considered one of the great success stories of modern medicine. In the beginning of the pandemic when AIDS was first recognized in 1981, and linked to HIV in 1983, all that could be offered to patients suffering from the complications of AIDS was palliative care and treatment of opportunistic infections.

The first antiretroviral drug that was used for its activity against HIV was zidovudine, a nucleoside reverse transcriptase inhibitor (NRTI). Zidovudine obtained accelerated approval in 1987 by the US Food and Drug Administration (FDA) but failed to give a sustained virological suppression when used as a single drug (monotherapy) as HIV

quickly developed resistance to zidovudine.[4] A number of new nucleosides were introduced and the use of dual NRTI therapy was well established and seemed promising at first. However, suppression of the HIV viral load remained suboptimal with NRTI dual therapy. During the next decade the initial optimism on HIV treatment disappeared as the AIDS pandemic continued to grow and many lives were lost to AIDS. Eventually HIV treatment evolved rapidly with the introduction of the first protease inhibitors (PIs) and nonnucleoside reverse transcriptase inhibitor (NNRTIs) in the mid-1990s. These new antiretroviral drugs in combination with the NRTIs made it possible to use the much more effective triple therapy. Combination antiretroviral therapy (cART) consisting of at least three antiretroviral agents from two different drug classes was found to be essential for effective and sustained virological suppression of HIV.[4,5] The AIDS death rate in the United States fell by more than two-thirds within two years after the licensing of the first PI.[4,6] HIV infection has turned from a fatal disease into a chronic illness. For approximately one decade the standard of care for HIV infection included two NRTIs in combination with a PI or an NNRTI as third agent.

Progress in HIV treatment was made in simplifying the complex multidrug regimens and reducing side effects, which significantly improved adherence and reduced treatment failure. Although HIV treatment had made a huge step forward, some HIV-infected individuals who have had extensive prior antiretroviral therapy failed to sustain maximal viral suppression with the available combinations of antiretroviral agents due to the development of resistant virus. Also drug toxicity and tolerability limited the continued use of several antiretroviral agents in patients. Moreover the transmission of new HIV infections with multidrug resistant virus against the existing drug classes highlighted the urgent need for novel antiretroviral drugs, preferably against new HIV targets.[7] A multifaceted approach to antiretroviral therapy, using combinations of inhibitors that target different steps of the viral life cycle, was, and still is, considered the best potential for long-term control of HIV infection. It wasn't until the end of 2007 that the FDA and the European Medicines Agency (EMA) granted accelerated approval for raltegravir, the first of a new class of antiretroviral agents called HIV-1 integrase strand transfer inhibitors (INSTI), commonly referred to as integrase inhibitors.[8,9]

Raltegravir, the first of a new class

Infection with HIV-1 requires a few essential steps in the viral replication. One of these steps is integration of viral DNA into the host cell genome by the HIV-1 specific enzyme called integrase. Blocking the strand transfer activity for this enzyme limits viral replication and thereby the infection of new cells. Raltegravir selectively inhibits integrase and represented a new therapeutic target for the treatment of HIV infection.[10]

Raltegravir was the first approved HIV-1 integrase inhibitor by the FDA and EMA and is to be administered orally in a dosage 400 mg twice daily.[11,12] Initially, raltegravir was licensed in combination with other antiretroviral agents for treatment-experienced patients ≥ 18 years with evidence of HIV-1 replication despite ongoing antiretroviral therapy. The good safety profile and its potent and rapid antiretroviral activity has quickly extended the use of raltegravir from salvage therapy to first-line treatment. In 2009, the FDA and EMA changed raltegravir's approval to include antiretroviral treatment-naive HIV-1-infected patients.[8,9,13]

Role of raltegravir in HIV management

Immediately after the introduction of raltegravir with its new mechanism of action, it played an important role in HIV management. It presented an effective therapeutic option for pretreated HIV-infected patients with multidrug-resistant virus and limited treatment options.[14,15] Despite its expanded approval in treatment-naive patients, the role of raltegravir as first-line agent in HIV management was not so clear in clinical practice. In the December 2009 update of the Guidelines for the Use of Antiretroviral Agents in HIV-1-Infected Adults and Adolescents from the Department of Health and Human Services raltegravir was recommended together with tenofovir/emtricitabine as one of the four preferred regimens in treatment-naive patients.[16] Nonetheless, clinicians in the Netherlands considered raltegravir less appealing for initial therapy because of its twice daily dosage regimen. In previous years much effort had been put into simplifying the HIV treatment to improve adherence. With the introduction of the first once-a-day three-drug-combination tablet consisting of efavirenz, tenofovir and emtricitabine (Atripla) in 2006, the twice daily dosage of raltegravir can be seen as a disadvantage.[8] The QDMRK study, a phase III study which compared the efficacy, safety, and pharmacokinetics of 800 mg raltegravir once a day versus 400 mg twice a day in treatment-naive HIV-infected patients, was in progress at that time and the results were highly anticipated.[17,18]

Apart from the new mechanism of action and the potent virological efficacy of raltegravir, one of its benefits is a good safety profile. At that time long-term data with clinical experience using raltegravir were not yet available. Although, this was just a matter of time as phase II and III studies were ongoing and preliminary data were reassuring. Furthermore, raltegravir was seen as an antiretroviral drug that could potentially be of great value in special patient populations, such as in children, and in pregnant women to prevent MTCT. Additional studies were needed to confirm this.

HIV and drug-drug interactions

The introduction of effective antiretroviral therapy has increased the life expectancy of HIV-infected individuals.[19] In high-income countries, approximately 33% of all adults living with HIV were aged 50 years and over in 2012.[20] In 2015, more than 50% of all HIV-infected individuals living in the USA will be 50 years of age or older.[19,21] Additionally, it is believed that HIV infection, and quite possibly its treatment, may be contributing to the acceleration of the aging process by several years when compared to uninfected individuals.[22] The older HIV population is increasingly experiencing common medical conditions associated with aging, such as hypertension, dyslipidemia, cognitive impairment, diabetes, osteoporosis, and malignancies. Some of these conditions are more prevalent in HIV-infected individuals compared to uninfected individuals from the same age. As a consequence an increasing number of patients on antiretroviral treatment need medication for various comorbidities which significantly enhances the potential for drug-drug interactions.[23]

For example, HIV-infected patients, especially patients ≥ 50 years of age, are at increased risk of cardiovascular disease (CVD).[21,24] Dyslipidemia, which is highly prevalent among HIV-infected patients, contributes to this increased risk. Statins lower plasma low-density lipoprotein (LDL) cholesterol levels and are frequently being used as lipid-lowering therapy in HIV-infected patients.[25,26] However, the concomitant use of statins and antiretroviral agents, in particular PIs and NNRTIs, may lead to clinically relevant pharmacokinetic drug-drug interactions with potentially severe statin-induced toxicity as a result.[27-29]

The introduction of cART has reduced the risk of AIDS-defining malignancies and dramatically prolonged survival. But as a result the HIV population is increasingly more at risk for development of non-AIDS-defining malignancies that typically occur at older ages. Although the type of cancer that HIV-infected patients are diagnosed with is changing, the need for treatment with chemotherapy in combination with cART is increasingly common. Concomitant use of cART with chemotherapy is complicated due to drug-drug interactions and overlapping toxic effects.[30,31]

Depression is the most common mental health disorder among HIV-patients with a lifetime prevalence that is approximately 2-fold higher than among HIV-uninfected individuals.[32,33] Depression is associated with an increased risk of treatment failure and viral resistance of antiretroviral agents due to adherence problems.[34] Therefore treating depression with antidepressant therapy is important to improve health outcomes in those living with HIV.

Of the 35 million people living with HIV worldwide, approximately 4 - 5 million are coinfected with hepatitis C (HCV).[2] Several antivirals to treat HCV should be avoided or

used with great caution with commonly used antiretroviral agents due to pharmacokinetic interactions. [35]

Besides conventional medication for comorbidities, approximately 60% of HIV-infected patients use complementary and alternative medicines to treat HIV-related symptoms and side effects of antiretroviral therapy. But even herbal medicines may cause clinically significant interactions with antiretroviral agents with potential drug failure as a result.[36-39]

These are a few examples of common medical conditions within the HIV-infected population and the subsequent use of various therapeutic drug classes besides cART. Polypharmacy and managing these potential drug-drug interactions is considered the next therapeutic challenge in HIV.[40]

The use of clinical pharmacology

Clinical pharmacology is the science that studies the effect of drugs in humans with a focus on the translation and application of basic pharmacological principles into clinical practice. Two major principles in clinical pharmacology that are involved in the relationship between dose, drug exposure, and response in patients, are the pharmacokinetics and pharmacodynamics of a drug. Pharmacokinetics can be defined as what the body does to the drug and is comprised of the absorption, distribution, metabolism, and elimination profile. Pharmacodynamics is what the drug does to the body and can be divided into efficacy or therapeutic response, and toxicity. Clinical pharmacology plays a critical role in the treatment of HIV infection and can connect the gap between the medical science of antiretroviral drugs and their use in daily clinical practice.

Although many antiretroviral drugs are now available, a limited number of combinations have been proven to be effective in individual patients. An understanding of the interindividual variation in response, both efficacy and toxicity, of antiretroviral drugs has evolved over time leading to individualization of antiretroviral therapy based on the pharmacological characteristics of antiretroviral agents. The need for individualized approaches to cART has been further increased due to the presence of comorbidities in especially the older HIV population. Most HIV-infected patients take at least three antiretroviral agents, but may also take a variety of medication for concomitant illnesses whether or not related to HIV. With the use of multiple antiretroviral agents, it is critical to understand the pharmacokinetics of these agents to avoid or manage drug-drug interactions.[41] Many antiretroviral agents, in particular PIs and NNRTIs, are not only substrates but also inhibitors or inducers of cytochrome P450 or other hepatic enzymes and drug transporters. Probably the best known example of a perpetrator of pharmacokinetic drug-drug interactions within the HIV treatment, is the use of ritonavir. Ritonavir is a potent inhibitor of the CYP3A4 enzyme,

which is an important liver enzyme responsible for the hepatic metabolism of many therapeutic agents. Although ritonavir is being used for its positive effect as booster for the PIs to improve their pharmacokinetic properties, its negative impact on the occurrence of adverse drug-drug interactions is generally known. Polypharmacy could be a reason for clinicians to start with or switch to an alternative antiretroviral regimen with little propensity to interact with concomitant medication.

Pharmacokinetic drug-drug interactions as well as intra- and interpatient pharmacokinetic variability are some of the main causes for suboptimal drug exposure and is an important reason for treatment failure in HIV-infected patients. Therapeutic drug monitoring (TDM) is managing the therapeutic regimen of an individual patient by measuring drug concentrations, usually in blood. In HIV infection, TDM has been used to optimize and individualize cART response.[42-46] Especially certain patient groups who are at increased risk for pharmacokinetic variability resulting in potential low or elevated plasma concentrations could benefit from TDM. These special patient populations include pediatric and pregnant patients, patients with renal and hepatic impairment, and patients with complex drug-drug interactions.[42,44,47-49] Pregnancy is associated with considerable physiological changes which may influence the pharmacokinetic profile of antiretroviral agents and lead to decreased drug exposure. [50,51] To monitor the effect of pharmacokinetic drug-drug interactions, drug measurements of an antiretroviral drug before and after introduction of an interacting agent can establish patient-specific targets and guide subsequent dose adjustments.[52]

In conclusion, it is widely acknowledged that better understanding of the clinical pharmacology, including the pharmacokinetics of antiretroviral drugs is essential for their safe and effective use in HIV-infected patients.

Clinical pharmacology of raltegravir

Raltegravir (Isentress®) became available at the end of 2007 as film-coated tablets containing 400 mg raltegravir (as potassium). The recommended dose of raltegravir in adult HIV-infected patients is 400 mg orally twice daily with or without food.[53,54]

Raltegravir absorption is rapid, with a time to reach maximum plasma concentration of 1 to 3 hours, depending on the food intake. Raltegravir solubility, and oral absorption, improves at higher gastrointestinal pH values. The apparent terminal half-life of raltegravir is approximately 9 hours and steady-state plasma concentrations are generally reached in approximately 2-3 days.[55,56]

Raltegravir is primarily metabolized by glucuronidation via UDP-glucuronosyltransferase (UGT)1A1 in the liver, with minor contributions from UGT1A3 and UGT1A9.[57] *In vitro*

characterization of raltegravir transport by drug transporters indicates that raltegravir is subject to P-glycoprotein (P-gp)-mediated efflux.[58,59] Raltegravir does not appear to influence UGT enzymes. Unlike other antiretroviral drug classes, such as PIs and NNRTIs, raltegravir is not a substrate of CYP450 enzymes and does not inhibit or induce CYP450 enzymes.[53]

Approximately 7-14% of a raltegravir dose is excreted unchanged in the urine. No clinically important effects were observed on the pharmacokinetic profile of raltegravir in patients with moderate hepatic insufficiency (Child Pugh score 7-8) or severe renal insufficiency (creatinine clearance <30 mL/min/1.73 m^2).[60] The pharmacokinetics of raltegravir displays considerable inter- and intraindividual variability.[61,62]

Initial pharmacokinetic/pharmacodynamic analyses based on phase II and III clinical studies did not suggest a particular threshold for a raltegravir plasma concentration associated with reduced efficacy. In 2012 the pharmacokinetic data of the QDMRK study (800 mg once daily versus 400 mg twice daily) showed that the trough level (C_{12h}) is considered the most important parameter to evaluate with respect to raltegravir's virological efficacy, with a suggested threshold of 0.020 mg/L.[17]

Raltegravir: drug-drug interactions

Managing potential drug-drug interactions in HIV-infected patients may be challenging for clinicians and pharmacists, especially when multiple interacting agents are used. A potential strategy is to choose an antiretroviral regimen with little propensity to interact with concomitant medication. Based on the available theoretical data and drug-interaction studies, raltegravir is not known to inhibit or induce CYP450 enzymes or UGT enzymes. Although the metabolic profile of raltegravir or its transport via drug transporters might not be fully clarified, raltegravir could be considered a preferred antiretroviral agent if potential pharmacokinetic interactions are a concern. However, theoretical data is not always sufficient to predict pharmacokinetic interactions as unexpected drug-drug interactions with raltegravir have been observed in previous studies.[63,64]

Some guidelines and articles on the management of cancer in HIV-infected patients recommend to use raltegravir as an alternative for NNRTIs and PIs to avoid potential pharmacokinetic drug-drug interactions via the CYP450 pathway.[30,31,65,66] Nonetheless, little is known on the pharmacokinetics of raltegravir in this setting, including the potential influence of severe chemotherapy-induced intestinal toxicity on the absorption and total exposure to raltegravir.

Aim of the thesis

The overall aim of this thesis was to study the clinical pharmacology of the HIV-1 integrase inhibitor raltegravir to optimize its safe and effective use in HIV-infected patients in clinical practice. **Part 1** of the thesis focuses on pharmacokinetic drug-drug interactions between raltegravir and other frequently used concomitant therapeutic agents or alternative medication for coexisting medical conditions. **Part 2** of the thesis presents the pharmacokinetics and TDM of raltegravir in special patient populations.

Part 1: Drug-drug interactions

To recommend the safe use of raltegravir with other frequently used or otherwise important concomitant medication, four pharmacokinetic drug-drug interaction studies were conducted in healthy volunteers.

Chapter 2 describes the two-way pharmacokinetic drug-drug interaction between raltegravir and atorvastatin. In addition to this, we investigated the tolerability of the treatment combination and whether raltegravir influenced the short-term lipid-lowering effect of atorvastatin. Raltegravir and atorvastatin share a similar metabolic pathways via UGT and P-gp which might interfere with both their pharmacokinetic profiles.

Chapter 3 evaluates the two-way pharmacokinetic drug-drug interaction and tolerability of concomitant administration of citalopram, a selective serotonin reuptake inhibitor (SSRI) for the treatment of depression, and raltegravir in healthy volunteers.

Boceprevir was in 2011 introduced as a newly developed NS3 serine protease inhibitor for the treatment of chronic HCV genotype 1 infection.[67] Boceprevir should be avoided or used with great caution with commonly used antiretroviral agents due to pharmacokinetic interactions via the CYP450 pathway.[68,69] In order to recommend raltegravir as a preferred agent for combined HIV/HCV treatment with boceprevir, we designed a pharmacokinetic drug-drug interaction study between raltegravir and boceprevir as presented in **Chapter 4**.

A popular herbal product used worldwide by HIV-infected patients is Ginkgo biloba extract, which is used for its claimed beneficial effects on concentration, memory, depressive disorders, and dementia.[70] There is *in vitro* and animal evidence that Ginkgo biloba modulates UGT enzymes.[71-74] Therefore we performed the herb-drug pharmacokinetic interaction study between Ginkgo biloba and raltegravir (**Chapter 5**).

Part 2: Pharmacokinetics in special patient populations

The second part of this thesis focuses on the pharmacokinetics of raltegravir and the application of TDM in special patient populations.

The use of cART in pregnant HIV-infected women is important in the prevention of MTCT. Raltegravir could play an important role when a rapid decline in maternal plasma HIV RNA is needed to prevent MTCT during delivery or as an alternative antiretroviral drug in complex treatment-experienced HIV-infected pregnant women. We studied the effect of pregnancy on the pharmacokinetics of raltegravir and its safety, and efficacy in HIV-infected pregnant women (**Chapter 6**).

TDM could be of value in HIV-infected patients at risk for complex pharmacokinetic drug-drug interactions, such as HIV-infected patients who need to use chemotherapeutic agents.[30,31] In **Chapter 7** we describe a short case series of three HIV-infected patients with advanced stage non-Hodgkin lymphoma and use of chemotherapy in whom TDM of raltegravir was performed as part of our regular patient care. We used a limited plasma sampling method to estimate the exposure to raltegravir.

Raltegravir was the first INSTI approved by the FDA (December 2011) and EMA (December 2012) for treatment of HIV infection in the pediatric population.[11,12] Two age-appropriate formulations suitable for infants and young children were introduced: chewable tablets and granules which are administered as oral suspension. In **Chapter 8** we discuss our experience with TDM and dose optimalization of raltegravir chewable tablets in a 4 year-old HIV-infected patient, as well as provide a review of the available literature and information on the pharmacokinetics of raltegravir in children.

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PART 1

Drug-drug interactions

Pharmacokinetic drug-drug interaction study between raltegravir and atorvastatin 20 mg in healthy volunteers

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Journal of AIDS 2015; 69(1): 44–51

Abstract

Background

Dyslipidemia is highly prevalent among patients with HIV infection and contributes to an increased risk of cardiovascular disease. We investigated the influence of a frequently used statin, atorvastatin, on the pharmacokinetics of the HIV integrase inhibitor raltegravir and vice versa.

Methods

Open-label, crossover 3-period phase I trial in 24 healthy volunteers. Subjects took raltegravir 400 mg two times a day for 7 days, atorvastatin 20 mg once a day for 7 days, and the combination of atorvastatin 20 mg once a day + raltegravir 400 mg two times a day for 7 days with 2-week washout periods in between. Intensive steady-state 12- and 24-hour pharmacokinetic blood sampling was performed. Geometric mean ratios of the test treatment (combination raltegravir + atorvastatin) versus the reference treatment (raltegravir or atorvastatin alone) and 90% confidence intervals were calculated for the area under the plasma concentration-time curve (AUC). Fasting lipid profiles were obtained to assess short-term lipid-lowering effect of atorvastatin with or without concomitant raltegravir use.

Results

Twenty-four healthy volunteers (11 males) were enrolled. All but 1 subject completed the trial and no serious adverse events were reported. Geometric mean ratios (90% confidence interval) were 1.01 (0.68-1.51) for raltegravir $AUC_{0\text{-}12h}$ and 1.00 (0.90-1.11) for atorvastatin $AUC_{0\text{-}24h.}$ The $AUC_{0\text{-}24h}$ metabolite-to-parent ratio for atorvastatin lactone, ortho-hydroxy and para-hydroxy atorvastatin did not change during concomitant raltegravir use. The effect of atorvastatin on low-density lipoprotein cholesterol was not significantly different when combined with raltegravir versus atorvastatin alone (p=0.638).

Conclusions

Atorvastatin 20 mg has no clinically relevant effect on the pharmacokinetics of raltegravir and vice versa. The combination was well tolerated and can be administered without dose adjustments.

Introduction

The introduction of effective antiretroviral therapy has increased the life expectancy of HIV-infected individuals. By 2015, it is projected that more than 50% of all HIV-infected individuals living in the USA will be 50 years of age or older.[1] HIV-infected patients, especially patients older than or equal to 50 years of age, are at increased risk of comorbidities such as cardiovascular disease (CVD).[1,2] Dyslipidemia is highly prevalent among patients with HIV infection and contributes to the increased cardiovascular risk in this patient population. Although data on prevention of CVD in HIV-infected patients are limited, available evidence suggests to use intervention strategies in the HIV-infected population similar to those for the general population. International guidelines on the treatment of HIV infection include recommendations on the use of statins for the treatment of HIV-associated dyslipidemia and prevention of artherosclerotic disease.[3-5] Statins lower plasma low-density lipoprotein (LDL) cholesterol levels by inhibition of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase and are widely used as lipid-lowering therapy in HIV-infected patients.[6-8] A retrospective cohort study of 700 patients with HIV showed that atorvastatin and rosuvastatin were preferable to pravastatin because of greater declines in total cholesterol.[9] Currently, atorvastatin is one of the most commonly prescribed statins for HIV-infected patients. In addition, statins exhibit anti-inflammatory effects and are currently of interest for their potential immune-modulatory properties in HIV-infected patients.[10-12]

A complicating factor in the concomitant use of antiretroviral agents and lipid-lowering drugs is the occurrence of drug-drug interactions, specifically between statins and HIV protease inhibitors or non-nucleoside reverse transcriptase inhibitors.[13-17] Raltegravir, an HIV integrase inhibitor, does not influence cytochrome P450 (CYP) enzymes and has in general little propensity to interact with other medication. In contrast to protease inhibitors and efavirenz, raltegravir has a beneficial lipid profile. Switching from ritonavir-boosted protease inhibitors to raltegravir does not only improve plasma lipids but also led to significant changes in several cardiovascular biomarkers associated with inflammation, insulin resistance, and hypercoagulability.[18] Therefore, raltegravir could be one of the preferred antiretroviral agents in HIV-infected patients with dyslipidemia and statin use, especially in older patients with comorbidities and the subsequent use of concomitant medications.

The concomitant use of raltegravir and atorvastatin has not been investigated yet in a pharmacokinetic study, although there is, at least theoretically, a risk for a drug-drug interaction. Raltegravir is primarily metabolized by glucuronidation via UDP-glucuronosyl-transferase (UGT) 1A1 in the liver, with minor contributions from UGT1A3 and UGT1A9.[19] It is not known to influence CYP enzymes, UGT enzymes or organic anion-transporting

polypeptide (OATP)-1B1.[20,21] Raltegravir is subject to minor P-glycoprotein (P-gp)-mediated efflux.[22] Atorvastatin and its metabolites are to various extent substrates for CYP3A4, UGT1A1, UGT1A3, and P-gp. The active uptake transporter OATP1B1 facilitates the uptake of atorvastatin into the hepatocytes, which is the site of action for statins.[23,24] When administered in high dose, atorvastatin increases the bioavailability of digoxin, most probably by inhibition of P-gp. There is no evidence that atorvastatin influences the metabolism of UGT substrates.[23] Because raltegravir and atorvastatin share similar metabolic pathways through UGT and P-gp, a pharmacokinetic interaction cannot be excluded.

The primary objective of this study was to assess the effect of steady-state raltegravir 400 mg twice daily on the pharmacokinetics of steady-state atorvastatin 20 mg once daily and vice versa in healthy volunteers. Secondary objectives included the safety and tolerability of the treatment combination and the lipid-lowering effect of short-term atorvastatin use in the presence and absence of raltegravir.

Methods

Design

This open-label 3-period randomized crossover phase I trial in 24 healthy volunteers was conducted from May to July 2013 at the Radboud University Medical Center, Nijmegen, the Netherlands. The study was designed to determine the effect of steady-state raltegravir on the pharmacokinetics of steady-state atorvastatin and vice versa by intrasubject comparison.

Healthy volunteers were equally randomized to one of the following treatment sequences: ABC; ACB; BCA; BAC; CAB; or CBA. The treatments regimens were (A), raltegravir 400 mg twice daily for 7 days; (B), atorvastatin 20 mg once daily for 7 days; and (C), raltegravir 400 mg twice daily and atorvastatin 20 mg once daily for 7 days. Every treatment period was followed by a washout period of 14 days. Blood samples for pharmacokinetic assessment were collected at day 7 of each treatment period during a 12-hour period for raltegravir and a 24-hour period for atorvastatin after observed intake of the study medication. The trial was approved by the Investigational Review Board of the Radboud University Medical Center, Nijmegen, The Netherlands. The trial was conducted in accordance with Good Clinical Practice and the Declaration of Helsinki and registered at ClinicalTrials.gov (NCT01779687). All participants signed informed consent before screening evaluations.

Study population

Healthy male and female participants between the age of 18 and 55 and with a body mass index of 18-30 kg/m^2 were eligible for enrolment. Included participants had to be in a

good age-appropriate health condition as established by physical examination, medical history, electrocardiography and biochemical, hematologic, and urinalysis testing within 4 weeks before day 1. Main exclusion criteria were a positive HIV, hepatitis B and C test result and use of any medication except for acetaminophen from 2 weeks preceding dosing.

Study drug and dosing

The approved dose of raltegravir (Isentress; Merck Sharp & Dohme Ltd, Hoddesdon, United Kingdom) is 400 mg twice daily.[20,25] The clinical dosage of atorvastatin (Lipitor; Pfizer, New York, NY) ranges from 10 to 80 mg once daily orally.[26] In this study, atorvastatin 20 mg tablets were taken once daily in the morning during atorvastatin treatment periods. A treatment duration of 7 days for all treatment periods was chosen to reach steady-state plasma concentrations of the study drugs and to allow sufficient time to observe any competition for a particular metabolic pathway with concomitant use. On the days of pharmacokinetic sampling, both raltegravir and atorvastatin were taken on an empty stomach. A standardized breakfast, consisting of 1 glass of milk and 2 slices of buttered wheat bread with 48+ cheese and cervelat, was served at 2 hours after dosing. Intake of medication at the clinical trial unit was supervised and recorded by the study personnel. Tablets were counted to assess adherence. In addition, drug intake of atorvastatin at home was monitored by use of microelectronic monitoring system (MEMS) caps (Aardex Ltd, Zug, Switzerland), which records the opening of the medication bottle. Subjects were asked to write down the exact times of medication intake in a booklet. Because the raltegravir medication bottles were incompatible with MEMS caps, we were unable to monitor the raltegravir intake electronically.

Study procedures

Pharmacokinetic assessment was performed at steady-state conditions at day 7 of each treatment period. Blood samples for pharmacokinetic analysis were collected into lithium-heparinized tubes from predose until 12 hours after intake of the study drug with an additional sample at 24 hours after intake of atorvastatin. Blood samples for analysis of atorvastatin and the metabolites atorvastatin lactone, ortho-hydroxy atorvastatin, and para-hydroxy atorvastatin were immediately put on ice and centrifuged within 30 minutes at 1,900 x *g* at 4°C. Plasma was transferred to polypropylene tubes and kept on ice for a maximum of 3 hours until storage at -80°C awaiting further bioanalysis. Blood samples for analysis of raltegravir were centrifuged for 5 minutes at 1,900 x *g* at 20°C. Plasma was transferred to polypropylene tubes and stored at -40°C until further bioanalysis.

Safety assessment consisted of monitoring adverse events and laboratory evaluations (biochemistry and hematology). Adverse events were graded according to the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events (version 1.0, December 2004) and causality assessment with the study drugs was performed.

Participants were to refrain from sports and strenuous exercise during the treatment periods to avoid potential exercise induced myalgia and creatine kinase (CK) elevations. The lipid-lowering effect of atorvastatin was evaluated by measuring serum lipid profiles after 12 hours overnight fasting on days 1 and 7 of each treatment period. A lipid profile in serum consisted of total cholesterol, triglycerides, high-density lipoprotein (HDL) cholesterol, non-HDL cholesterol and LDL cholesterol (calculated by means of the Friedewald equation).[27]

Bioanalytical methods

Atorvastatin, atorvastatin lactone, orthy-hydroxy atorvastatin, and para-hydroxy atorvastatin in plasma were quantified using a validated liquid chromatography-tandem mass spectrometry bioanalytical method with a linear calibration range of 0.0500 – 20.0 ng/mL for all analytes at Analytical Biochemical Laboratory BV (Assen, The Netherlands). Concentrations of raltegravir in plasma were analyzed using a validated reversed-phase high-pressure liquid chromatography with fluorescence detection. The linear calibration range in plasma was 0.014-10.0 mg/L. The raltegravir assay was performed at the laboratory of the Pharmacy of the Radboud University Medical Center (Nijmegen, The Netherlands) and externally validated through the International Interlaboratory Quality Control Program for Measurement of Antiretroviral Drugs in Plasma and by the Clinical Pharmacology Quality Assurance and Quality Control program.[28,29]

Pharmacokinetic analysis

Pharmacokinetic parameters were determined using a noncompartmental model in WinNonlin/Phoenix version 6.3 (Pharsight Corporation, St. Louis, MO). Based on the individual plasma concentration-time data, the following pharmacokinetic parameters of raltegravir, atorvastatin, atorvastatin lactone, orthy-hydroxy atorvastatin, and para-hydroxy atorvastatin were determined: the area under the plasma concentration-time curve from 0 to 12 hours (raltegravir) or 24 hours (atorvastatin) after intake using the trapezoidal rule ($AUC_{0\text{-}tau}$), the trough concentration (C_{12h}/C_{24h}) defined as the sample taken at 12 and 24 hours, respectively after intake, the maximum plasma concentration of the drug (C_{max}), the time to reach C_{max} (t_{max}) and the apparent elimination half-life ($t_{1/2}$). Pharmacokinetic parameters are reported as geometric means with 95% confidence intervals (CIs). Geometric mean ratios (GMRs) of the pharmacokinetic parameters of the test treatment (combination raltegravir + atorvastatin) versus the reference treatment (raltegravir or atorvastatin alone) and 90% CIs were calculated after log-transformation of within-subject ratios using a mixed-effects bioequivalence module in WinNonlin/Phoenix. Atorvastatin metabolite-to-parent ratios (mean ± SD) of $AUC_{0\text{-}24h}$ were calculated for both treatments.

Statistical analysis

The data obtained in this study were analyzed according to an equivalence approach that is recommended for pharmacokinetic interaction studies by the Food and Drug Administration and European Medicines Agency (EMA) guidelines.[30-32] For atorvastatin and its metabolites AUC GMRs with 90% CI within the range of 0.80-1.25 are considered to indicate no significant interaction between atorvastatin and raltegravir. For raltegravir, AUC GMRs with 90% CI within the range of 0.74-1.35 are considered to indicate no significant interaction if the GMR lies within the conventional acceptance range of 0.80-1.25. According to the "Guideline on the Investigation of Bioequivalence" (EMA), the wider equivalence range could be considered for highly variable drugs (intrasubject coefficient of variation >30%), such as raltegravir, if this range in pharmacokinetic parameters is not considered clinically relevant. Sample size calculation (beta = 0.2, alpha = 0.1) was based on an assumed intrasubject SD of 0.5 for raltegravir AUC, an equivalence range of 0.74-1.35, and the hypothesis that there is no difference in AUC for the reference and test treatment. The required number of participants was 20, and to account for dropouts a total number of 24 participants were included. Statistical analyses were carried out using IBM SPSS Statistics software version 20. To compare the mean change (95% CI) in fasting serum lipid profile (day 7 - 1) between 2 treatments a paired t-test was performed.

Results

Baseline characteristics

Twenty-four healthy volunteers (13 females, 11 males) were enrolled. Subjects were white (n=23) or mixed-race (n=1). One subject withdrew consent and did not complete the reference treatment period with raltegravir alone because of reasons not related to the study. Median (range) age and body mass index were 31 (18-55) years and 20.9 (18.3-28.9) kg/m^2, respectively. The subjects were in good general health, according to medical history, physical examination, vital signs, and laboratory data. Adherence to the study treatment was good. One subject took an extra tablet atorvastatin 20 mg in the evening on days 1 and 4 during the treatment period of atorvastatin with raltegravir. These deviations did not lead to exclusion of the subject from pharmacokinetic analysis. All other subjects took all doses of atorvastatin and raltegravir according to tablet count, booklet, MEMS caps recordings and blood concentrations.

Pharmacokinetics

The plasma concentration versus time curves and the steady-state pharmacokinetic parameters of raltegravir alone and with concomitant use of atorvastatin 20 mg once daily are shown in Figure 1A and Table 1. Mean exposure to raltegravir, which is expressed as $AUC_{0\text{-}12h}$, was similar for raltegravir coadministered with atorvastatin relative to raltegravir alone: the

GMR with 90% CI of $AUC_{0\text{-}12h}$ was 1.01 (0.68-1.51). Raltegravir pharmacokinetics was highly variable which is best seen in the large 90% CI around the GMRs in Table 1 and graphically in Figure 1B. Figure 1B shows considerable variation in the amount and direction of the individual changes in $AUC_{0\text{-}12h}$ of raltegravir alone compared with the combination with atorvastatin. The GMR of the main pharmacokinetic parameter raltegravir $AUC_{0\text{-}12h}$ fell within the range of 0.80-1.25, and the 90% CI partly overlaps the range of 0.74-1.35 that reflects the variability.

Figure 2 shows the plasma concentration versus time curves of atorvastatin (A) and the metabolites atorvastatin lactone (B), ortho-hydroxy atorvastatin (C), and para-hydroxy atorvastatin (D) when atorvastatin 20 mg once daily was administered alone or with raltegravir 400 mg twice daily. The steady-state pharmacokinetic parameters of atorvastatin and its metabolites and the GMRs are described in Table 2. Relative to administration of atorvastatin alone, the coadministration of raltegravir did not have an effect on the pharmacokinetic parameters of atorvastatin, atorvastatin lactone, ortho- hydroxy, and para-hydroxy atorvastatin. The GMRs of the exposure ($AUC_{0\text{-}24h}$) to atorvastatin and the major metabolites were close to 1.0 and the 90% CI fell entirely within the range of 0.80-1.25. At steady state mean (±SD) metabolite-to-parent ratios of $AUC_{0\text{-}24h}$ for treatment with atorvastatin alone versus treatment with raltegravir were 0.80 (±0.27) versus 0.77 (±0.30) for atorvastatin lactone, 1.24 (±0.38) versus 1.19 (±0.37) for ortho-hydroxy atorvastatin and 0.17 (±0.06) in both treatments for para-hydroxy atorvastatin. Figure 3 shows the individual atorvastatin metabolite-to-parent ratios of $AUC_{0\text{-}24h}$.

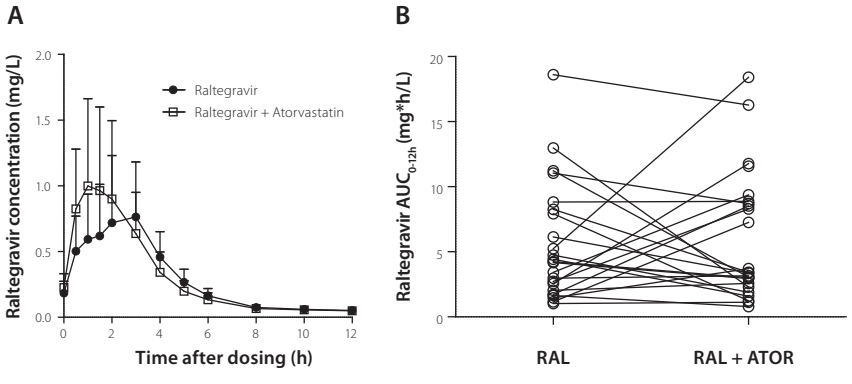

Figure 1 Geometric mean (+ upper 95% CI) raltegravir plasma concentration-time profiles (A) and individual $AUC_{0\text{-}12h}$ (B) at steady state when raltegravir 400 mg twice daily was administrated alone or with atorvastatin 20 mg once daily.

Table 1 Comparison of steady-state pharmacokinetic parameters of raltegravir 400 mg twice daily alone and with coadministration of atorvastatin 20 mg once daily in healthy volunteers.

Pharmacokinetic parameter	Raltegravir alone n=23		Raltegravir + atorvastatin n=24		Raltegravir + atorvastatin: raltegravir alone	
	Geometric mean	95% CI	Geometric mean	95% CI	GMR	90% CI
$AUC_{0\text{-}12h}$ (mg·h/L)	4.13	(2.91-5.87)	4.23	(2.92-6.14)	1.01	(0.68-1.51)
C_{max} (mg/L)	1.29	(0.83-1.99)	1.48	(0.96-2.28)	1.14	(0.70-1.86)
t_{max} [a] (h)	1.55	(0.55-3.00)	1.50	(0.63-2.00)		
C_{12h} (mg/L)	0.052	(0.035-0.077)	0.049	(0.039-0.062)	0.96	(0.69-1.32)
$t_{1/2}$ [b] (h)	3.28	(2.64-4.09)	3.35	(2.86-3.93)	0.97	(0.77-1.21)

[a] For t_{max}, median + interquartile range is reported.
[b] $t_{1/2}$ could not be determined in 4 and 6 subjects for respectively raltegravir alone and raltegravir+ atorvastatin.
$AUC_{0\text{-}12h}$, area under the plasma concentration-time curve up to 12 hours after intake; CI, confidence interval; C_{max}, maximum plasma concentration; C_{12h}, plasma concentration 12 hours after intake; GMR, Geometric Mean Ratio; $t_{1/2}$, apparent elimination half-life; t_{max}, time to reach C_{max}.

Safety and tolerability

The study medication was generally well tolerated. No serious adverse events were reported and there were no discontinuations due to adverse events. Twenty-two subjects reported a total of 121 adverse events. Twenty-five adverse events (21%) reported by a total of 14 subjects were considered possible (n=20) or probable (n=5) related to the study medication. All possible and probable drug-related adverse events were mild (toxicity grade 1 or 2), resolved without sequela, and were not related to a specific treatment period. The reported adverse events were myalgia (n=6, 4 subjects), abdominal pain/ bloated feeling (n=4), fatigue (n=3, 2 subjects), CK elevation grade 2 (n=3), headache (n=2), nausea (n=2) and diarrhoea, dry mouth, pruritis, general malaise and transaminase elevation (all reported once). Nonsymptomatic CK elevation classified as grade 2 (2.1-4.0 times the upper limit of normal) was reported once during atorvastatin treatment and twice during treatment with atorvastatin and raltegravir. Myalgia without CK elevation was reported 3 times during raltegravir treatment or its washout period, twice during atorvastatin treatment, and once when raltegravir was combined with atorvastatin.

Lipid-lowering effects

Table 3 summarizes the mean (95% CI) change (day 7 - 1) in serum lipid profile observed after short-term treatment with atorvastatin 20 mg once daily alone or together with raltegravir. Raltegravir did not significantly influence the short-term lipid-lowering effects of atorvastatin on total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, and non-HDL cholesterol ($p > 0.05$). In our healthy study population a distinct lipid-lowering effect was observed after only 6 doses of atorvastatin 20 mg once daily: mean (95% CI) change in LDL cholesterol (mmol/L) was -0.14 (-0.26 to -0.02) for raltegravir alone compared with -1.17 (-1.38 to -0.97) for atorvastatin alone and -1.22 (-1.40 to -1.03) for atorvastatin with raltegravir ($p < 0.001$).

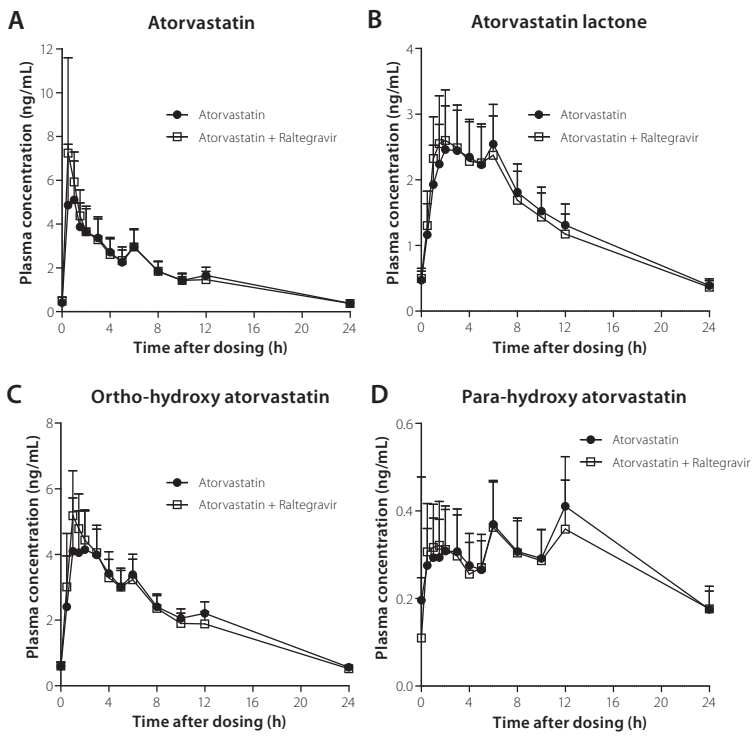

Figure 2 Geometric mean (+ upper 95% CI) plasma concentration-time profiles of atorvastatin (A) and the metabolites atorvastatin lactone (B), ortho-hydroxy atorvastatin (C), and para-hydroxy atorvastatin (D) at steady state when atorvastatin 20 mg once daily was administered alone or with raltegravir 400 mg twice daily.

Table 2 Comparison of steady-state pharmacokinetic parameters of atorvastatin, atorvastatin lactone, ortho-hydroxy atorvastatin, para-hydroxy atorvastatin after administration of atorvastatin 20 mg once daily alone and coadministration with raltegravir 400 mg twice daily in healthy volunteers.

Analyte and pharmacokinetic parameter	Atorvastatin alone n=24		Atorvastatin + Raltegravir n=23		Atorvastatin + Raltegravir : Atorvastatin alone	
	Geometric mean	95% CI	Geometric mean	95% CI	GMR	90% CI
Atorvastatin						
$AUC_{0\text{-}24h}$ (ng·h/mL)	43.7	(36.3-52.6)	44.6	(35.9-55.6)	1.00	(0.90-1.11)
C_{max} (ng/mL)	9.28	(7.32-11.8)	9.95	(7.68-12.9)	1.05	(0.84-1.30)
t_{max} [a] (h)	0.52	(0.50-1.0)	0.50	(0.50-1.0)		
C_{24h} (ng/mL)	0.382	(0.305-0.479)	0.391	(0.304-0.501)	0.99	(0.86-1.15)
$t_{1/2}$ (h)	6.92	(6.24-7.67)	6.97	(6.15-7.90)	1.00	(0.91-1.10)
Atorvastatin lactone						
$AUC_{0\text{-}24h}$ (ng·h/mL)	33.3	(27.2-40.8)	32.4	(25.6-40.8)	0.96	(0.87-1.06)
C_{max} (ng/mL)	3.00	(2.43-3.70)	2.96	(2.31-3.79)	0.97	(0.86-1.09)
t_{max} [a] (h)	2.0	(1.5-6.0)	2.0	(1.0-3.0)		
C_{24h} (ng/mL)	0.388	(0.307-0.491)	0.369	(0.286-0.477)	0.93	(0.81-1.07)
$t_{1/2}$ (h)	7.17	(6.64-7.73)	7.29	(6.65-7.99)	1.01	(0.94-1.07)
Ortho-hydroxy atorvastatin						
$AUC_{0\text{-}24h}$ (ng·h/mL)	51.7	(44.7-59.9)	50.0	(42.9-58.3)	0.97	(0.90-1.04)
C_{max} (ng/mL)	6.21	(4.89-7.90)	6.21	(5.00-7.71)	1.00	(0.82-1.20)
t_{max} [a] (h)	1.0	(0.52-2.0)	1.0	(1.0-1.5)		
C_{24h} (ng/mL)	0.561	(0.489-0.636)	0.528	(0.469-0.594)	0.94	(0.84-1.05)
$t_{1/2}$ (h)	7.44	(6.85-8.09)	7.37	(6.70-8.12)	0.99	(0.91-1.07)
Para-hydroxy atorvastatin						
$AUC_{0\text{-}24h}$ (ng·h/mL)	7.13	(5.71-8.91)	6.95	(5.38-8.97)	0.96	(0.88-1.06)
C_{max} (ng/mL)	0.470	(0.365-0.605)	0.442	(0.331-0.590)	0.94	(0.82-1.07)
t_{max} [a] (h)	12	(2.3-12)	6.0	(1.5-12)		
C_{24h} (ng/mL)	0.175	(0.142-0.217)	0.179	(0.137-0.234)	1.00	(0.87-1.14)

[a] For t_{max}, median + interquartile range is reported.

$AUC_{0\text{-}24h}$, area under the plasma concentration-time curve up to 24 hours after intake; CI, confidence interval; C_{max}, maximum plasma concentration; C_{24h}, plasma concentration 24 hours after intake; GMR, Geometric Mean Ratio; $t_{1/2}$, apparent elimination half-life; t_{max}, time to reach C_{max}.

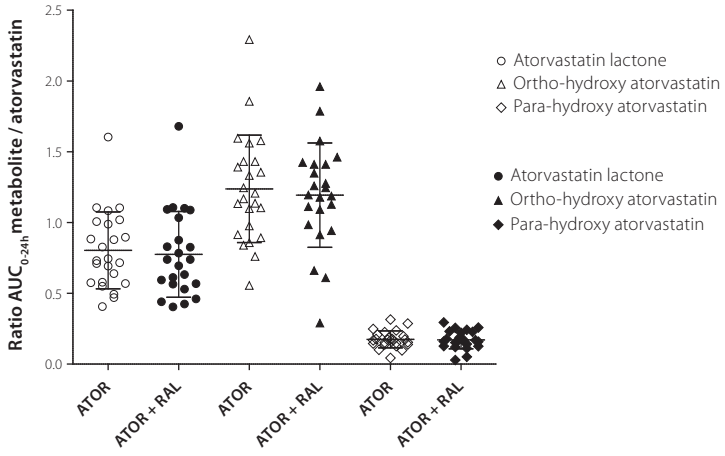

Figure 3 Individual and mean (± SD) metabolite-to-parent ratios of $AUC_{0\text{-}24h}$ (ng·h/mL) of atorvastatin with the metabolites atorvastatin lactone, ortho-hydroxy atorvastatin, and para-hydroxy atorvastatin when atorvastatin 20 mg once daily was administered alone or with raltegravir 400 mg twice daily.

Table 3 Mean change (95% CI) in plasma lipid profile of short-term treatment with atorvastatin 20 mg once daily with and without coadministration of raltegravir.

Lipid parameter (mmol/L)	Atorvastatin alone	Atorvastatin + Raltegravir	*p*
Cholesterol	-1.31 (-1.55 to -1.08)	-1.37 (-1.56 to -1.18)	0.629
HDL cholesterol	-0.09 (-0.13 to -0.06)	-0.05 (-0.10 to -0.01)	0.199
LDL cholesterol	-1.17 (-1.38 to -0.97)	-1.22 (-1.40 to -1.03)	0.638
Triglycerides	-0.22 (-0.37 to -0.08)	-0.22 (-0.41 to -0.34)	0.997
Non-HDL cholesterol	-1.31 (-1.56 to -1.07)	-1.32 (-1.49 to -1.14)	0.977

Discussion

In HIV-infected patients the use of lipid-lowering therapy with statins is complicated by drug-drug interactions with antiretroviral agents, specifically with HIV protease inhibitors or non-nucleoside reverse transcriptase inhibitors.[13-17] The purpose of this study was to evaluate the 2-way pharmacokinetic drug-drug interaction and tolerability of concomitant administration of atorvastatin 20 mg once daily and the HIV integrase inhibitor raltegravir 400 mg twice daily in healthy volunteers. Our findings suggest that atorvastatin 20 mg has no clinically relevant effect on the pharmacokinetics of raltegravir. Raltegravir does not influence the pharmacokinetics of atorvastatin and its metabolites and did not seem to influence the short-term lipid-lowering effect of atorvastatin. These results are relevant for the increasing number of HIV-infected patients who are at risk for CVD and have dyslipidemia that requires therapy with potent statins such as atorvastatin.

The metabolism of atorvastatin is complex with CYP3A4 being the major enzyme responsible for the formation of the 2 active metabolites: ortho-hydroxy atorvastatin acid and para-hydroxy atorvastatin acid. About 70% of the circulating inhibitory activity for HMG-CoA reductase is attributable to these active metabolites. The majority of clinically relevant drug-drug interactions with atorvastatin involve inhibition or induction of CYP3A4. Raltegravir is not an inducer or inhibitor of CYP450 and could be an alternative for other antiretroviral agents, such as protease inhibitors, if drug-drug interactions are a concern. Atorvastatin acid (the parent drug), para- and ortho-hydroxy atorvastatin are in equilibrium with their inactive lactone forms.[33] UGT1A1 and UGT1A3 are involved in the acid to lactone conversion, whereas esterases mediate the hydrolysis of atorvastatin lactone to the open acid form of atorvastatin.[24,34] Because raltegravir is primarily metabolized by UGT1A1 and to a lesser extent UGT1A3 we considered it relevant to analyze the 2 active hydroxyl metabolites and the lactone form of atorvastatin acid. The mean exposure ($AUC_{0\text{-}24h}$) to atorvastatin, atorvastatin lactone, ortho-hydroxy or para-hydroxy atorvastatin was not influenced by concomitant raltegravir use. Metabolite-to-parent ratios remained unaffected, and no competition for this particular UGT metabolic pathway with concomitant use was observed. The mean exposure to raltegravir ($AUC_{0\text{-}12h}$) was similar for treatment with raltegravir alone compared with raltegravir combined with atorvastatin and resulted in a GMR of 1.01. The pharmacokinetics of raltegravir is highly variable which was observed in our study as well.[35,36] The 90% CIs partly overlap the predefined range of 0.74-1.35 for no clinically relevant interactions.

A limitation of our study is that the included participants were predominantly white individuals. Therefore, our study population does not reflect the demographics of the HIV-infected population around the world and its genetic variability. A more important limitation of our study could be that we used atorvastatin 20 mg while the maximum

registered daily dose of atorvastatin is 80 mg. Only the highest dose (80 mg) of atorvastatin, which is not often used in clinical practice, increases the bioavailability of digoxin (AUC increased by 15%), most probably by inhibition of P-gp. Raltegravir is only a weak substrate of P-gp and does not have a small therapeutic window like digoxin, making it less likely that inhibition of P-gp has a clinically significant effect on the pharmacokinetics of raltegravir. Another limitation could be that the lipid-lowering effect was evaluated after a relatively short duration of atorvastatin use. However, in our study, atorvastatin 20 mg once daily during 1 week significantly decreased LDL cholesterol, as well as total cholesterol and non-HDL cholesterol compared with no treatment with atorvastatin, suggesting we allowed sufficient time to evaluate the lipid-lowering effects.

The treatment combination of atorvastatin and raltegravir was well tolerated. A total of 6 mild cases of myalgia were reported by 4 subjects, and 3 subjects had mild-to-moderate CK elevation with no symptoms. These adverse events were not related to a specific treatment period and did not occur more frequent during concomitant atorvastatin and raltegravir use. Although uncommon, cases of myopathy and rhabdomyolysis have been reported with raltegravir use.[16,37,38] Raltegravir-based therapy has been reported to be associated with a higher prevalence of symptomatic skeletal muscle toxicity, which seemed to not be concentration- or time-dependent, and not associated with elevated CK.[39] However, significant CK elevations were seen with raltegravir use, but in these patients symptoms were uncommon, not severe, and occurred in patients with identifiable risk factors.[40] Although a causal relationship with raltegravir has not been clearly established, raltegravir should be used with caution in patients who have had myopathy or rhabdomyolysis in the past or have any predisposing issues including other medicinal products associated with these conditions. All statins carry the potential risk of myopathy and in rare cases progression to fatal rhabdomyolysis. High atorvastatin doses as well as certain pharmacokinetic drug-drug interactions, particularly those leading to higher concentrations in the peripheral blood and muscle cells, increase the risk of muscle toxicity. Atorvastatin-treated patients experiencing myopathy were found to have higher plasma concentrations of the hydroxymetabolites and cyclic lactones.[41] Because exposure to atorvastatin and metabolite-to-parent ratios in this study were not influenced by concomitant raltegravir use, an increase in statin-induced muscle toxicity due to a pharmacokinetic interaction with raltegravir is not likely to occur. Although our study did not show any safety issues, the short-term design without the use of high-dose atorvastatin and its healthy study population preclude a different monitoring approach for muscle toxicity than currently advised in the product labeling of both drugs.

In conclusion, our findings suggest that atorvastatin 20 mg has no clinically relevant effect on the pharmacokinetics of raltegravir. Raltegravir does not influence the pharmacokinetics of atorvastatin and has no effect on its short-term lipid-lowering effect. The combination can be administered with no dose adjustments. Coadministration of raltegravir and atorvastatin 20 mg was safe and well tolerated in our study in healthy HIV-negative subjects.

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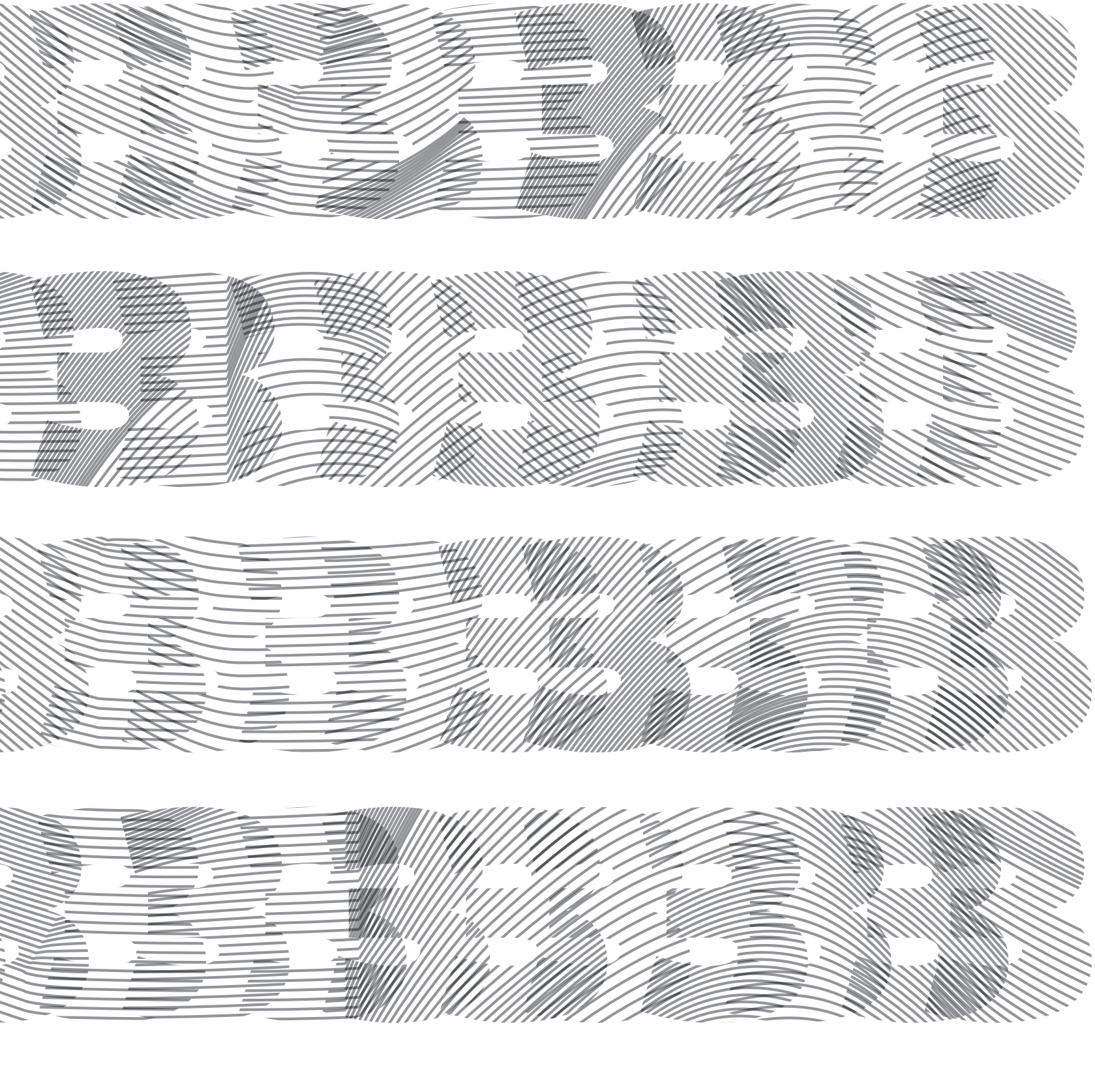

Pharmacokinetic drug-drug interaction study between raltegravir and citalopram

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Antiviral Therapy, accepted for publication

Abstract

Background

Depression is the most common mental health disorder among HIV-infected patients. When treating HIV-infected patients with a selective serotonin reuptake inhibitor (SSRI), potential drug-drug interactions with antiretroviral agents have to be taken into account. We investigated the two-way pharmacokinetic drug-drug interaction and tolerability of concomitant administration of the SSRI citalopram and the HIV-1 integrase inhibitor raltegravir in healthy volunteers.

Methods

An open-label, crossover, two-period trial was conducted in 24 healthy volunteers. Subjects received the following treatments: citalopram 20 mg once daily for 2 weeks followed by the combination with raltegravir 400 mg twice daily for 5 days and after a washout period raltegravir 400mg twice daily for 5 days. Intensive steady-state pharmacokinetic blood sampling was performed. Geometric mean ratios (GMRs) of the combination versus the reference treatment and 90% CIs were calculated for the area under the plasma concentration-time curve (AUC). CYP2C19 genotyping was performed because it influences N-demethylation of citalopram to desmethylcitalopram.

Results

A total of 22 healthy volunteers completed the trial. GMRs (90% CI) were 1.00 (0.98, 1.03) for citalopram $AUC_{0\text{-}24h,}$ 0.99 (0.88, 1.12) for desmethylcitalopram $AUC_{0\text{-}24h}$ and 0.77 (0.50, 1.19) for raltegravir $AUC_{0\text{-}12h}$. Raltegravir plasma concentration 12 h after intake (C_{12h}) did not change with concomitant use of citalopram. Within each CYP2C19 phenotype subgroup the citalopram metabolite-to-parent ratio, which is a measure for metabolic enzyme activity, was not influenced by concomitant raltegravir use.

Conclusions

Raltegravir does not influence the pharmacokinetics of citalopram and desmethylcitalopram. Citalopram did not change the pharmacokinetics of raltegravir in a clinically meaningful way. The combination was well tolerated and can be administered without dose adjustments.

Introduction

Depression is the most common mental health disorder among HIV-infected patients. The lifetime prevalence of depression in patients infected with HIV is approximately twofold higher than among HIV-uninfected individuals.[1-4] Depression is a risk factor for poor adherence to antiretroviral therapy and is associated with an increased risk of treatment failure, disease progression and mortality.[5-7] Recognizing and treating depression is important in order to improve quality of life and health outcomes in those living with HIV.[2,8,9]

Antidepressant therapy is effective in most HIV-infected patients with major depression and treatment options are similar to HIV-negative patients. Selective serotonin reuptake inhibitors (SSRIs) and tricyclic antidepressants are equally effective, but SSRIs have demonstrated more favourable side effect profiles and are generally better tolerated.[10,11] Compliant SSRI use is associated with improved adherence to antiretroviral agents, leading to improved HIV viral control and increased $CD4^+$ T-cell counts.[12] Citalopram is a widely used SSRI in the general population and has a relatively favorable drug interaction profile compared to other SSRIs.[11] This suggests that citalopram may be a good choice for the treatment of depression in HIV-infected patients who are at risk for experiencing drug-drug interactions.[13]

Biotransformation of citalopram is mainly hepatic by cytochrome P450 enzymes (CYP) 2C19, CYP3A4 into the main metabolite desmethylcitalopram, which is further metabolized by CYP2D6 to didesmethylcitalopram.[13-18] At steady state, plasma concentrations of desmethylcitalopram and didesmethylcitalopram are approximately 30-50% and 5-10% that of the parent compound, respectively. The metabolites do not likely contribute significantly to the clinical effects of citalopram, since they are present in lower concentrations and have been shown *in vitro* to be much weaker inhibitors of serotonine reuptake than citalopram.[19] The genotype of CYP2C19 plays an important role in the extent of N-demethylation of citalopram to desmethylcitalopram *in vivo*.[20,21] Citalopram is a weak CYP2D6 inhibitor and has weak or no effect on CYP1A2, CYP2C19 and CYP3A4. Desmethylcitalopram is a slightly more potent inhibitor of CYP2D6 and may mediate a mild interaction with other drugs metabolized by CYP2D6.[13,14] An *in vitro* study revealed that citalopram is a weak inhibitor of P-glycoprotein (P-gp).[22,23]

Raltegravir is the first registered HIV-1 integrase strand transfer inhibitor (INSTI) and guidelines recommend its use with tenofovir and emtricitabine in treatment-naïve HIV-infected patients.[24] Raltegravir is generally well tolerated and has minimal potential to interact with concomitant medication because it does not influence CYP or UDP-glucuronosyltransferase (UGT) enzymes.[25] Raltegravir is subject to minor P-gp-mediated efflux and is primarily metabolized by UGT1A1. Depression was added to the product labelling

as a precaution for use after identification of several post-marketing cases. In four patients with ongoing depression and use of antidepressants an association between starting raltegravir and exacerbation of depression was described.[26] The mechanism by which raltegravir may have contributed to the observed psychiatric decompensation remains unknown. According to the authors, a potential cause could be one or more as yet unidentified drug-drug interactions with raltegravir and the antidepressant agents used. In one of the cases citalopram was used.

With the combined use of citalopram and raltegravir, no major pharmacokinetic interaction via UGT and CYP enzymes is theoretically expected. A minor interaction may occur through inhibition of P-gp-mediated transport of raltegravir by citalopram. However theoretical data is not always sufficient to predict pharmacokinetic interactions as unexpected drug-drug interactions with raltegravir have been observed in previous studies.[27,28]

The objective of this study was to assess the two-way pharmacokinetic interaction between raltegravir and citalopram in healthy volunteers, and to evaluate the safety and tolerability of the treatment combination.

Method

Study design

This open-label, two-period, randomized, crossover, phase I trial in 24 healthy volunteers was conducted from February to April 2014 at the Radboud university medical center, Nijmegen, The Netherlands. The study was designed to determine the effect of raltegravir 400 mg twice daily on the pharmacokinetics of citalopram 20 mg once daily and vice versa by intrasubject comparison.

The healthy volunteers were randomized into two groups of 12 subjects. The treatment regimen for group 1 was citalopram 10 mg once daily for three days followed by dose escalation to 20 mg once daily for two weeks (reference citalopram). Citalopram was continued with raltegravir 400 mg twice daily for 5 days (test combination). Citalopram was tapered off and after a washout period raltegravir 400 mg twice daily was given for 5 days (reference raltegravir). Group 2 started with the raltegravir reference treatment and after a washout period continued with the same citalopram reference treatment and combination treatment as group 1. Pharmacokinetic assessment was performed on three occasions on the last day of each treatment period. The trial was approved by the Investigational Review Board of the Radboud university medical center, Nijmegen, The Netherlands. The trial was conducted in accordance with Good Clinical Practice and the

Declaration of Helsinki, and registered at ClinicalTrials.gov (NCT01978782). All participants signed informed consent prior to screening evaluations.

Study population

Healthy male and female participants between the age of 18 and 55 and with a body mass index (BMI) of 18 to 30 kg/m^2 were eligible for enrolment. Included participants had to be in a good, age-appropriate health condition as established by physical examination, medical history, electrocardiography and biochemical, hematologic, and urinalysis testing within 4 weeks prior to day 1. Main exclusion criteria were a positive HIV, hepatitis B and C test result, presence of long QT syndrome or prolonged QT time and use of any medication except for acetaminophen from 2 weeks preceding dosing.

Study drug and dosing

The approved dosage of raltegravir (Isentress, Merck Sharp & Dohme Ltd, Hoddesdon, UK) is 400 mg twice daily.[25,29] The clinical dosage of citalopram for depression in patients from 18-65 years old is 20 mg once daily orally which can be increased to a maximum of 40 mg once daily depending on the effect. We studied the effect of 20 mg citalopram once daily and included dose escalation at start, as well as tapering off before discontinuation of citalopram to minimize the risk of adverse events (AEs). A treatment duration of two weeks for citalopram and 5 days for raltegravir was chosen to allow sufficient time to reach steady-state plasma concentrations on the day of pharmacokinetic assessment. On the days of pharmacokinetic sampling, both raltegravir and citalopram were taken on an empty stomach. A standard breakfast (480 kCal; 26 g fat) was served at 2 h after dosing. Intake of medication at the clinical research centre was supervised and recorded by the research nurses. Tablets were counted to assess adherence. In addition, drug intake of citalopram at home was monitored by use of microelectronic monitoring system (MEMS) caps (Aardex Ltd, Zug, Switzerland), which records the opening of the medication bottle. Subjects were asked to write down the exact times of medication intake in a booklet.

Study procedures

Intensive pharmacokinetic sampling was performed at plasma steady-state conditions. Additional trough samples were collected during treatment to monitor adherence and to ensure that steady-state plasma concentrations had been reached on the day of pharmacokinetic assessment. Blood samples were collected into lithium-heparinized tubes during a 12-h period for raltegravir and a 24-h period for citalopram after observed intake of the study medication. Blood samples were centrifuged for 5 minutes at 1900 x *g* at 20°C. Plasma was transferred to polypropylene tubes and stored at -40°C until further bioanalysis. All participants who received study drugs were included in the safety data set. Safety assessment consisted of monitoring AEs and laboratory evaluations (biochemistry and hematology). Cardiovascular safety assessment, consisting of blood pressure measurement

and electrocardiogram (ECG) recording, was performed predose and 3 h after the first intake of 10 mg and 20 mg citalopram, and repeated at steady state. ECG recordings were evaluated by the medical investigator for potential prolongation of QT intervals. Adverse events were graded according to the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events (version 1.0, December 2004) and causality assessment with the study drugs was performed. Genotyping of CYP2C19 for the presence of the alleles **2* (681G>A), **3* (636G>A), and **17* (806C>T) was performed by the Department of Clinical Chemistry at Erasmus Medical Center (Rotterdam, The Netherlands) as previously described.[30,31] The CYP2C19 genotypes were classified into four phenotypes: poor metabolizer (PM) carrying two loss-of-function alleles (*2/*2, *2/*3, *3/*3); intermediate metabolizer (IM) carrying one loss-of-function allele (*1/*2, *1/*3, *2/*17); extensive metabolizer (EM) carrying normal function alleles (*1/*1, *1/*17), and ultra rapid metabolizer (UM) for alleles (*17/*17).

Bioanalytical methods

The citalopram and desmethylcitalopram assay was performed at the laboratory of the Pharmacy of Maastricht University Medical Centre (Maastricht, The Netherlands) by use of a validated reversed-phase ultra performance liquid chromatography method with fluorescence detection. The linear calibration range in plasma was 1.0 to 200 µg/L. Concentrations of raltegravir in plasma were analyzed using a validated reversed-phase high-pressure liquid chromatography with fluorescence detection as previously described.[32] The linear calibration range in plasma was 0.014-10.0 mg/L. The raltegravir assay was performed at the laboratory of the Pharmacy of the Radboud university medical center (Nijmegen, The Netherlands) and externally validated through the International Interlaboratory Quality Control Program for Measurement of Antiretroviral Drugs in Plasma, as well as by the Clinical Pharmacology Quality Assurance and Quality Control Program (CPQA).[33,34]

Pharmacokinetic analysis

Pharmacokinetic parameters were determined using a noncompartmental model in WinNonlin/Phoenix version 6.3 (Pharsight Corporation, St. Louis, MO, USA). Based on the individual plasma concentration-time data, the following pharmacokinetic parameters of raltegravir, citalopram and desmethylcitalopram were determined: the area under the plasma concentration-time curve from 0 to 12 h (raltegravir) or 24 h (citalopram and desmethylcitalopram) after intake using the trapezoidal rule ($AUC_{0\text{-}tau}$), the trough concentration defined as the plasma concentration of raltegravir 12 hours after intake (C_{12h}) or the plasma concentration of citalopram/desmethylcitalopram 24 hours after intake (C_{24h}), the maximum plasma concentration of the drug (C_{max}), the time to reach C_{max} (t_{max}), the apparent elimination half-life ($t_{1/2}$), the apparent oral clearance (CL/F), and the apparent volume of distribution (V/F). Pharmacokinetic parameters are reported as

geometric means with 95% CIs. Geometric mean ratios (GMRs) of the pharmacokinetic parameters of the test treatment (combination raltegravir + citalopram) versus the reference treatment (raltegravir or citalopram alone) and 90% CIs were calculated using a mixed-effects bioequivalence module in WinNonlin/Phoenix. Citalopram metabolite-to-parent ratios expressed as mean ± standard deviation (SD) of $AUC_{0\text{-}24h}$ were calculated when citalopram was administered alone versus concomitant raltegravir use for all subjects and CYP2C19 phenotype subgroups.

Statistical analysis

The data obtained in this study were analyzed according to an equivalence approach that is recommended for pharmacokinetic interaction studies by FDA and EMA guidelines.[35-37] For citalopram and desmethylcitalopram AUC GMRs with 90% CIs within the range of 0.80 to 1.25 are considered to indicate no significant interaction with raltegravir. For raltegravir AUC GMR with 90% CIs within the range of 0.74 to 1.35 are considered to indicate no significant interaction if the GMR lies within the conventional acceptance range of 0.80 to 1.25. According to the 'Guideline on the Investigation of Bioequivalence' (EMA) the wider equivalence range could be considered for highly variable drugs (intrasubject coefficient of variation >30%), such as raltegravir, if this range in pharmacokinetic parameters is not considered clinically relevant. Sample size calculation (beta=0.2, alpha=0.1) was based on an equivalence range of 0.74-1.35 assuming no difference in AUC of raltegravir with or without citalopram and an intrasubject standard deviation of 0.5 for raltegravir AUC. The required number of participants was 20 and to account for dropouts a total number of 24 participants were included.

Results

Baseline characteristics

In total, 24 healthy volunteers (11 females, 13 males) were enrolled and 22 subjects completed the study. One subject decided to withdraw consent because of mild (grade 1) AEs after intake of a single dose of 10 mg citalopram. One subject withdrew consent after completing the reference treatment with raltegravir due to personal reasons. Subjects were Caucasian (n=22) or mixed-race (n=2). Median (range) age and BMI were 47 (18-53) years and 24.8 (20.9-29.3) kg/m^2, respectively. Twenty-two subjects had evaluable citalopram curves. According to CYP2C19 genotyping these 22 subjects were classified into the following phenotypes: CYP2C19 PM (n=1), CYP2C19 IM (n=7), and CYP2C19 EM (n=14). Adherence to the study treatment was good according to tablet count, booklet and MEMS caps recordings. There were no deviations in drug intake in 21 subjects. Two subjects missed 1-2 doses and one subjects took one extra dose of study medication.

Pharmacokinetics

Raltegravir plasma concentration versus time curves and steady-state pharmacokinetic parameters of raltegravir 400 mg twice daily alone and coadministered with citalopram 20 mg once daily are shown in Figure 1 and Table 1. Coadministration of citalopram decreased raltegravir geometric mean peak plasma concentration (C_{max}) and exposure ($AUC_{0\text{-}12h}$) to raltegravir by 36% and 23%, respectively, compared to administration of raltegravir alone. The median time to reach C_{max} of raltegravir was 2.0 hours irrespective of citalopram treatment. The GMR of the raltegravir $AUC_{0\text{-}12h}$ fell below the predefined interval of 0.80-1.25 according to the equivalence approach and the 90% CI was large and overlapped the lower bound of the predefined interval of 0.74-1.35 for highly variable drugs. GMR of C_{max} as well as the lower bound of the 90% CI fell below the lower level of the 0.74-1.35 interval. The geometric mean C_{12h} and apparent elimination half-life ($t_{1/2}$) were similar after concomitant use of citalopram and raltegravir compared to raltegravir alone with GMRs close to 1.0.

Raltegravir pharmacokinetics exhibit a high degree of inter- and intrasubject variability, which apart from the large CIs in Table 1 is best seen graphically in Figure 2. Figure 2 shows the individual subject changes in $AUC_{0\text{-}12h}$ (Figure 2A) and C_{max} (Figure 2B) of treatment with raltegravir alone versus coadministration with citalopram. Despite an average decrease in the AUC and C_{max} of raltegravir when coadministered with citalopram, an increase in AUC and C_{max} was observed in, respectively, 45% (n=10) and 36% (n=8) of the subjects with paired pharmacokinetic curves (n=22).

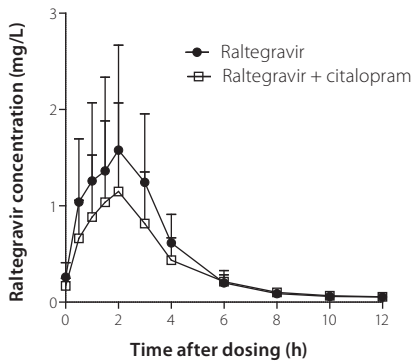

Figure 1 Geometric mean (+ upper 95% CI) raltegravir plasma concentration-time profiles at steady state when raltegravir 400 mg twice daily was administrated alone or with citalopram 20 mg once daily.

Table 1 Comparison of steady-state pharmacokinetic parameters of raltegravir 400 mg twice daily alone and with coadministration of citalopram 20 mg once daily in healthy volunteers.

Pharmacokinetic parameter	Raltegravir alone (n=23)		Raltegravir + citalopram (n=22)		Raltegravir + citalopram: raltegravir alone	
	Geometric mean	95% CI	Geometric mean	95% CI	GMR	90% CI
$AUC_{0\text{-}12h}$ (mg·h/L)	6.82	(4.64-10.0)	5.27	(3.43-8.10)	0.77	(0.50-1.19)
C_{max} (mg/L)	2.45	(1.60-3.76)	1.58	(0.93-2.68)	0.64	(0.38-1.09)
t_{max} [a] (h)	2.0	(1.0-3.0)	2.0	(1.5-3.0)		
C_{12h} (mg/L)	0.054	(0.037-0.080)	0.056	(0.043-0.073)	1.03	(0.71-1.50)
$t_{1/2}$ [b] (h)	2.65	(2.28-3.09)	2.89	(2.43-3.44)	1.09	(0.94-1.28)
CL/F (L/h	58.6	(39.9-86.2)	75.9	(49.4-117)	1.29	(0.84-1.99)
V/F [b] (L)	191	(120-305)	246	(142-428)	1.21	(0.77-1.89)

[a] For t_{max}, median + interquartile range is reported.

[b] $t_{1/2}$ and V/F could not be determined in 3 and 4 subjects for raltegravir alone and raltegravir + citalopram, respectively.

Abbreviations: $AUC_{0\text{-}12h}$, area under the plasma concentration-time curve up to 12 h after intake; CI, confidence interval; C_{max}, maximum plasma concentration; C_{12h}, plasma concentration 12 h after intake; CL/F, apparent oral clearance; GMR, geometric mean ratio; $t_{1/2}$, apparent elimination half-life; t_{max}, time to reach C_{max}; V/F, apparent volume of distribution.

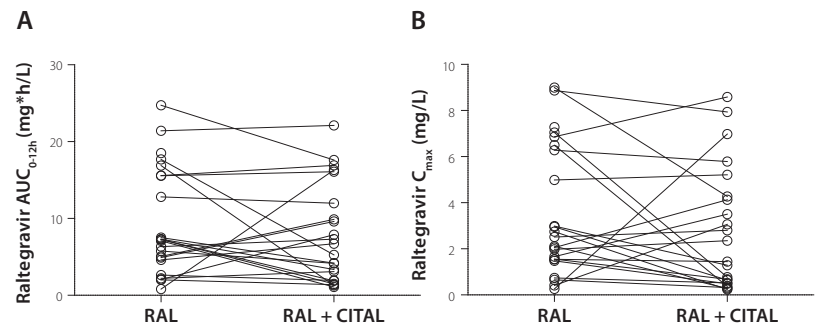

Figure 2 Individual $AUC_{0\text{-}12h}$ (A) and C_{max} (B) of raltegravir at steady state when raltegravir 400 mg twice daily was administrated alone (RAL) or with citalopram 20 mg once daily (RAL + CITAL).

Figure 3 shows the plasma concentration-time curves of citalopram and desmethylcitalopram when citalopram 20 mg once daily was administered alone or with raltegravir 400 mg twice daily. The steady-state pharmacokinetic parameters of citalopram and desmethylcitalopram and the GMRs are described in Table 2. Coadministration of raltegravir did not have an effect on the pharmacokinetic parameters of citalopram and desmethylcitalopram. The GMRs of the exposure ($AUC_{0\text{-}24h}$) to both citalopram and desmethylcitalopram, were close to 1.0 and the 90% CI fell entirely within the range of 0.80 to 1.25. The same applies to the other calculated parameters in Table 2.

Table 2 Comparison of steady-state pharmacokinetic parameters of citalopram and desmethylcitalopram after administration of citalopram 20 mg once daily alone and with coadministration of raltegravir 400 mg twice daily in healthy volunteers.

Analyte and pharmacokinetic parameter	**Citralopram alone (n=22)**		**Citalopram + raltegravir (n=22)**		**Citalopram + raltegravir : citalopram alone**	
	Geometric mean	**95% CI**	**Geometric mean**	**95% CI**	**GMR**	**90% CI**
Citalopram						
$AUC_{0\text{-}24h}$ (µg·h/L)	1140	(996-1304)	1144	(998-1311)	1.00	(0.98-1.03)
C_{max} (µg/L)	59.0	(52.2-66.6)	57.8	(50.7-65.8)	0.98	(0.95-1.01)
t_{max} [a] (h)	4.0	(3.0-4.0)	3.1	(3.0-4.0)		
C_{24h} (µg/L)	37.4	(32.3-43.2)	38.6	(33.6-44.4)	1.03	(1.00-1.07)
$t_{1/2}$ (h)	28.1	(25.8-30.5)	31.6	(29.2-34.2)	1.13	(1.04-1.22)
CL/F (L/h)	17.6	(15.3-20.1)	17.5	(15.3-20.0)	1.00	(0.97-1.02)
V/F (L)	711	(615-822)	798	(687-926)	1.12	(1.03-1.22)
Desmethylcitalopram						
$AUC_{0\text{-}24h}$ (µg·h/L)	372	(333-415)	370	(333-411)	0.99	(0.88-1.12)
C_{max} (µg/L)	17.4	(15.6-19.3)	16.8	(15.1-18.7)	0.97	(0.86-1.09)
t_{max} [a] (h)	10	(6.0-10)	10	(10-10)		
C_{24h} (µg/L)	13.9	(12.4-15.6)	14.5	(13.0-16.1)	1.04	(0.92-1.18)
CL/F (L/h)	53.8	(48.2-60.0)	54.1	(48.6-60.1)	1.01	(0.89-1.14)

[a] For t_{max}, median + interquartile range is reported.

Abbreviations: $AUC_{0\text{-}24h}$, area under the plasma concentration-time curve up to 24 hours after intake; CI, confidence interval; C_{max}, maximum plasma concentration; C_{24h}, plasma concentration 24 hours after intake; CL/F, apparent oral clearance; GMR, geometric mean ratio; $t_{1/2}$, apparent elimination half-life; t_{max}, time to reach C_{max}; V/F, apparent volume of distribution.

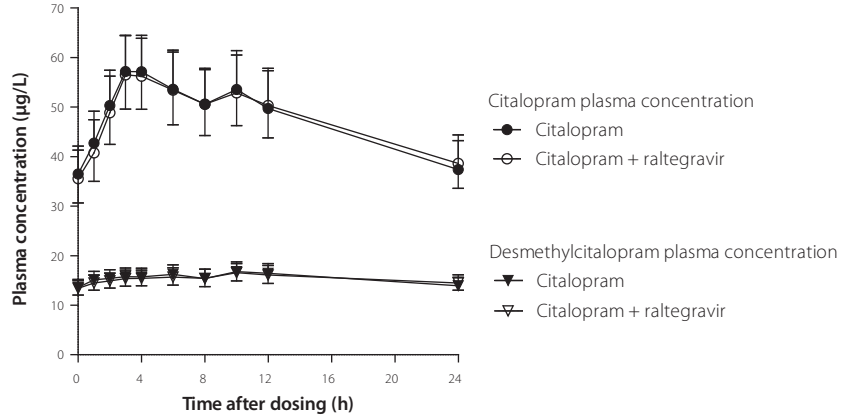

Figure 3 Geometric mean (+95% CI) plasma concentration-time profiles of citalopram and the metabolite desmethylcitalopram at steady state when citalopram 20 mg once daily was administered alone or with raltegravir 400 mg twice daily.

At steady state mean (±SD) metabolite-to-parent ratios of $AUC_{0\text{-}24h}$ (n=22) did not change with concomitant raltegravir use and was 0.34 (±0.91) for citalopram alone versus 0.34 (±0.94) for the combination. Mean (±SD) $AUC_{0\text{-}24h}$ metabolite-to-parent ratios were 0.22 versus 0.21 for CYP2C19 PM (n=1), 0.25 (±0.054) versus 0.25 (±0.060) for CYP2C19 IM (n=7), and 0.39 (±0.065) versus 0.39 (±0.066) for CYP2C19 EM (n=14) for the reference versus the test treatment. The subgroups CYP2C19 IM and PM have a reduced mean desmethylcitalopram-to-citalopram $AUC_{0\text{-}24h}$ ratio compared to CYP2C19 EM. Within each phenotype subgroup the metabolite-to-parent ratio was not influenced by raltegravir use.

Safety and tolerability

The study medication was well tolerated and no serious AEs or grade 3/4 events were reported. A total of 18 of the 24 subjects who received study medication reported 66 AEs that were considered to be possibly (94%) or probably (6%) related to the study medication. The treatment-related AEs were mild in severity and categorized as toxicity grade 1 (86%) and grade 2 (14%). Most frequently reported AEs (≥ 5% incidence and > 2 subjects) were headache (n=19, 8 subjects), drowsiness (n=7, 7 subjects), increased sweating of hands (n=6, 3 subjects), dizziness (n=3, 3 subjects) and nausea (n=3, 3 subjects). The majority of the AEs (n=47) started during the citalopram 10 mg lead-in phase and the 2-week reference treatment with citalopram 20 mg. Seven AEs were reported during the 5 days of combination treatment and 8 AEs started during the 5 days of raltegravir reference treatment. Four AEs were reported when citalopram was tapered off or during the

washout period just after discontinuing the study medication. There were no clinically relevant changes in safety laboratory measurements and cardiovascular safety assessment (vital signs and ECG).

Discussion

Raltegravir concomitant use had no effect on the pharmacokinetics of citalopram and its main metabolite desmethylcitalopram. Relative to treatment with raltegravir alone, coadministration of citalopram resulted in an average decrease in exposure to raltegravir by 23%, which is not considered to be of clinical importance. These results are relevant and reassuring for HIV-infected patients with a depressive disorder who require treatment with an SSRI, such as citalopram.

The average decrease of raltegravir $AUC_{0\text{-}12h}$ (23%) when combined with citalopram, is largely due to a mean reduction in C_{max} of 36%. Lower peak plasma levels can be a result of a decrease in oral bioavailability. Efflux mechanisms such as P-gp play an important role in oral drug absorption. Inducers of P-gp could decrease the oral bioavailability and reduce C_{max} values of P-gp substrates. *In vitro* characterization of raltegravir transport by drug transporters indicates that raltegravir is a weak P-gp substrate.[38] However, this does not explain the mean reduction in C_{max} of raltegravir when combined with citalopram, as *in vitro* research showed that citalopram is a weak P-gp inhibitor.[22,23]
Although in our study a mean decrease in C_{max} was observed, approximately one third of the subjects showed an increase in raltegravir C_{max} with citalopram use. The difference in C_{max} and $AUC_{0\text{-}12h}$ of raltegravir in the presence or absence of citalopram is more likely due to the high variability in raltegravir pharmacokinetics instead of an effect caused by citalopram. The extensive intra- and interindividual variability in the pharmacokinetics of raltegravir seen in our study is described by others also and consistent with the known pharmacokinetic profile of raltegravir.[39-41] A major contributor to the variability in raltegravir pharmacokinetics is pH dependent absorption and dissolution of raltegravir in the gastro-intestinal tract.[42]

An important consideration when interpreting the results is whether the changes in pharmacokinetic parameters are clinically relevant. The magnitude of the observed effect on the AUC of raltegravir (23%) is not regarded to be of clinical importance. Similar effects on the pharmacokinetics of raltegravir are described with drug-interacting agents in the product information leaflet without special recommendations to adjust the dosage of raltegravir.[25] Although no clear relationship between pharmacokinetic parameters and the efficacy of raltegravir has been established for the registered twice-daily dose regimen, the C_{trough} level is considered the most important parameter to evaluate with respect to virological efficacy.[43] The geometric mean plasma concentration taken 12 h after intake of the last dose (C_{12h}) in our study was similar after concomitant use of citalopram and raltegravir compared to raltegravir alone and well above the suggested threshold of 0.020 mg/L from the QDMRK study.[43] The pharmacokinetic parameters of raltegravir observed in our study are comparable to data in HIV-infected patients reported by Markowitz et al.[44]

The results of this study showed that raltegravir has no influence on the pharmacokinetics of citalopram and its main metabolite desmethylcitalopram. It confirms that raltegravir has no influence on CYP2C19, CYP3A4 or CYP2D6, which are involved in citalopram bio-transformation and does not support a role of a drug-drug interaction in the described post-marketing cases with exacerbation of depression after initiating raltegravir.[26]

Pharmacogenetic factors can cause intersubject variability in plasma levels of citalopram and its metabolites. It is known that the metabolism of citalopram is affected by CYP2C19 polymorphism.[20,21] The citalopram metabolite-to-parent ratio of $AUC_{0\text{-}24h}$, which is the most specific measure for enzyme activity, was reduced in CYP2C19 IMs and PMs compared to CYP2C19 EMs. Because our drug-drug interaction study is based on intrasubject comparison and no influence on CYP-enzymes was expected by raltegravir, any genetic differences in citalopram metabolism between subjects are expected to be of minor importance when interpreting the results. This is reflected by our findings that the GMR of the pharmacokinetic parameters of citalopram and desmethylcitalopram was close to 1.0. Furthermore citalopram metabolite-to-parent ratios of $AUC_{0\text{-}24h}$ were not influenced by concomitant raltegravir use.

The use of citalopram and raltegravir was well tolerated and no serious AEs were reported during the conduct of this study. The reported AEs related to the study medication were mild, transient and mostly attributed to citalopram use. This was to be expected as citalopram is associated with certain AEs, such as somnolence and dizziness, in especially the first two weeks of treatment.

There are a few limitations to our study. Our study population was predominantly Caucasian which does not represent the ethnic variability in the global HIV population. Another limitation could be that the combination treatment was started directly after the reference treatment with citalopram. We confirmed that steady-state conditions had been reached on the day of the first pharmacokinetic curve before raltegravir was added by comparing predose plasma concentrations of citalopram and desmethylcitalopram with C_{trough} levels taken two days in advance.

In summary, we evaluated the two-way pharmacokinetic drug-drug interaction and tolerability of concomitant administration of citalopram 20 mg once daily and raltegravir

400 mg twice daily in healthy volunteers. Raltegravir does not influence the pharmacokinetics of citalopram and its main metabolite desmethylcitalopram. Coadministration of citalopram resulted in an average decrease in exposure to raltegravir by 23% which is not considered to be of clinical importance. The combination of raltegravir and citalopram was well tolerated and can be administered without dose adjustments.

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Lack of a clinically significant drug-drug interaction in healthy volunteers between the hepatitis C virus protease inhibitor boceprevir and the HIV integrase inhibitor raltegravir

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Clinical Infectious Diseases 2013; 56(2): 300-6

Abstract

Background

Patients coinfected with human immunodeficiency virus (HIV) and hepatitis C virus (HCV) are likely to use both HIV and HCV treatment. Drug-drug interactions have been demonstrated between boceprevir, an HCV protease inhibitor, and frequently prescribed antiretroviral drugs, such as efavirenz and boosted HIV protease inhibitors. Concomitant administration of boceprevir with these drugs should be avoided. This study was designed to investigate the absence of a drug-drug interaction between boceprevir and raltegravir, an HIV integrase inhibitor.

Methods

This was an open-label, randomized, 2-period, crossover phase 1 trial in 24 healthy volunteers. All subjects were randomly assigned to receive boceprevir 800 mg every 8 hours for 9 days plus a single dose of raltegravir 400 mg on day 10 followed by a washout period and a single dose of raltegravir 400 mg on day 38, or the same medication in reverse order. Blood samples for pharmacokinetics were collected and pharmacokinetic parameters were calculated.

Results

The geometric mean (GM) of raltegravir area under the concentration-time curve $(AUC)_{0\text{-}12h}$ and maximum plasma concentration (C_{max}) for raltegravir + boceprevir versus raltegravir alone were 4.27 (95% confidence interval [CI], 3.22-5.66) versus 4.04 (95% CI, 3.09-5.28) mg·h/L and 1.06 (95% CI, 0.76-1.49) versus 0.93 (95% CI, 0.70-1.23) mg/L, respectively. GM ratio estimates of raltegravir $AUC_{0\text{-}12h}$ and C_{max} for raltegravir + boceprevir versus raltegravir alone were 1.04 (90% CI, 0.88-1.22) and 1.11 (90% CI, 0.91-1.36), respectively. The GM of boceprevir $AUC_{0\text{-}8h}$, C_{max}, and C_{8h} were 5.45 (95% CI, 5.11-5.81) mg·h/L, 1.88 (95% CI, 1.72-2.06) mg/L, and 0.09 (95% CI, 0.07-0.11) mg/L, respectively. These data are comparable to those from historical controls.

Conclusions

Due to the absence of a clinically significant drug interaction, raltegravir can be recommended for combined HIV/HCV treatment including boceprevir.

Introduction

The prevalence of hepatitis C virus (HCV) and human immunodeficiency virus (HIV) coinfection ranges from ±10% to 70% in Europe and North America.[1] Since the introduction of combination antiretroviral therapy (cART), the life expectancy of HIV-infected patients has improved dramatically. Since then, liver-related deaths have become the most frequent cause of non-AIDS-related deaths, to which HCV coinfection makes a substantial contribution.[2]

The NS3 serine protease inhibitors boceprevir and telaprevir have been approved since 2011 for use in patients with chronic HCV genotype 1 infection. When added to the standard of care, sustained virological response (SVR) rates improve by 25%-31% shown in HCV monoinfected patients.[3,4] In total SVR rates around 68-75% are seen.

According to US guidelines, first-line cART for HIV-infected patients should consist of the 2 nucleoside reverse transcriptase inhibitors (NRTIs) tenofovir and emtricitabine, in combination with the nonnucleoside reverse transcriptase inhibitor efavirenz, the ritonavir-boosted HIV protease inhibitors atazanavir or darunavir, or the integrase inhibitor raltegravir.[5]

As HIV/HCV-coinfected patients are likely to use both HIV and HCV treatment, including the HCV protease inhibitors, simultaneously, it is important to know if drug-drug interactions occur. At this moment it is not recommended to coadminister boceprevir with darunavir/ritonavir, lopinavir/ritonavir, or efavirenz because decreased concentrations of boceprevir, as well as decreased concentrations of the boosted HIV protease inhibitors, have been found.[6,7] Because the combination with atazanavir/ritonavir did not substantially influence boceprevir concentrations, although atazanavir levels were lower, coadministration of these drugs can be considered on a case-by-case basis.[7] The only remaining first-line antiretroviral agent that can be added to an NRTI backbone is raltegravir. Raltegravir is a substrate of UDP-glucuronosyltransferase (UGT) and does not influence cytochrome P450 (CYP) enzymes; boceprevir is a substrate of aldo-keto reductase and CYP3A and inhibits CYP3A. Hence, no significant interaction between boceprevir and raltegravir is expected, but pharmacokinetic drug-drug interaction studies are lacking.

This pharmacokinetic study in healthy volunteers was performed to confirm that a clinically significant drug-drug interaction between raltegravir and boceprevir is absent.

Materials and methods

Study design

This open-label, 2-period, randomized, crossover phase 1 trial was conducted from August to November 2011 at the Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands. The study was designed to determine the effect of steady-state boceprevir on the pharmacokinetics of a single dose of raltegravir by intrasubject comparison. The secondary objective was to examine the effect of a single dose of raltegravir on the pharmacokinetics of steady-state boceprevir (by comparison with historical controls) and to study the safety of a single-dose raltegravir coadministered with steady-state boceprevir. Healthy volunteers were equally randomized to 2 treatment groups. Group A received a single dose of 400 mg of raltegravir on day 10. After a washout period of 2 weeks, the participants took 800 mg of boceprevir every 8 hours with food for 9 days (days 29-37). On day 38 they received a single dose of 400 mg of raltegravir and 2 doses of 800 mg of boceprevir (1 together with raltegravir and 1 dose 8 hours later). Group B received the same regimens but in reversed order. On days 10 and 38, a 12-hour pharmacokinetic curve was recorded.

Procedures

The trial was approved by the Investigational Review Board of the Radboud University Nijmegen Medical Centre. The trial was conducted in accordance with Good Clinical Practice and the Declaration of Helsinki. All participants signed informed consent prior to screening evaluations.

Study population

Healthy male and female subjects aged 18-55 years and with a body mass index (BMI) of 18-30 kg/m2 (extremes included) were eligible for enrolment. Included participants had to be in a good, age-appropriate health condition as established by physical examination, medical history, electrocardiography, and biochemical, hematologic, and urinalysis testing within 4 weeks prior to day 1. Main exclusion criteria were a history of sensitivity or idiosyncrasy to medicinal products or excipients; a positive HIV, hepatitis B or C test result; or the use of any medications (for 2 weeks preceding dosing) except for acetaminophen.

Study drug and dosing

The approved dose of boceprevir (Victrelis, Merck Sharp & Dohme) is 800 mg every 8 hours with food.[6] In this study, subjects took 4 capsules of 200 mg of boceprevir at approximately 08:00 hours, 16:00 hours, and 0:00 hours with a meal or a snack. A treatment duration of 10 days was chosen to reach steady state and to assess potential effects on metabolizing enzymes or drug transporters. Raltegravir (Isentress, Merck Sharp & Dohme) was administered as a single dose of 400 mg on day 10 and day 38 together with a standardized breakfast consisting of 2 slices of wheat bread (1 slice with cheese and 1 with sliced sausage) and 1 glass of milk.

Intake of medication at the clinical trial unit was supervised and recorded by the study personnel. Drug intake of boceprevir at home was monitored by use of microelectronic monitoring system (MEMS) caps (Aardex, Zug, Switzerland), which records the opening of the medication bottle. In addition, the weight of the bottles containing the boceprevir capsules was recorded on each visit day during boceprevir treatment to assess adherence. Subjects were asked to write down the exact times of medication intake in a booklet.

Pharmacokinetic sampling and safety assessments

Blood samples for assessment of pharmacokinetic parameters of raltegravir were collected during a 12-hour period after intake of a single dose of 400 mg of raltegravir on days 10 and 38. Blood samples were collected into heparinized tubes and centrifuged for 10 minutes at 1900 x *g* at 20°C. Plasma was transferred to polypropylene tubes and stored at -40°C until further bioanalysis.

Blood samples for assessment of pharmacokinetic parameters of boceprevir were collected during an 8-hour period after intake of 800 mg of boceprevir on day 10 or 38. In addition, blood samples were taken predose on days 1, 3, 6, and 8 (group B) and on days 28, 31, 34, and 36 (group A). Blood samples for boceprevir were collected into prechilled potassium-ethylenediaminetetraacetic acid-containing tubes and centrifuged for 15 minutes at 1500 x *g* at 4°C within 30 minutes after blood collection. Plasma (1.5 mL) was transferred to prechilled cryovials containing 75 µL of 85% phosphoric acid, mixed by a vortex mixer and stored at ≤–20°C within 1 hour of sample collection.

Bioanalytic methods

Concentrations of raltegravir in plasma were analyzed by use of a validated reversed-phase high-pressure liquid chromatography (HPLC) method with fluorescence detection.[8] The linear calibration ranges in plasma were from 0.014 mg/L to 10.0 mg/L. The raltegravir assay was performed at the laboratory of the Pharmacy of the Radboud University Nijmegen Medical Centre and was externally validated through the International Interlaboratory Quality Control Program for Measurement of Antiretroviral Drugs in Plasma, as well as by the Proficiency Testing Program of the ACTG/IMPAACT group.[9,10]

Boceprevir (SCH503034) is an approximately equal mixture of 2 diastereomers; SCH534128, the active diastereomer and SCH534129, which is inactive. The predominant metabolic pathway produces inactive stereoisomers, together called SCH629144.[11] Concentrations of boceprevir were determined as the sum of concentrations of the 2 diastereomers SCH534128 and SCH534129. Concentrations of SCH629144 were obtained as the sum of concentrations of 4 analytes, namely, SCH783004, SCH783005, SCH783006, and SCH783007.

The overall lower limit of quantification (LLOQ) was 0.0048 mg/L for boceprevir and 0.0025 mg/L for SCH629144. The calibration range for SCH534128 and SCH534129 and for the 4 metabolites were from the LLOQ to 5.20 mg/L, 4.80 mg/L, and 2.50 mg/L, respectively. Concentrations of both diastereomers and its metabolites in collected plasma samples were determined using HPLC-tandem mass spectrometry at PPD Global Central Labs (Middleton, Wisconsin).

Pharmacokinetic analysis

Based on the individual plasma concentration-time data, the following pharmacokinetic parameters of raltegravir were determined: the area under the concentration-time curve from 0 to 12 hours after intake ($AUC_{0\text{-}12h}$), maximum plasma concentration (C_{max}), time of C_{max} (t_{max}), the bioavailability adjusted volume of distribution (V/F), apparent oral clearance (CL/F), and the apparent elimination half-life ($t_{1/2}$). For boceprevir (both diastereomers and metabolites) the same parameters were determined plus the concentration at 8 hours after intake (C_{8h}); AUC was determined from 0 to 8 hours after intake ($AUC_{0\text{-}8h}$). All pharmacokinetic parameters were calculated by noncompartmental methods using the linear log trapezoidal rule.

Statistical analysis

The data obtained in this study were analyzed according to an equivalence approach that is recommended for pharmacokinetic interaction studies.[12,13] The main pharmacokinetic parameter to be evaluated in this respect was the exposure to raltegravir, as expressed in the $AUC_{0\text{-}12h}$. The required sample size was calculated (power of 80%) assuming no difference in $AUC_{0\text{-}12h}$ of raltegravir with or without boceprevir and an intrasubject coefficient of variation of 22.5% of raltegravir AUCs. The required number of participants was 20. Taking dropouts into account, in total 24 subjects were included in the study.

The geometric mean ratio estimates of all determined pharmacokinetic parameters of raltegravir with boceprevir versus raltegravir alone, except for t_{max}, were calculated using the mixed model analysis, with the Kenward-Roger approach for the evaluation of the fixed effects. In addition, the nonparametric Wilcoxon signed-rank test was done for t_{max} values between the 2 regimens. Geometric mean ratio estimates with 90% confidence interval (CI) entirely within the range of 0.80-1.25 were considered to indicate no significant interaction. Pharmacokinetic parameters of boceprevir (diastereomers and metabolites) were compared with historical data from healthy volunteers.

Statistical analyses were carried out using SPSS software version 16.0 or higher (SPSS, Chicago, Illinois) and SAS 9.2. Descriptive statistics were calculated using Excel 2007 software (Microsoft Corporation) or WinNonlin version 5.3 (Pharsight Corporation).

Results

Baseline characteristics

Twenty-four healthy volunteers (12 males) were included in the study. Subjects were of white (n = 22), black (n = 1), or mixed Asian/white (n = 1) ethnicity. The mean age and BMI were 38 years (range, 20-55 years) and 23 kg/m^2 (18-27 kg/m^2).

Twenty-two subjects (10 males) completed the trial. One subject had to discontinue due to nonadherence to the study protocol and another subject because of elevated alanine aminotransferase. Both dropouts completed the raltegravir alone treatment and remained included in the demographics, safety, and pharmacokinetic analyses.

Compliance

The compliance to boceprevir treatment was good. All but 1 subject (21/22) took all doses of boceprevir and raltegravir according to pill count, diary, and MEMS caps recordings. Only 1 subject missed 1 dose of boceprevir. Seven subjects (1-3 times per subject) took the dose of boceprevir outside a 2-hour time frame (07:00-09:00 hours/15:00-17:00 hours/ 23:00-01:00 hours).

Pharmacokinetics

Pharmacokinetic parameters were calculated on all available data from the 24 subjects included in the trial. The plasma concentration versus time curves of raltegravir alone and of raltegravir with boceprevir are shown in Figure 1. The pharmacokinetic parameters of raltegravir with and without boceprevir are shown in Table 1. For raltegravir coadministered with boceprevir relative to raltegravir alone, the geometric mean ratio estimates of $AUC_{0\text{-}12h}$

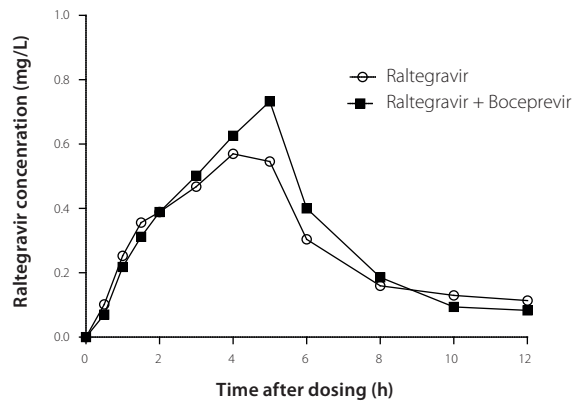

Figure 1 Geometric mean plasma concentrations of raltegravir following a single dose of 400 mg raltegravir in the presence and absence of steady-state boceprevir.

Table 1 Comparison of single-dose pharmacokinetic parameters of raltegravir with or without coadministration of multiple doses of boceprevir in healthy volunteers

	n	RAL + BOC GM (95% CI)		n	RAL GM (95% CI)		n[a]	Geometric Mean Ratio Estimate (90% CI)	
$AUC_{0\text{-}12h}$ (mg·h/L)	22	4.27	(3.22-5.66)	24	4.04	(3.09-5.28)	22	1.04	(0.88-1.22)
C_{max} (mg/L)	22	1.06	(0.76-1.49)	24	0.93	(0.70-1.23)	22	1.11	(0.91-1.36)
t_{max} [b] (h)	22	5.00	(1.00-12.00)	24	4.00	(1.00-12.03)	22		
V/F (L)	19	261.2	(176.6-386.2)	19	335.3	(234.8-478.9)	17	0.75	(0.58-0.98)
CL/F (L/h)	19	82.5	(58.0-117.3)	19	81.4	(54.8-120.9)	17	0.99	(0.73-1.35)
$t_{1/2}$ (h)	15	1.83	(1.42-2.34)	13	1.80	(1.31-2.47)	10	0.98	(0.74-1.30)

[a] The number of paired samples per parameter is given.

[b] For t_{max}, median + range is reported; the result of the Wilcoxon signed-rank test was P = .312.

Abbreviations: $AUC_{0\text{-}12h}$, area under the plasma concentration-time curve 0-12 hours after intake; BOC, boceprevir; CI, confidence interval; CL/F, apparent oral clearance; C_{max}, maximum plasma concentration; RAL, raltegravir; $t_{1/2}$, elimination half-life; t_{max}, time to reach C_{max}; V/F, volume of distribution.

and C_{max} were 1.04 (90% CI, 0.88-1.22) and 1.11 (90% CI, 0.91-1.36). The geometric mean ratio estimates with 90% CI of the main pharmacokinetic parameter raltegravir $AUC_{0\text{-}12h}$ fell entirely within the range of 0.80 to 1.25, which indicates no significant interaction with boceprevir. It is suggested that boceprevir does not influence the other pharmacokinetic parameters of raltegravir.

The plasma concentrations versus time curves of boceprevir, the active diastereomer SCH534128, and the inactive diastereomer SCH534129 after multiple doses of boceprevir are shown in Figure 2. The pharmacokinetic parameters of boceprevir, the diastereomers, and the metabolites together as SCH629144 are given in Table 2. The $AUC_{0\text{-}8h}$ of boceprevir in this study was 5.45 mg·h/L and in historical controls the $AUC_{0\text{-}8h}$ of boceprevir was 5.41 mg·h/L.[6] No differences in exposure to boceprevir or the individual diastereomers were observed compared with historical controls.

Adverse events and safety assessments

No serious adverse events were reported. In total, 90 adverse events were reported by 22 subjects after intake of study medication. The most frequently reported adverse experiences that were possibly, probably, or definitely drug-related are shown in Table 3. Two adverse events (creatine kinase elevation and myalgia) were reported as grade 4 of intensity. All other adverse events were grade 1 or 2 of intensity. No additional side effects were seen when raltegravir was added to steady-state boceprevir.

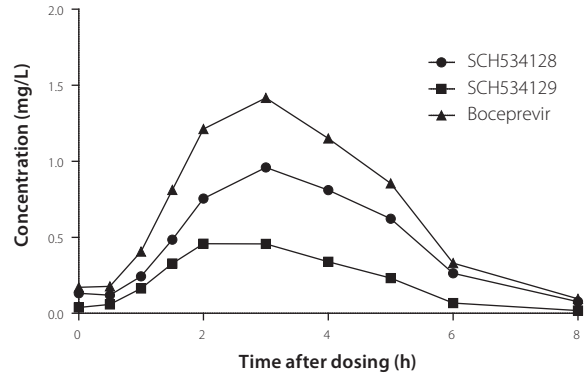

Figure 2 Geometric mean plasma concentrations of boceprevir, SCH534128, and SCH534129 after multiple doses of boceprevir 800 mg and a single dose of raltegravir 400 mg.

Table 2 Pharmacokinetic parameters of multiple doses of boceprevir in healthy volunteers

		boceprevir		SCH534128 (active)		SCH534128 (inactive)		SCH629144 (metabolites)	
	n[a]	GM	(95% CI)	GM	(95% CI)	GM	(95% CI)	GM	(95% CI)
$AUC_{0\text{-}8h}$ (mg·h/L)	22	5.45	(5.11-5.81)	3.74	(3.50-4.01)	1.69	(1.56-1.82)	22.69	(19.94-25.83)
C_{max} (mg/L)	22	1.88	(1.72-2.06)	1.23	(1.13-1.35)	0.65	(0.59-0.73)	5.19	(4.53-5.95)
t_{max} [b] (h)	22	3.00	(1.50-5.00)	3.00	(1.50-5.00)	2.00	(1.50-5.00)	4.00	(2.00-5.00)
C_{8h} (mg/L)	22	0.09	(0.07-0.11)	0.07	(0.06-0.08)	0.02	(0.01-0.02)	1.30	(1.04-1.62)
V/F (L)	22	224.5	(201.6-250.0)	334.2	(298.7-373.9)	671.6	(587.7-767.4)	73.9	(64.1-85.2)
CL/F (L/h)	22	143.0	(133.9-152.7)	207.0	(193.1-221.9)	467.1	(431.9-505.2)	30.7	(26.7-35.1)
$t_{1/2}$ (h)	20	1.10	(1.04-1.17)	1.14	(1.07-1.21)	1.01	(0.94-1.08)	1.65	(1.49-1.84)

[a] n = 13 for the SCH629144 $t_{1/2}$.

[b] For t_{max}, median (range) has been reported.

Abbreviations: $AUC_{0\text{-}8h}$, area under the plasma concentration-time curve 0-8 hours after intake; C_{8h}, concentration at 8 hours after intake; CI, confidence interval; CL/F, apparent oral clearance; C_{max}, maximum plasma concentration; GM, geometric mean; $t_{1/2}$, elimination half-life; t_{max}, time to reach Cmax; V/F, volume of distribution.

Table 3 Most frequently reported treatment drug-related adverse events in number of subjects reporting this adverse event

Adverse event	Raltegravir (n=24) (%)	Washout raltegravir (n=12) (%)	Boceprevir (n=22) (%)	Boceprevir + raltegravir (n=22) (%)	Washout boceprevir + raltegravir (n=10) (%)	Total (n=24) (%)
Dysgeusia			17 (77)			17 (77)
Headache		2 (17)	4 (18)	3 (14)		8 (33)
Atypical lymphocytes			3 (14)			3 (14)
Nausea			3 (14)			3 (13)
Xerostomia			3 (14)			3 (13)
Elevated ALT			2 (9)		1 (10)	3 (13)
Myalgia		1 (8)	2 (9)			2 (8)
Sore throat			2 (9)			2 (8)
Dyspepsia			2 (9)			2 (8)
Elevated AST			1 (5)		1 (10)	2 (8)
Diarrhea			1 (5)	1 (5)		2 (8)

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase.

Discussion

No significant difference was observed for the most important pharmacokinetic parameter of raltegravir, $AUC_{0\text{-}12h}$, between raltegravir alone and raltegravir in combination with boceprevir. Exposure to boceprevir in the presence of raltegravir was comparable to historical controls. Because boceprevir interacts with many other first-line antiretroviral drugs, it is relevant to know that boceprevir combined with raltegravir is a good treatment option for HIV/HCV-coinfected patients because of the absence of a clinically significant drug-drug interaction.

Boceprevir and telaprevir have shown some extensive drug-drug interactions with various drugs and drug classes. Several drug combinations with boceprevir or telaprevir should be avoided or should be used with great caution. These HCV protease inhibitors can be the perpetrator or victim of such interactions. Since there is an association between boceprevir and telaprevir exposure and HCV decline, a reduction in plasma concentrations might lead to a decreased efficacy of treatment or even to resistance.[14-16] An explanation for the large number of drug-drug interactions with boceprevir and telaprevir, is that both HCV protease inhibitors are substrates and inhibitors of the CYP3A enzyme, which is

responsible for the metabolism of numerous drugs.[6,7,17,18] Besides that, they are also substrates and inhibitors of P-glycoprotein (P-gp), an efflux transporter that plays a significant role in the absorption and elimination of many drugs.[6,7,17,18]

At this moment, there are a number of studies performed on potential drug-drug interactions with the HCV protease inhibitors and antiretroviral drugs. Boceprevir did not influence the AUC of the NRTI tenofovir.[19] The nonnucleoside reverse transcriptase inhibitor efavirenz is known to induce CYP3A enzymes and P-gp transporters and boceprevir AUC and trough concentrations were reduced by 19% and 44%, respectively, in combination with efavirenz; this combination should be avoided.[19] When the HIV protease inhibitors boosted with ritonavir are coadministered with telaprevir or boceprevir, higher concentrations of the HCV protease inhibitors were theoretically expected (due to CYP3A inhibition by ritonavir), but controversially, concentrations were found to be lower. Trough concentrations of boceprevir were 18%, 35%, and 57% lower in combination with boosted atazanavir, darunavir, and lopinavir, respectively.[20] In addition, decreased concentrations of the HIV protease inhibitors were found when taken with boceprevir.

Until now, the effect of boceprevir on raltegravir or vice versa was not known, but a drug-drug interaction was not expected based on the pharmacokinetic characteristics of both drugs. Boceprevir is metabolized by 2 distinctive pathways, mainly through ketone reduction by aldo-keto reductase (AKR1C2 and AKR1C3) and to a lesser extent by CYP3A4 and CYP3A5.[6,7] Because the biotransformation and clearance of boceprevir involves 2 different enzymatic pathways, it is less likely to be subject to significant drug-drug interactions with concomitant medication affecting only 1 of these pathways. Boceprevir is a strong inhibitor of CYP3A4 and CYP3A5.[6,7] Raltegravir is not a substrate of CYP and does not influence CYP-mediated metabolism of other agents.[21,22] It is a P-gp substrate, and is metabolized by UGT but does not itself influence UGT-mediated metabolism of other agents.[21-23]

Because raltegravir is not a CYP3A substrate and thus will not be affected by the strong inhibition of CYP3A by boceprevir, and because raltegravir is metabolized by UGT but boceprevir is not known to influence UGT, a major drug-drug interaction is unlikely with this combination. A minor interaction may occur through inhibition of P-gp mediated transport of raltegravir by boceprevir.

However, even when no drug interaction is expected theoretically, it may be recommended to collect sufficient clinical evidence to support this hypothesis because unexpected interactions with antiretroviral agents have been observed in the past. This is also true for raltegravir; for instance, there is a 17% decrease in atazanavir $AUC_{0\text{-}12h}$ when combined with raltegravir, and combined use of tenofovir and raltegravir leads to 49% increase in raltegravir AUC.[24,25]

Since raltegravir has been demonstrated to be a drug with a low interaction profile and in general is the victim and not the perpetrator of drug-drug interactions, the primary objective of this study was to determine the effect of multiple doses of boceprevir on the pharmacokinetics of a single dose of raltegravir. Because influence of raltegravir on boceprevir was considered unlikely, and to reduce exposure of the drugs to healthy volunteers, we chose to perform a 1-way interaction study and therefore compared the pharmacokinetic data on boceprevir found in our study with data from historical controls. In light of other unexpected findings from drug-drug interaction studies with boceprevir that are known at this moment, a 2-way interaction study would be preferred in order to compare the boceprevir pharmacokinetics with and without raltegravir intraindividually.[20] Our study was conducted in healthy volunteers, limiting our interpretation in HIV/HCV-coinfected patients. There are few data on the pharmacokinetics of a single dose of 400 mg raltegravir in the target population. The pharmacokinetics of boceprevir are not different in HCV-positive or -negative patients, but in patients with cirrhosis higher plasma concentrations of boceprevir are found.[6,7] It is, however, not likely that higher concentrations of boceprevir and/or raltegravir will affect the possibility of an interaction between these drugs.

Phase 2 clinical trials with boceprevir in HIV-coinfected patients is ongoing and interim results seem to be promising.[26] Twelve weeks after therapy with boceprevir added to pegylated interferon-alfa and ribavirin, 60.7% of patients had an undetectable HCV load versus 26.5% of patients on standard of care only. In the study with boceprevir, patients were not allowed to use efavirenz and the number of patients on ritonavir-boosted HIV protease inhibitors or raltegravir was small. Unfortunately, drug concentration data have not yet been presented and, therefore, up till now, it remains unknown if reduced drug concentrations have contributed to HIV or HCV breakthroughs.

In conclusion, coadministration of multiple-dose boceprevir with raltegravir did not meaningfully affect single-dose raltegravir exposure. Steady-state boceprevir exposure after coadministration with a single dose of raltegravir was comparable to the exposure of boceprevir administered alone as reported for historical controls. Due to the absence of a clinically significant drug-drug interaction, raltegravir can be recommended for combined HIV/HCV treatment including boceprevir. In the groups of healthy volunteers participating in this study, coadministration of single-dose raltegravir to steady-state boceprevir was safe and well tolerated.

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Effect of Ginkgo biloba on the pharmacokinetics of raltegravir in healthy volunteers

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Antimicrobial Agents and Chemotherapy 2012; 56(10): 5070-5

Abstract

Medicinal herbs may cause clinically relevant drug interactions with antiretroviral agents. Ginkgo biloba extract is a popular herbal product among HIV-infected patients because of its positive effects on cognitive function. Raltegravir, an HIV integrase inhibitor, is increasingly being used as part of combined antiretroviral therapy. Clinical data on the potential inhibitory or inductive effect of Ginkgo biloba on the pharmacokinetics of raltegravir were lacking, and concomitant use was not recommended. We studied the effect of Ginkgo biloba extract on the pharmacokinetics of raltegravir in an open-label, randomized, two-period, crossover phase I trial in 18 healthy volunteers. Subjects were randomly assigned to a regimen of 120 mg of Ginkgo biloba twice daily for 15 days plus a single dose of raltegravir (400 mg) on day 15, a washout period, and 400 mg of raltegravir on day 36 or the test and reference treatments in reverse order. Pharmacokinetic sampling of raltegravir was performed up to 12 h after intake on an empty stomach. All subjects (9 male) completed the trial, and no serious adverse events were reported. Geometric mean ratios (90% confidence intervals) of the area under the plasma concentration-time curve from dosing to infinity ($AUC_{0-\infty}$) and the maximum plasma concentration (C_{max}) of raltegravir with Ginkgo biloba versus raltegravir alone were 1.21 (0.93 to 1.58) and 1.44 (1.03 to 2.02). Ginkgo biloba did not reduce raltegravir exposure. The potential increase in the C_{max} of raltegravir is probably of minor importance, given the large intersubject variability of raltegravir pharmacokinetics and its reported safety profile.

Introduction

Approximately 60% of HIV-infected patients use complementary and alternative medicines to treat HIV-related symptoms and side effects of conventional antiretroviral therapy. Herbal medicines can cause clinically significant interactions with antiretroviral agents with potential drug failure as a result.[1,2] Among the most popular herbal products used worldwide is Ginkgo biloba extract, which is made from the leaves of the Ginkgo biloba tree. It is used for the treatment of peripheral vascular disease and is frequently taken for its claimed beneficial effects on concentration, memory, depressive disorders and dementia.[3] Because cognitive impairment is one of the most feared complications among HIV-infected patients, the popularity of Ginkgo biloba within this patient group is easily explained.[4] Although Ginkgo biloba extract has potential beneficial effects, self-medication with Ginkgo biloba may lead to undesirable drug interactions with regular medication. For example, a study in healthy subjects showed that plasma concentrations of midazolam (a CYP3A probe) were significantly reduced after Ginkgo biloba intake.[5] If this were also true with antiretroviral agents, it could place individual patients at risk for virological failure.

In past years, a few articles have been published about the potential negative effects of Ginkgo biloba on the pharmacokinetics of antiretroviral agents. One case report described virological failure in an HIV-infected patient taking an efavirenz-based regimen due to concomitant use of Ginkgo biloba. Although the underlying mechanism remained unclear, terpene lactones in Ginkgo biloba may lower plasma efavirenz levels by the induction of CYP2B6, CYP3A4 and/or P-glycoprotein (P-gp).[6] Unlike the inductive effects of Ginkgo biloba on the pharmacokinetics of midazolam, Ginkgo biloba extract did not change the exposure of lopinavir (a protease inhibitor and CYP3A substrate) when used with low-dose ritonavir. The use of low-dose ritonavir, a potent CYP3A inhibitor, may have offset the effect of Ginkgo biloba on the pharmacokinetics of lopinavir.[5]

In vivo data of Ginkgo biloba with other antiretroviral drug classes, such as HIV integrase inhibitors, like raltegravir, are lacking. According to current guidelines, raltegravir in combination with tenofovir/emtricitabine is recommended as one of the preferred regimens for antiretroviral-naïve patients.[7,8] Raltegravir targets the HIV-1 integrase enzyme and prevents the integration of viral DNA into the genome of the host cell. Raltegravir has shown sustained antiretroviral activity, is generally well tolerated and has little propensity to interact with other drugs. The primary route of metabolism is glucuronidation via UDP-glucuronosyltransferase 1A1 (UGT1A1) in the liver, with minor contributions from UGT1A3 and UGT1A9.[9-11] *In vitro* studies suggest that raltegravir is a weak P-gp substrate.[12] There is in vitro and animal evidence that Ginkgo biloba modulates UGT enzymes. Ginkgo biloba extract and ginkgolides induce the expression of UGT1A1 in human primary

hepatocytes, although inhibitory effects on UGT enzymes of Ginkgo biloba extract, or one of its components, have been described, as well.[13-16] Other investigations showed that long-term use of Ginkgo biloba inhibits P-gp-mediated drug transport, but contrary results have also been reported.[3,17-20]

Given the inconclusive data on the potential inhibitory or inductive effect of Ginkgo biloba extract on glucuronidation or P-gp-mediated transport, Ginkgo biloba may theoretically influence the exposure to raltegravir. Therefore, concomitant use is currently not recommended. The objectives of this study were to assess the effect of steady-state Ginkgo biloba on the pharmacokinetics of a single dose of raltegravir in healthy volunteers and to evaluate the safety and tolerability of the combination.

Materials and methods

Study design

This open-label, randomized, two-period, crossover, single-centre, phase I trial was conducted from December 2010 to March 2011 at the Radboud University Nijmegen Medical Centre (Nijmegen, The Netherlands). The study was designed to examine the effect of multiple doses of Ginkgo biloba on the pharmacokinetics of a single dose of raltegravir by intrasubject comparison. The secondary objective was to evaluate the safety of the combined use of raltegravir and Ginkgo biloba.

Eighteen healthy volunteers (9 females and 9 males) were stratified according to gender in group A en B. In group A, participants received 120 mg of Ginkgo biloba twice daily for 14 days, followed by a single dose on day 15, together with a single dose of 400 mg of raltegravir. After a washout period of 3 weeks, the participants received a single dose of 400 mg of raltegravir on day 36. In group B, participants received the test and reference treatments in reverse order. They received a single dose of 400 mg of raltegravir on day 15, followed by a washout period of 1 week. On day 22 they started Ginkgo biloba treatment (120 mg twice daily for 14 days). On day 36, participants took a single dose of Ginkgo biloba together with a single dose of 400 mg of raltegravir. The trial was approved by the Investigational Review Board of the Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands. The trial was conducted in accordance with good clinical practice and the Declaration of Helsinki. The study was registered at http://www.ClinicalTrials.gov (NCT01246804).

Study population

Healthy male and female participants between the age of 18 and 55 years and with a body mass index of 18 to 30 kg/m^2 (extremes included) were eligible for enrolment. The

included participants had to be in a good, age-appropriate health, as established by physical examination, medical history, electrocardiography, and biochemical, hematologic, and urinalysis testing within 4 weeks prior to day 1. The subjects had to be able and willing to sign an informed consent form prior to screening evaluations. The main exclusion criteria were a history of sensitivity or idiosyncrasy to medicinal products or excipients, a positive HIV or hepatitis B or C test result, or therapy with any drug (for 2 weeks preceding dosing), except for acetaminophen. Other exclusion criteria were participation in a drug trial or blood donation within 60 days prior to day 1 of the study. Pregnant and breastfeeding females were also excluded.

Study drug and dosing

Ginkgo biloba extract is a complex mixture of chemical constituents, and the actual contents may vary depending on the part of the plant being processed, the season, or the manufacturing process.[3] The industry standard for powdered extracts, as used in this study, is 24% flavonoids (quercetin, kaempferol, and isorhamnetin, among others) and 6% terpene lactones (ginkgolides and bilobalide) by weight. In this trial, the commercial product Tavonin (a tablet with 40 mg of Ginkgo biloba extract) was used because of its standardized composition of 9.6 mg of flavonoids and 2.4 mg of terpene lactone. Tavonin (Dr. Willmar Schwabe GmbH, Karlsruhe, Germany) is licensed in The Netherlands for the treatment of occlusive peripheral arterial disease. The Ginkgo biloba dose used in this trial (120 mg twice daily) is the recommended dosage for the prevention of cognitive decline and memory support.[21,22] A treatment period of 14 days of Ginkgo biloba was chosen to reach steady state and asses potential effects on metabolizing enzymes or drug transporters. Raltegravir (Isentress; Merck Sharp & Dohme Limited, Hoddesdon, United Kingdom) was administered as a single dose of 400 mg on an empty stomach followed by a standardized breakfast 2 h after intake. The breakfast consisted of a glass of milk and two slices of wheat bread with cheese and cervelat.

Pharmacokinetic sampling and safety assessments

Blood samples for assessment of the pharmacokinetic parameters of raltegravir were collected during a 12-hour period at 0 (predose), 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10 and 12 h after intake of a single dose of 400 mg of raltegravir on days 15 and 36. Blood samples were collected into heparinized tubes and centrifuged for 10 minutes at 2930 x *g* at 20°C. Plasma was transferred to polypropylene tubes and stored at -40°C until further bioanalysis. Blood samples for serum biochemistry and hematology were taken on days 15 and 36, as well as before and during Ginkgo biloba treatment (days 1 and 8 of treatment). Subjects were asked about the presence of adverse events at each visit day. Screening for drugs of abuse in urine was performed on days 15 and 36; blood glucose and urinalysis were carried out on day 36. Pregnancy was checked by performing a human chorionic gonadotropin (hCG) blood test on all female subjects on days 15 and 36.

Compliance

All intake of medication at the clinical trial unit was supervised and recorded by the study personnel. Intake of Ginkgo biloba tablets at home was monitored by use of micro-electronic monitoring system (MEMS) caps (Aardex Ltd., Zug, Switzerland), which record the opening of the medication bottle. In addition, Ginkgo biloba tablets were counted on each visit day during Ginkgo biloba treatment to assess adherence. Subjects were asked to write down the exact times of intake in a booklet.

Bioanalysis of raltegravir in plasma

The concentrations of raltegravir in plasma were analyzed by use of a validated reversed-phase high-pressure liquid chromatography (HPLC) method with fluorescence detection. Sample preparation consisted of a liquid-liquid extraction by adding 500 µL of acetate buffer (pH 4.0; 0.2 M), 5 mL of hexane/dichloromethane (1:1 [vol/vol]), and 50 µL of internal standard (lormetazepam in methanol-water [1:1 {vol/vol}]) to 500 µL of plasma. The samples were mixed on a vortex mixer for 5 min, followed by centrifugation at 11500 x *g* for 5 min. After freezing at -40°C for 5 min, the organic supernatant was decanted and evaporated at 37°C under a stream of nitrogen gas. The residue was reconstituted in 200 µL of eluent (acetonitrile-phosphate buffer, pH 4.8; 20 mM; 35:65 [vol/vol]). Forty microliters of the reconstituted solution was injected onto a SymmetryShield RP 18 column (3.5 µm; 100 by 4.6 mm). The flow rate was set at 1.5 mL/min. Raltegravir was detected by the use of a fluorescence detector ($\lambda_{excitation}$, 240 nm; $\lambda_{emission}$, 412 nm). The lower limit of quantification was 0.014 mg/L. The linear calibration ranges in plasma were from 0.014 to 10 mg/L. The validation results displayed accuracies of the quality control samples of 100%, 102% and 107% at plasma concentrations of 0.060, 0.400, and 4.00 mg/L. At the same concentrations, the precision values (within-day coefficients of variation [CV]) were 3.7%, 1.8%, and 0%, respectively. The raltegravir assay was performed at the laboratory of the Pharmacy of Radboud University Nijmegen Medical Centre (Nijmegen, The Netherlands) and was externally validated through the International Interlaboratory Quality Control Program for Measurement of Antiretroviral Drugs in Plasma.[23]

Pharmacokinetic analysis

Pharmacokinetic parameters for raltegravir were calculated by noncompartmental methods using the WinNonlin software package (version 5.2; Pharsight, Mountain View, CA) and the linear log trapezoidal rule. Based on the individual plasma concentration-time data, the following pharmacokinetic parameters of raltegravir were determined: the area under the plasma concentration-time curve from dosing to infinity ($AUC_{0-\infty}$) (in mg·h/L), the area under the plasma concentration-time curve from 0 to 12 h after intake (AUC_{0-12h}) (in mg·h/L), the maximum plasma concentration of the drug (C_{max}) (in mg/L), the time to reach C_{max} (t_{max}) (in h), the apparent volume of distribution (V/F) (in L), the apparent oral clearance (CL/F) (in L/h) and the apparent elimination half-life ($t_{1/2}$) (in h).

Sample size and statistical analysis

For the identification of a clinically relevant drug interaction, the bioequivalence approach was used, as described previously.[24] The main pharmacokinetic parameter to be evaluated in this respect is the exposure to raltegravir, expressed as the AUC. Sample size calculation was performed using the method for two-period designs of Diletti et al.[25] The required sample size was calculated (power of 80%) assuming no difference in the AUC of raltegravir with or without Ginkgo biloba and an estimated intrasubject coefficient of variation of the log-transformed AUC values for raltegravir of 20%. The required number of participants was 16. Taking dropouts into account, a total of 18 subjects were included. Geometric mean ratios (GMRs) with 90% confidence intervals (CI) were calculated for $AUC_{0-\infty}$, AUC_{0-12h}, C_{max}, and $t_{1/2}$ after log transformation of within-subject ratios of raltegravir combined with Ginkgo biloba verus raltegravir alone. GMRs with 90% CI falling entirely within the range of 0.80 to 1.25 were considered to indicate no significant interaction. Statistical and descriptive analysis were carried out using SPSS for Windows, version 16.0.1 (SPSS, Chicago, IL), and Microsoft Office Excel 2007.

Results

Baseline characteristics

A total of 18 subjects (9 male and 9 female, all Caucasian) were enrolled in the study and received treatment. The mean (range) age, body weight, and body mass index were 38 (22 to 55) years, 72 (52 to 93) kg and 23 (19 to 28) kg/m^2, respectively. The subjects were in good general health, according to medical histories, physical examinations, vital signs, and laboratory data. All included subjects completed the trial and were available for statistical evaluation.

Compliance

The compliance of the Ginkgo biloba treatment of all 18 subjects was good, as indicated by their statements about the intake of the drug doses as noted in the booklets, the number of Ginkgo biloba tablets counted on each visit day, and the MEMS caps (data not shown). Only two subjects admitted to have missed one Ginkgo biloba dose.

Pharmacokinetics

The pharmacokinetic parameters and the plasma concentration versus time curves of raltegravir in the presence and absence of steady-state Ginkgo biloba are shown in Table 1 and Figure 1. Ginkgo biloba increased the maximum plasma concentration (C_{max}) and the area under the plasma concentration-time curve from dosing to infinity ($AUC_{0-\infty}$) of raltegravir. The apparent elimination half-life of raltegravir did not appear to be influenced by Ginkgo biloba. The median time to reach C_{max} of raltegravir was 2.0 h irrespective of

Table 1 Comparison of single-dose pharmacokinetic parameters of raltegravir with or without coadministration of multiple doses of Ginkgo biloba in healthy volunteers

Pharmacokinetic parameter	Raltegravir alone		Raltegravir + Ginkgo biloba		Raltegravir + Ginkgo biloba / Raltegravir alone	
	Geometric mean	95% CI	Geometric mean	95% CI	GMR	90% CI
$AUC_{0-\infty}$ (mg·h/L)	6.35[b]	(4.39-9.18)	7.44	(5.10-10.9)	1.21[b]	(0.93-1.58)
AUC_{0-12h} (mg·h/L)	5.93	(4.21-8.34)	7.33	(5.01-10.7)	1.24	(0.97-1.58)
C_{max} (mg/L)	2.08	(1.39-3.12)	3.01	(2.00-4.52)	1.44	(1.03-2.02)
t_{max} [a] (h)	2.00	(1.13-3.00)	2.00	(1.50-2.00)		
CL/F (L/h)	63.0[b]	(43.6-91.1)	53.8	(36.8-78.5)		
V/F (L)	288[b]	(193-429)	220	(148-325)		
$t_{1/2}$ (h)	3.17[b]	(2.61-3.86)	2.83	(2.42-3.32)	0.93[b]	(0.73-1.17)

[a] For t_{max}, median + interquartile range is reported.
[b] Two subjects were excluded because the $t_{1/2}$ could not be determined.
$AUC_{0-\infty}$, area under the plasma concentration-time curve from dosing to infinity; AUC_{0-12h}, area under the plasma concentration-time curve up to 12 h after intake; CI, confidence interval; CL/F, apparent oral clearance; C_{max}, maximum plasma concentration; GMR, Geometric Mean Ratio; $t_{1/2}$, apparent elimination half-life; t_{max}, time to reach C_{max}; V/F, volume of distribution.

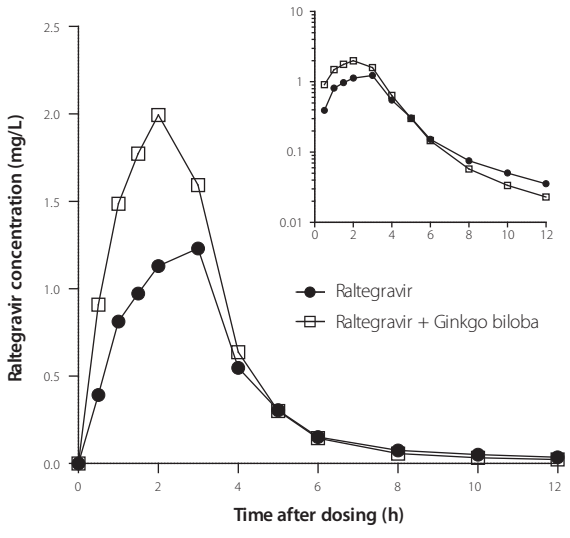

Figure 1 Geometric mean plasma concentrations following a single dose of 400 mg of raltegravir in the presence and absence of steady-state Ginkgo biloba (semilog scale on the inset).

Ginkgo biloba treatment. For raltegravir coadministered with Ginkgo biloba relative to raltegravir alone, the GMRs (90% confidence intervals) was 1.21 (0.93- to 1.58) for $AUC_{0-\infty}$, 1.44 (1.03 to 2.02) for C_{max}, and 0.93 (0.73 to 1.17) for $t_{1/2}$ (Table 1). Two subjects were excluded from the calculation of the GMRs of $AUC_{0-\infty}$ and $t_{1/2}$ because the $t_{1/2}$ could not be determined in these subjects. Figure 2 shows the individual subject changes in the C_{max} and $AUC_{0-\infty}$ of raltegravir alone and coadministered with Ginkgo biloba. Although a small majority of the subjects showed an increase of C_{max} upon coadministration, considerable variation in the amount and direction of the effect was seen, as well as variation between individuals. This also applies to the observed individual changes in the $AUC_{0-\infty}$. The coefficient of variation of the $AUC_{0-\infty}$ values of raltegravir alone and raltegravir with Ginkgo biloba were 66% and 51%, respectively.

Adverse events and safety assessments

The study medication was generally well tolerated, and no serious adverse events were reported. There were no discontinuations due to adverse events, and all subjects completed the trial. Sixteen subjects reported a total of 32 adverse events. Five (16%) adverse events were considered possibly drug-related (all were classified as grade I). Diarrhea was reported by two subjects during Ginkgo biloba treatment and one subject reported a transient headache which was possibly related to raltegravir and/or Ginkgo biloba. One subject developed a grade I triglyceride elevation after administration of raltegravir which returned to normal within 7 days. One subject had a mild elevated gamma-glutamyltransferase (γ-GT), which remained elevated during the study.

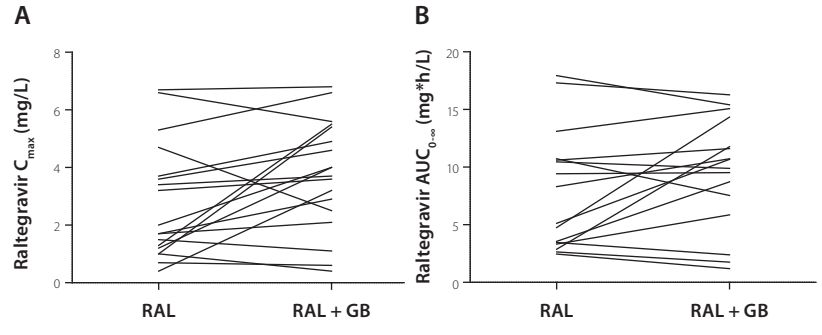

Figure 2 Individual changes in the maximum plasma concentration (A) and the area under the concentration-time curve (B) of raltegravir alone versus raltegravir coadministrated with Ginkgo biloba (GB).

Two subjects were excluded from figure 2B because $AUC_{0-\infty}$ could not be determined.

Discussion

Herb-drug interactions are an important consideration in HIV-infected patients because herbs are frequently used, often not reported, and a potential cause for drug failure. A popular herbal product among HIV-infected individuals is Gingko biloba extract, despite a lack of evidence for effectiveness or safety within this special patient group. Because human and preclinical data were inconclusive with regard to Ginkgo biloba's potential to modulate UGT and P-gp activity, a drug-interaction study with raltegravir in healthy volunteers was carried out.

Steady-state Ginkgo biloba increased the mean exposure to raltegravir ($AUC_{0-\infty}$) by 21% and the C_{max} by 44%. However, the large 90% confidence interval for the GMRs partly overlaps the predefined range of 0.80 to 1.25 in the bioequivalence approach and reflects the variability in the individual changes in the $AUC_{0-\infty}$ and C_{max} of raltegravir.[24] Based on our findings, raltegravir exposure after a single dose was not clinically significantly reduced by concomitant use of Ginkgo biloba extract at steady state, making it less likely that Ginkgo biloba is an UGT inducer as suggested in *in vitro* research.[13] The observed mean increase in the C_{max} by concomitant use of Ginkgo biloba is more likely to be caused by a change in oral bioavailability than by inhibition of the metabolism of raltegravir, because the apparent elimination half-life of raltegravir remained unaffected. The slight increase of raltegravir $AUC_{0-\infty}$ when combined with Ginkgo biloba, is largely due to the observed increase in the C_{max} (Figure 1). The difference in the C_{max} of raltegravir alone versus raltegravir with Ginkgo biloba within subjects could be (partly) due to the normal intrasubject variability in raltegravir pharmacokinetics instead of an effect caused by Ginkgo biloba. It is known that raltegravir pharmacokinetics exhibits considerable intra-en intersubject variability.[26,27] A possible explanation for the increase in the C_{max} and bio-availability of raltegravir when combined with Ginkgo biloba could be the inhibition of P-gp by Ginkgo biloba. *In vitro* characterization of raltegravir transport by drug transporters indicates that raltegravir is a weak P-gp substrate.[12] P-gp is an active ATP-dependent efflux pump and is encoded by the ABCB1 gene. Efflux mechanisms, such as P-gp, are responsible for transporting a broad range of compounds out of the intestinal epithelial cells back into the intestinal lumen, and plays an important role in oral drug absorption. The effect of chronic use of Ginkgo biloba extract on the pharmacokinetics of the P-gp substrate talinolol was studied in healthy volunteers. The observed increase in the C_{max} by 33% and AUC by 21 % without any significant alterations in the t_{max} and $t_{1/2}$ of talinolol supports our hypothesis.[19,20] The variation in change of the oral bioavailability of raltegravir and subsequent C_{max} values could be a reflection of individual variation in the inhibitory potential of P-gp by Ginkgo biloba. The expression and transport activities of P-gp may differ between individuals due to genetic variation in the highly polymorphic ABCB1 gene.[28,29] Therefore, the extent of the inhibition of P-gp may vary accordingly. Although

inhibition of P-gp-mediated efflux of raltegravir by Ginkgo biloba is an interesting hypothesis, one must be cautious in translating findings obtained from *in vitro* experiments directly to the clinical setting. Raltegravir transport by P-gp is not yet confirmed in human studies. There are currently no clinical data indicating that the pharmacokinetic profile of raltegravir may be affected by selective P-gp inducers or inhibitors.

It is well known that the pharmacokinetics of raltegravir displays large intersubject variability, which was observed in our study, as well (CV in $AUC_{0-\infty}$, 66%).[26,27] Contributing factors, in general, are differences in absorption due to food intake or pH effects, genetic polymorphisms associated with altered UGT1A1 activity, and potential drug interactions. In this study, raltegravir was administered in a fasted state to minimize intersubject variability. Nevertheless, differences in gastric pH and therefore absorption of raltegravir probably led to variability in pharmacokinetic parameters between subjects, and maybe within subjects, as well. Individuals with decreased UGT1A1 expression caused by UGT1A1*28 polymorphism (approximately 7 to 19% of the Caucasian population is homozygous for UGT1A1*28), are known to have moderately elevated plasma levels of raltegravir. However, this increase in plasma levels is not considered to be of clinical importance.[30,31] Pharmacogenetic testing was not performed in our study, and UGT1A1*28 polymorphism might have contributed to the intersubject variability, as well. In two subjects, the elimination half-life of raltegravir could not be determined because of secondary peaks in the plasma concentration-time curve. These secondary peaks are frequently observed in pharmacokinetic studies of raltegravir and can be attributed to either delayed absorption or enterohepatic circulation, which is not uncommon for UGT substrates.[10,26] The pharmacokinetic parameters observed in this study were compared with data from Wenning et al., as these healthy subjects (all with UGT1A1*1/*1 genotype) were exposed to similar study conditions, i.e., a single intake of a 400-mg raltegravir tablet on an empty stomach.[31] No major differences between our data and these historical controls could be observed.

The combined use of chronic Ginkgo biloba and a single dose of raltegravir was well tolerated. No serious events were reported during the trial. The reported adverse events related to the study medication were mild and transient. In clinical practice, raltegravir is well tolerated, with no dose-related toxicities indentified so far. Given that there have been no acute safety findings associated with peak raltegravir concentrations, the somewhat higher C_{max} values for raltegravir when coadministered with Ginkgo biloba compared with intake of raltegravir alone are not expected to lead to any meaningful clinically significant safety concerns.

Our study was undertaken in healthy volunteers, limiting our interpretation in HIV-infected individuals with concomitant medication and comorbidity. Because of the inconsistencies

and controversies in the literature regarding the exact action of Ginkgo biloba extract on metabolizing enzymes or drug transporters and the variation in effect seen in this study, it is not possible to draw any definite conclusions. However this study does provide data to support the idea that raltegravir can be used safely for the management of HIV infection when taken in combination with Ginkgo biloba. No decrease in $AUC_{0-\infty}$ of raltegravir was observed, and the increase in maximum plasma concentrations are not considered to be of clinical importance, due to the normal intersubject variability of raltegravir pharmacokinetics and the good safety profile of raltegravir.

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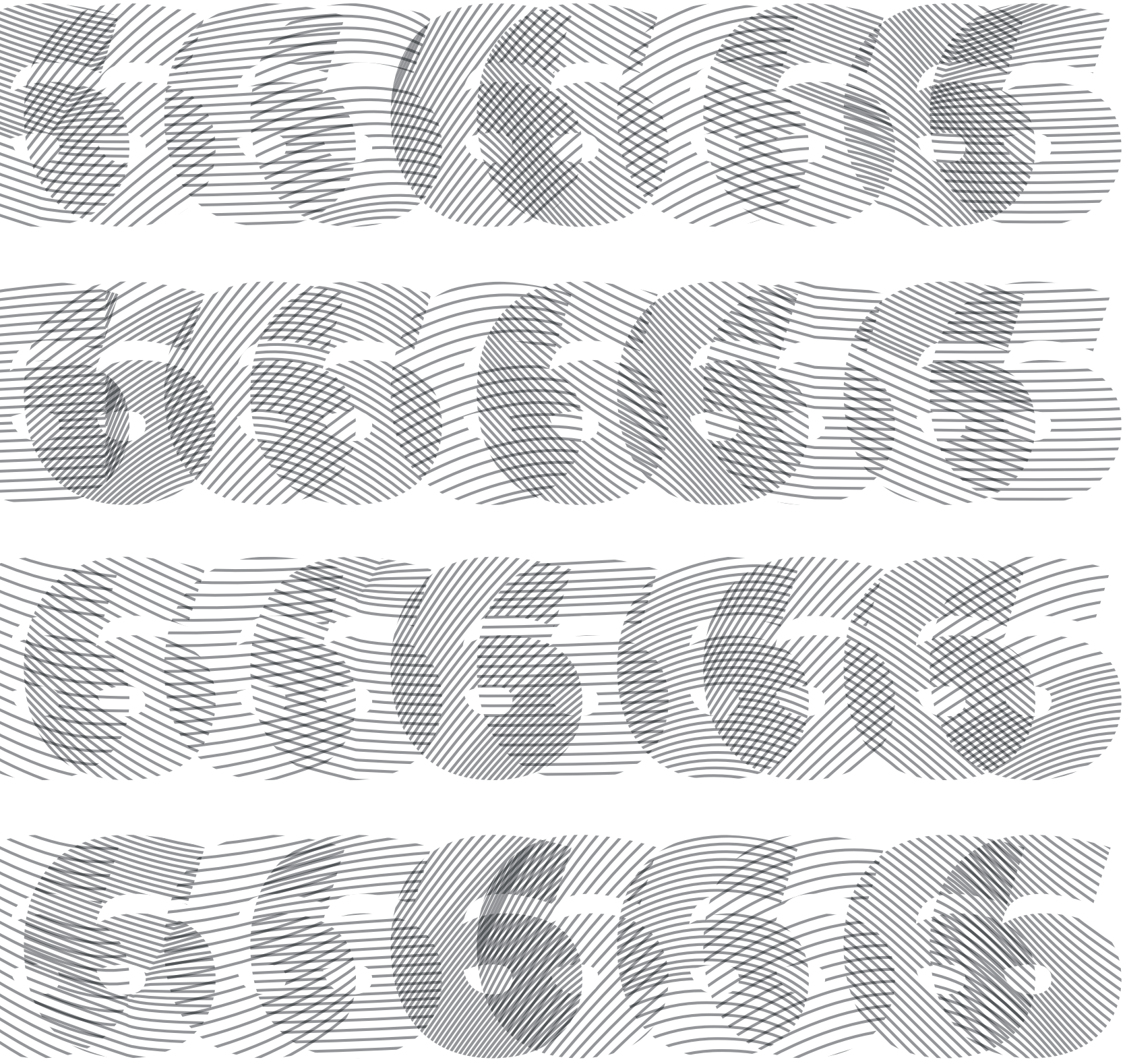
Pharmacokinetics in special patient populations



Raltegravir in HIV-1-infected pregnant women: pharmacokinetics, safety, and efficacy

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Clinical Infectious Diseases 2015; 61(5): 809-16

Abstract

Background

The use of raltegravir in human immunodeficiency virus (HIV)–infected pregnant women is important in the prevention of mother-to-child HIV transmission, especially in circumstances when a rapid decline of HIV RNA load is warranted or when preferred antiretroviral agents cannot be used. Physiological changes during pregnancy can reduce antiretroviral drug exposure. We studied the effect of pregnancy on the pharmacokinetics of raltegravir and its safety and efficacy in HIV-infected pregnant women.

Methods

An open-label, multicenter, phase 4 study in HIV-infected pregnant women receiving raltegravir 400 mg twice daily was performed (Pharmacokinetics of Newly Developed Antiretroviral Agents in HIV-Infected Pregnant Women Network). Steady-state pharmacokinetic profiles were obtained in the third trimester and postpartum along with cord and maternal delivery concentrations. Safety and virologic efficacy were evaluated.

Results

Twenty-two patients were included, of which 68% started raltegravir during pregnancy. Approaching delivery, 86% of the patients had an undetectable viral load (<50 copies/mL). None of the children were HIV-infected. Exposure to raltegravir was highly variable. Overall area under the plasma concentration-time curve (AUC) and plasma concentration at 12 hours after intake (C_{12h}) in the third trimester were on average 29% and 36% lower, respectively, compared with postpartum: Geometric mean ratios (90% confidence interval) were 0.71 (0.53–0.96) for $AUC_{0\text{-}12h}$ and 0.64 (0.34–1.22) for C_{12h}. The median ratio of raltegravir cord to maternal blood was 1.21 (interquartile range, 1.02–2.17; n = 9).

Conclusions

Raltegravir was well tolerated during pregnancy. The pharmacokinetics of raltegravir showed extensive variability. The observed mean decrease in exposure to raltegravir during third trimester compared to postpartum is not considered to be of clinical importance. Raltegravir can be used in standard dosages in HIV-infected pregnant women.

Introduction

An estimated 1.4 million pregnant women infected with human immunodeficiency virus (HIV) give birth annually worldwide, of which the majority live in sub-Saharan Africa.[1] Mother-to-child HIV transmission (MTCT) is the most common route of HIV infection among infants and children. Each day, approximately 1000 infants acquire HIV due to MTCT during pregnancy, delivery or breastfeeding.

Combination antiretroviral therapy (cART) is the standard of care for the prevention of perinatal transmission. The main goal of cART is maximal suppression of HIV replication. Its implementation together with other effective interventions has led to dramatic declines in the number of perinatally HIV-infected children from 15-40% to <2%. Absent or delayed prenatal care, acute primary infection in late pregnancy, and the continued increase in incidence of HIV infection in women of childbearing age are among the most important obstacles to fully eliminate perinatal transmission in the United States and other resource-rich countries.[2]

In current US and European treatment guidelines for HIV type 1 (HIV-1) infection in pregnancy, preferred combined antiretroviral agents include 2 nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs) in combination with the protease inhibitors lopinavir or atazanavir boosted with ritonavir or the nonnucleoside reverse transcriptase inhibitor nevirapine. Regimens including the HIV-1 integrase inhibitor raltegravir can be considered for use in special circumstances because information on the pharmacokinetics and the safety of raltegravir in pregnancy is limited. Examples of these special circumstances could be pregnant women who present late in care (>28 weeks gestational age) or whose HIV RNA load is not undetectable at the third trimester.[2-4] HIV integrase inhibitors such as raltegravir have been shown to rapidly reduce HIV RNA load, with shorter times to achieve virological suppression compared with agents from other drug classes.[5] Case reports and small case series suggest that raltegravir could play an important role when a rapid decline in maternal plasma HIV RNA is needed to prevent MTCT during delivery or as an alternative antiretroviral drug in complex treatment-experienced HIV-infected pregnant women.[6-17] In a pilot study including 28 pregnant HIV-infected women, which was presented as abstract at a conference, the use of raltegravir seemed safe in both women and infants.[18]

Pregnancy is associated with considerable physiological changes such as changes in gastrointestinal, hepatic, and renal function as well as alterations in the expression and activity of transport proteins and metabolic enzymes. Pregnancy may influence the pharmacokinetic profile of antiretroviral agents and lead to decreased drug exposure. Suboptimal drug exposure can result in HIV RNA rebound, the selection of resistant virus and an increased risk of HIV-1 transmission to the infant.[19,20]

Published information on the pharmacokinetics of raltegravir during pregnancy is limited.[21,22] Watts et al describe a 50% reduction in median exposure to raltegravir during pregnancy versus postpartum and a large variability in raltegravir pharmacokinetics. The authors report that 92% of women had an HIV RNA load of <400 copies/mL at delivery and none of the infants were confirmed to be infected. Additional well-controlled studies are needed to confirm that raltegravir can be used safely in this special patient population. We studied the effect of pregnancy on the pharmacokinetics of raltegravir and its safety and efficacy in pregnant HIV-infected women.

Methods

Study design and participants

This multicentre, phase 4 study was designed as a nonrandomized, open-label trial in HIV-infected pregnant women and coordinated by the PANNA (Pharmacokinetics of Newly Developed Antiretroviral Agents in HIV-Infected Pregnant Women) network study group. The PANNA network is a European network of 19 hospitals in seven countries with the primary aim to collect pharmacokinetic data during pregnancy on antiretroviral agents for which no or limited data are available (www.pannastudy.com). We enrolled HIV-infected pregnant women (aged ≥18 years) who were on a cART regimen containing raltegravir 400 mg twice daily. Patients were eligible for inclusion if they were on raltegravir treatment for at least 2 weeks prior to the first pharmacokinetic assessment in the third trimester of pregnancy. Exclusion criteria were a medical history or current condition that might interfere with drug absorption, distribution, metabolism, or excretion (such as renal failure or hepatic failure), and grade III/IV anaemia (ie, hemoglobin <4.6 mmol/L or <7.4 g/dL). The study was conducted in compliance with the principles of the Declaration of Helsinki. Informed consent was obtained from each participant before undergoing any protocol-specified procedures. The study was approved by the appropriate medical ethical committee of each centre and by the national authorities where applicable. The trial is registered at ClinicalTrials.gov (identifier NCT00825929).

Procedures

Inclusion screening consisted of clinical evaluations (medical history and physical examination) and laboratory assays (serum biochemistry, hematology, qualitative urinalysis, HIV-1 RNA load, and CD4 cell count). Blood samples for safety and efficacy assessments were obtained on pharmacokinetic sampling days and analyzed at local laboratories. Adverse events were recorded at each visit and graded according to the Division of AIDS toxicity table (2004). Infant birth weight, gestational age at birth, congenital abnormalities, and HIV infection status were collected. Safety outcomes were maternal adverse events and congenital abnormalities. Efficacy outcomes were an

undetectable HIV RNA load (<50 copies/mL) measured at or prior to delivery, and infant HIV infection status measured by HIV DNA polymerase chain reaction test.

Pharmacokinetic assessment took place in the third trimester (approximately at week 33) and at least 2 weeks postpartum (approximately 4-6 weeks postpartum). Blood samples for pharmacokinetic assessment were collected during a 12-hour period at 0 (predose), 0.5, 1, 2, 3, 4, 6, 8, and 12 hours after observed intake of 400 mg of raltegravir after a standard breakfast (650 kCal; 30 g fat). Where possible, umbilical cord blood and matching maternal blood samples were obtained at delivery to assess placental transfer. Plasma was separated and stored at -18°C or lower until shipment on dry ice to the laboratory of the pharmacy of the Radboud University Medical Center (Nijmegen, The Netherlands). Concentrations of raltegravir in plasma were analyzed using validated reversed-phase high-pressure liquid chromatography with fluorescence detection. The linear calibration ranges in plasma were 0.014-10.0 mg/L with a lower limit of quantification of 0.014 mg/L. The raltegravir assay was externally validated through the International Interlaboratory Quality Control Program for Measurement of Antiretroviral Drugs in Plasma as well as by the Clinical Pharmacology Quality Assurance and Quality Control Program (CPQA).[16,23]

Statistical analysis

Pharmacokinetic parameters were determined using a noncompartmental model in WinNonlin/Phoenix version 6.3 (Pharsight Corporation). Based on the individual plasma concentration-time data, the following pharmacokinetic parameters of raltegravir were determined: the area under the plasma concentration-time curve from 0 to 12 hours after intake using the trapezoidal rule ($AUC_{0\text{-}12h}$), the trough concentration (C_{12h}) defined as the sample taken at 12 hours, the maximum plasma concentration of the drug (C_{max}), the time to reach C_{max} (t_{max}), the apparent volume of distribution (V/F), the apparent oral clearance being the dose divided by $AUC_{0\text{-}12h}$ (CL_{ss}/F), and the apparent elimination half-life ($t_{1/2}$). Patients from whom a curve was taken during pregnancy were included in demographic, safety analyses, and descriptive statistics of the pharmacokinetic parameters. Pharmacokinetic parameters are reported as geometric means with 95% confidence intervals (CIs). We calculated geometric means ratios (GMRs) and 90% CIs of raltegravir pharmacokinetic parameters of third trimester versus postpartum using a mixed-effects model in WinNonlin/Phoenix. Cord blood/maternal blood plasma concentration ratios were determined and described.

Results

Twenty-two HIV-infected pregnant women receiving raltegravir 400 mg twice daily were enrolled in 10 European hospitals during 2010 to April 2014. The characteristics of the study population are presented in Table 1. Four patients (18%) were diagnosed with HIV after conception at 12, 16, 18 and 23 weeks of gestational age, respectively. Of the 18 pregnant women who were already aware of their HIV-positive status, 14 were on cART at the time of conception with a median duration of approximately five years (257 weeks). Seven patients (32%) were using raltegravir 400 mg twice daily prior to conception. If not used prior to conception raltegravir was started mainly during the second (27%) and third (32%) trimester of the pregnancy. Only two patients (9%) started a raltegravir-based regimen during the first trimester, of whom 1 patient was unaware of her pregnancy at that time. Various indications for raltegravir in this special patient population were presented: Raltegravir was either started as part of the first cART regimen to obtain a rapid decline in HIV RNA viral load with raltegravir as the fourth agent, added to the current regimen to optimize or intensify treatment in patients with a detectable viral load, or used as alternative to a preferred antiretroviral agent due to side effects (gastrointestinal or hyperbilirubinemia).

Concomitant HIV and non-HIV medication that could possibly influence raltegravir exposure was the use of ritonavir-boosted atazanavir in 4 patients, the use of acid reducing agents (ranitidine 150 mg twice daily or sodium alginate as needed) in 2 patients, the use of a calcium carbonate supplement in 2 patients, and the use of a magnesium supplement (in combination with atazanavir) in 1 patient. All potential drug-interacting agents were used during both pharmacokinetic assessments, limiting its influence on the comparison between the exposure in third trimester versus postpartum.

Pharmacokinetic assessment in the third trimester took place at a median gestational age of 33 weeks (interquartile range [IQR], 32-35 weeks). A total of 21 evaluable raltegravir pharmacokinetic curves were obtained. One pharmacokinetic profile sampling was stopped at 3 hours at the volunteer's request and these plasma concentrations could only be partly included in the analysis. Pharmacokinetic assessment postpartum took place at a median of 5 weeks (IQR, 4-6 weeks) and a minimum of 3 weeks after delivery in 18 evaluable pharmacokinetic postpartum curves. Four patients did not have a postpartum curve because they withdrew consent. The mean plasma concentration-time profile of raltegravir in the third trimester and postpartum are presented in Figure 1, and summary statistics of the pharmacokinetic parameters are listed in Table 2.

Table 1 Patient characteristics and pregnancy outcomes (n=22)

Characteristics	No. (%)
Age at delivery, y, median (IQR)	33 (29-36)
Race/ethnicity	
White	9 (41%)
Black	12 (55%)
Other	1 (5%)
Smoking	0 (0%)
Alcohol use	0 (0%)
Drug use	0 (0%)
ARV treatment at start of pregnancy	14 (64%)
ARV treatment duration before pregnancy, wk, median (IQR)	257 (110-440)
Start raltegravir	
Before conception	7 (32%)
1st trimester	2 (9%)
2nd trimester	6 (27%)
3rd trimester	7 (32%)
Concomitant ARVs	
NRTI	15 (68%) (11 [50%] tenofovir + emtricitabine; 3 [14%] tenofovir; 1 [5%] zidovudine + lamivudine)
Protease inhibitor[a]	13 (59%) (8 [36%] DRV/r; 3 [14%] ATV/r; 2 [9%] LPV/r)
NNRTI	2 (9%) etravirine
Entry inhibitor	2 (9%) maraviroc
Third trimester	
Gestational age, wk, median (IQR)	33 (32-35)
Weight, kg, median (IQR)	73 (67-79)
HIV RNA detectable (>50 copies/mL)	3 (14%) (74 copies/mL; 144 copies/mL; 242 copies/mL)
CD4 count[b], cells/uL, median (IQR)	622 (240-756)
Delivery	
Gestational age, wk, median (IQR)	38 (38-39)
Caesarian section[b]	11 (52%)
HIV RNA detectable closest to delivery (>50 copies/mL)	3 (14%) (144 copies/mL; 242 copies/mL; 290 copies/mL)
Time between HIV RNA measurement and delivery, wk, median (IQR)	3 (0-4)

Table 1 Continued

Characteristics	No. (%)
Postpartum (n=18)	
Time after delivery, wk, median (IQR)	5 (4-6)
Weight, kg, median (IQR)	64 (59-72)
HIV RNA detectable (>50 copies/mL)[c]	2 (12%) (99 copies/mL; 650 copies/ml)
CD-4 count, cells/uL, median (IQR)	585 (266-806)
Pregnancy outcomes	
Birth weight, g, median (IQR)	3115 (2628-3360)
Small for gestational age[d]	3 (14%)
Infant HIV DNA PCR test negative	22 (100%)

Data are presented as No. (%) unless otherwise specified.
Abbreviations: ARV, antiretroviral; ATV/r, atazanavir/ritonavir; DRV/r, darunavir/ritonavir; HIV, human immunodeficiency virus; IQR, interquartile range; LPV/r, lopinavir/ritonavir;
(N)NRTI, (non)nucleoside reverse transcriptase inhibitor; PCR, polymerase chain reaction.
[a] One subject stopped DRV/r before delivery and one subject switched from LPV/r to ATV/r during pregnancy (prior to pharmacokinetic assessments).
[b] Data available for 21 patients.
[c] Data available for 17 patients.
[d] Small for gestational age was determined as <10th percentile of the fetal-infant growth chart by Fenton.[25]

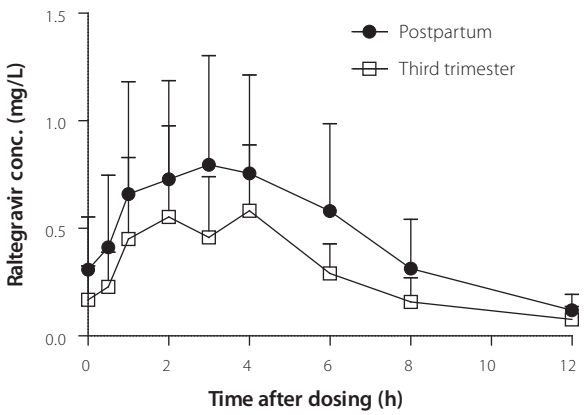

Figure 1 Geometric mean (+upper 95% confidence interval) raltegravir concentration-time profiles during the third trimester of pregnancy (open squares) and postpartum (filled circles).

Table 2 Pharmacokinetic parameters raltegravir during third trimester of pregnancy and postpartum

	Third trimester GM (95% CI) (n=21)		Postpartum GM (95% CI) (n=18)		GM ratio (90% CI) of third trimester: postpartum (n=17)	
$AUC_{0\text{-}12h}$, mg·h/L	5.00	(3.56-7.01)	7.11	(4.91-10.30)	0.71	(0.53-0.96)
C_{max}, mg/L	1.43	(0.93-2.22)	1.76	(1.10-2.80)	0.82	(0.55-1.23)
t_{max}, h[a]	1.98	(0-11.3)	2.03	(0-7.97)		
C_{12h}, mg/L	0.077	(0.043-0.137)	0.120	(0.074-0.193)	0.64	(0.34-1.22)
$t_{1/2}$, h	2.55[b]	(1.88-3.45)	2.53[c]	(1.91-3.36)	1.04	(0.73-1.47)
CL_{ss}/F, L/h	80.1	(57.0-112)	56.2	(38.8-81.4)	1.41	(1.04-1.90)
V/F, L	311[b]	(159-607)	205[c]	(115-367)	1.24	(0.67-2.27)

Abbreviations: AUC, area under the curve; CI, confidence interval; C_{12h}, plasma concentration 12 hours after intake, CL_{ss}/F, apparent oral clearance; C_{max}, maximum plasma concentration; GM, geometric mean; $t_{1/2}$, apparent elimination half-life; t_{max}, time to reach C_{max}; V/F, apparent volume of distribution
[a] t_{max} [median (minimum–maximum)].
[b] Available for 15 patients.
[c] Available for 14 patients.

Exposure to raltegravir, which is expressed as $AUC_{0\text{-}12h}$, was 29% lower in the third trimester versus postpartum by intrasubject comparison. C_{max} and C_{12h} were on average 18% and 36% lower during pregnancy. The apparent elimination half-life of raltegravir did not appear to be influenced by pregnancy. One patient in the third trimester (and none postpartum) had a C_{12h} plasma concentration below the suggested threshold of 0.020 mg/L, which was associated with failure to achieve an undetectable HIV RNA load in treatment-naive patients in the QDMRK study (phase 3 study to compare 800 mg once daily versus 400 mg twice daily raltegravir).[24] Raltegravir pharmacokinetics was highly variable which is best seen in the large 90% CIs around the GMR in Table 2 and graphically in Figure 2. Figure 2 shows the individual changes in $AUC_{0\text{-}12h}$ and C_{12h} of raltegravir in the third trimester of the pregnancy compared with postpartum. Although a mean decrease in raltegravir exposure (29%) and C_{12h} plasma concentrations (36%) in the third trimester was observed, considerable variation in the amount and direction of the effect is seen as well as variation between individual patients. Eleven out of 17 patients with complete paired pharmacokinetic curves (65%) showed a decrease in raltegravir exposure in third trimester compared to postpartum.

Nine umbilical cord blood samples were collected with matching maternal blood samples. The median time between the reported last dose and cord blood sampling, if available, was 10 hours (IQR, 7-11 hours); the median time between cord blood sample and maternal sample was 0 minutes (IQR, 0-4 min). The median ratio of raltegravir cord blood/maternal blood was 1.21 (IQR, 1.02-2.17; n=9).

No congenital abnormalities were reported. Five patients reported a total of 10 adverse events that were considered not to be or unlikely to be related to the cART given. Seven events were grade 1 or 2. Grade 3 neuropatic pain was reported as a serious adverse event not related to the use of raltegravir. Other grade 3 adverse events were severe anaemia due to hemorrhagic delivery and varicella lesions.

Twenty-two infants were born and they were all tested HIV negative. Three infants (14%) were small for gestational age (< 10th percentile of fetal-infant growth chart by Fenton[25]), which is higher than observed in the US for children born from HIV-infected women (7.3%).[26] Other pregnancy outcomes as well as the results of the maternal HIV RNA viral load measurements are shown in Table 1. In summary, 3 of 22 patients (14%) failed to achieve an undetectable HIV RNA viral load (<50 copies/mL) close to delivery (144, 242, and 290 copies/mL) when measured a median of 3 (IQR, 0-4) weeks before delivery. The patient with a C_{12h} level below the threshold of 0.020 mg/L in the third trimester had an HIV RNA viral load of 74 copies/mL measured in the third trimester and an undetectable viral load on the day of delivery. Adherence, based on self-reporting, was good in all patients.

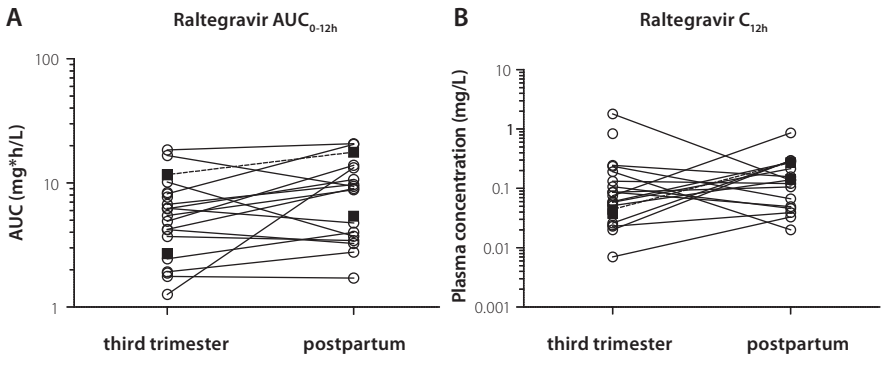

Figure 2 Individual raltegravir AUC_{0-12h} (A) and C_{12h} (B) parameters during the third trimester of pregnancy and postpartum

Symbols: Filled square ■ is a detectable (≥50 copies/mL) and an open circle ○ is an undetectable (<50 copies/mL) HIV RNA load close to delivery.

Discussion

In this study, we evaluated the effect of pregnancy on the pharmacokinetics of raltegravir and its safety and efficacy in 22 HIV-infected women. In the third trimester of pregnancy, systemic exposure (AUC) to raltegravir was on average 29% lower compared to postpartum. However, the pharmacokinetics of raltegravir was highly variable and exposure was not consistently decreased in third trimester compared with postpartum. Of the 17 women with paired pharmacokinetic curves, 6 (35%) had a higher AUC_{0-12h} in the third trimester. A similar effect of pregnancy on C_{12h} plasma levels was observed, leading to an average decrease of 36 % of the plasma levels seen postpartum. The magnitude of the observed effect is not considered to be of clinical importance. Similar effects of drug-interacting agents on the pharmacokinetics of raltegravir are described in the product information leaflet without special recommendation to adjust the dosage.[27] Viral suppression was good in our population with an HIV RNA load <400 copies/mL in all women and <50 copies/mL in 86% of women prior to delivery. The women (14%) who failed to have an undetectable viral load prior to delivery had adequate C_{12h} levels in third trimester. Only 1 patient had a C_{12h} level <0.020 mg/L in the third trimester, which is considered to be too low for adequate virological response in treatment-naive patients.[24] She had an undetectable viral load on the day of delivery.

The decrease in AUC (29%) in third trimester compared with postpartum was in line with the observations in a previous study with intensive pharmacokinetics of raltegravir during pregnancy from Watts et al.[21] They describe a more pronounced decrease of approximately 50% in AUC in the third trimester compared to postpartum. Given the high rate of viral suppression at delivery and the lack of a clear pharmacokinetic/pharmacodynamic relationship in nonpregnant adults, the authors suggest that a higher dose of raltegravir is not necessary during pregnancy. Watts et al reported a median AUC of 5.4 mg·h/L (n=41) in the third trimester, which is comparable to the geometric mean AUC in the third trimester (5.00 mg·h/L) found in our study.[21] The postpartum median AUC reported by Watts et al (11.6 mg·h/L [n=38] measured 3-14 weeks postpartum) was higher than the AUC we found (7.11 mg·h/L).[21] This difference probably causes the more pronounced decrease between the third trimester and postpartum found by Watts et al.[21] Raltegravir C_{12h} levels in the third trimester were comparable between Watts et al and our study (0.064 mg/L vs 0.077 mg/L, respectively).[21] The postpartum curves in our study are consistent with intensive pharmacokinetic profiles in nonpregnant HIV-infected patients in the twice-daily treatment arm of the QDMRK study.[24] The geometric mean AUC and C_{12h} (n=20) of raltegravir are 5.84 mg·h/L and 0.114 mg/L, respectively in the QDMRK study compared with 7.11 mg·h/L and 0.120 mg/L postpartum (n=18) in our study. This would suggest that the pharmacokinetic parameters collected at a median of 5 weeks postpartum in our study can be used as reference for the nonpregnant situation. The large inter- and

intrasubject variability in raltegravir pharmacokinetics observed in our study is well recognised by others in non-pregnant populations.[28,29] Intersubject variability in our study might have been caused by drug-drug interactions and differences in patient characteristics. These factors together with the time of postpartum pharmacokinetic assessment could also have contributed to the differences in pharmacokinetic parameters of raltegravir postpartum between Watt et al and our study.

There are many physiological changes during pregnancy that could alter distribution, metabolism and clearance of antiretroviral drugs used in pregnancy.[19,20] During pregnancy, the apparent volume of distribution increases with subsequent decreases in peak plasma concentrations, which was observed in our study as well. Alterations in drug elimination clearance during pregnancy can affect steady-state concentrations. Raltegravir is primarily metabolized by uridine diphosphate glucuronosyltransferase (UGT) 1A1. The potential effect of pregnancy on UGT1A1 activity has been evaluated and is believed to be increased during pregnancy.[19,30] Jeong et al suggest that the induction of UGT1A1 expression by rising progesterone levels in pregnant women may be responsible for the increase in clearance of UGT1A1 substrates.[31] This hypothesis is not supported by our study in which the apparent elimination half-life of raltegravir in the third trimester was similar to postpartum.

Raltegravir was well tolerated during pregnancy, and all of the children were tested HIV negative. Only 9 infants were exposed to raltegravir during the first trimester, with no birth defects reported. To assess prevalence rates of birth defects in infants exposed to raltegravir compared to nonexposed infants, more experience of raltegravir in human pregnancy is needed. Placental transfer of raltegravir is efficient with a median raltegravir cord blood to maternal plasma ratio of 1.21, in agreement with previous reports.[12,13,21,32,33] Unfortunately, the collection of neonatal blood samples to describe the washout pharmacokinetics and safety of in utero exposure to raltegravir was not part of this study. UGT1A1 neonatal enzyme activity is still immature after birth and leads to prolonged elimination of raltegravir after delivery. In newborns whose mothers were exposed to raltegravir during pregnancy, raltegravir is slowly metabolized with an elimination half-life that is highly variable.[9,12,33]

In conclusion, raltegravir was well tolerated during pregnancy in our study population. Raltegravir pharmacokinetics showed extensive inter- and intraindividual variability. Our findings show a mean decrease in exposure to raltegravir during third trimester compared with postpartum, which is not considered to be of clinical importance. Raltegravir in combination with other antiretroviral agents was effective in preventing MTCT by reducing and/or maintaining the HIV RNA viral load at an undetectable (<50 copies/mL) or low (<400 copies/mL) level. Our data support the use of raltegravir in standard dosages in HIV-infected pregnant women for the prevention of MTCT.

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Therapeutic drug monitoring of raltegravir in patients with HIV-associated non-Hodgkin lymphoma

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In preparation

Abstract

Non-Hodgkin lymphoma (NHL) is one of the most frequently diagnosed malignancies among human immunodeficiency virus (HIV)-infected individuals. Since the introduction of combination antiretroviral treatment most HIV-infected patients with NHL or other types of cancer can be treated with standard intensive high-dose chemotherapy similar to the HIV-negative population. Concomitant use of antiretroviral treatment with chemotherapy is complicated because of drug-drug interactions. Raltegravir, an HIV integrase strand-transfer inhibitor, has a favorable drug-interaction profile compared to other antiretroviral drug classes, such as protease inhibitors and non-nucleoside reverse-transcriptase inhibitors. Therefore, raltegravir is increasingly recommended as antiretroviral agent in HIV-infected patients with cancer who use chemotherapy. Despite its use in this setting, little is known about the pharmacokinetics of raltegravir in this special patient population. We present a case series of three HIV-infected patients who received chemotherapy for the treatment of advanced stage NHL in whom therapeutic drug monitoring of raltegravir was performed. Raltegravir trough plasma concentrations, which is seen as the most important parameter with respect to virological efficacy, was adequate (>0.020 mg/L) in all three patients. The observed decrease in exposure to raltegravir was not considered to be of clinical importance. However, chemotherapy-induced intestinal mucositis can influence the absorption of raltegravir. In these cases therapeutic drug monitoring by measuring raltegravir plasma concentrations could be a useful tool to monitor raltegravir treatment.

Introduction

Antiretroviral therapy has significantly decreased overall morbidity and mortality of AIDS-related complications in HIV-infected patients, including HIV-related hematological malignancies such as non-Hodgkin lymphoma (NHL). [1,2] The contribution of HIV-infected cases to the total number of NHL cases in the United States has declined over time from 12.5% in 1992-1995 to 3.6% in 2005-2009.[3] Nonetheless, the risk in HIV-infected patients for the development of NHL remains greatly elevated relative to the general population and is approximately 15 to 50-fold depending on the NHL subtype.[4,5] Most common subtypes within the HIV population are diffuse large B-cell lymphoma (DLBCL), Burkitt lymphoma and primary lymphomas of the central nervous system (CNS). NHL is still one of the most frequently diagnosed malignancies among HIV-infected individuals and comprises up to 23% of all cancer diagnoses within the HIV-infected population.[6] AIDS-defining malignancies together with a steep increase of non-AIDS-defining malignancies currently account for an estimated of one-third of all cause mortality among HIV-infected individuals.[7]

Since the introduction of combination antiretroviral therapy (cART) most HIV-infected patients with NHL or other types of cancer will be treated with standard intensive-dose chemotherapy similar to HIV-negative patients, with improved overall survival.[8-10] Recommended antiretroviral regimens in general include a minimum of three active antiretroviral agents from at least two different drug classes.[11] Most commonly used regimens consist of two nucleoside/nucleotide reverse-transcriptase inhibitors (NRTIs) and one antiretroviral agent of the following drug classes: nonnucleoside reverse-transcriptase inhibitors (NNRTIs), protease inhibitors (PIs) or integrase strand-transfer inhibitors (INSTIs). Concomitant use of cART with chemotherapy is complicated due to drug-drug interactions and overlapping toxic effects.[12,13] The drug interaction potential of antiretroviral agents in general is well documented but specific information on interactions with cytotoxic agents and practical guidelines for dose adjustments is very limited. The NRTI backbone used in HIV treatment has little propensity to interact with other agents but interactions might occur through change of renal drug clearance or overlapping nephrotoxicity and hematologic toxic effects. PIs and NNRTIs are extensively metabolized by cytochrome P450 (CYP) enzymes and induce or inhibit CYP enzymes. Because many chemotherapeutic agents are metabolized via the CYP450 pathway the potential for drug-drug interactions between chemotherapy and PIs and NNRTIs is high. Combination could result in drug accumulation and increased toxicity, or decreased exposure, reduced efficacy, and potential HIV drug resistance for antiretroviral agents.[12-14] Raltegravir, the first INSTI approved by the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA), has a favorable drug interaction profile because it does not influence CYP enzymes.[15,16] Given the concerns with drug-drug interactions or tolerability

with NNRTIs and PIs, clinicians may consider to start or switch to a raltegravir-based regimen in patients with chemotherapy.[12,13,17,18]

Although raltegravir is increasingly being recommended and used in clinical practice in HIV-infected patients with cancer, surprisingly little is known on the efficacy, safety and pharmacokinetics of raltegravir in this setting in comparison with other more frequently used antiretroviral agents.[16,18-21] To our knowledge, information on the pharmacokinetics of raltegravir in combination with various chemotherapeutic agents in HIV-infected patients is limited to a study presented on a conference in which they found an adequate trough plasma concentration of raltegravir in eight patients.[19] Whether chemotherapy-induced severe intestinal mucositis influences the absorption and total exposure to raltegravir has not been investigated.

This short case series describes three HIV-infected patients with advanced stage NHL who were treated with standard intensive chemotherapy and in whom therapeutic drug monitoring (TDM) of raltegravir was performed by means of a limited plasma sampling method to estimate the exposure to raltegravir.

Methods

The patients presented here were admitted to the department of hematology at the Radboud university medical center (Nijmegen, The Netherlands) in the years 2011-2013. The management of complex HIV-infected patients with NHL is performed by a multidisciplinary team of hematologists, infectious diseases specialists and clinical pharmacologists/hospital pharmacists with expertise on drug-drug interactions and TDM. The patients presented here received standard chemotherapy in accordance with national and international guidelines.[11] All three patients received raltegravir 400 mg twice daily orally according to the product labelling (Isentress, Merck Sharp & Dohme Ltd).[15,16] In our medical center TDM of raltegravir is available as regular patient care. Systemic exposure to raltegravir is best represented by the area under the plasma concentration-time profile (AUC) from 0 until 12 hours after intake of raltegravir. To reduce patient burden, practical issues and costs around 12-hour multiple blood sampling, an alternative limited sampling strategy was used in which an abbreviated AUC from 0 to 3 hours ($AUC_{0\text{-}3h}$) is calculated and extrapolated to $AUC_{0\text{-}12h}$ as described previously.[22]

Pharmacokinetic assessment of raltegravir was scheduled prior to and/or during chemotherapy as discussed within the multidisciplinary team. Pharmacokinetic sampling was performed at steady-state conditions at the following time-points: predose and 0.5, 1.0, 1.5, 2.0 and 3.0 hours after intake of raltegravir 400 mg on an empty stomach. Breakfast

was served 2 hours after intake of raltegravir. Blood samples were collected into lithium-heparinised tubes and centrifuged for 5 minutes at 1900 x *g* at 20°C. Plasma was transferred to polypropylene tubes and kept refrigerated until further bioanalysis. Concentrations of raltegravir in plasma were analyzed using a validated reversed-phase high-pressure liquid chromatography with fluorescence detection as previously described.[23] The linear calibration range in plasma was 0.014-10.0 mg/L. The raltegravir assay was performed at the laboratory of the Pharmacy of the Radboud university medical center (Nijmegen, The Netherlands) and externally validated through international Quality Assurance and Quality Control Programs for measurement of antiretroviral drugs in plasma.[20,24,25]

Based on the individual plasma concentration-time data the area under the plasma concentration-time curve from 0 to 3 hours ($AUC_{0\text{-}3h}$) was calculated using the trapezoidal rule. The $AUC_{0\text{-}12h}$ was estimated based on the following equation: $AUC_{0\text{-}12h} = 1.321*AUC_{0\text{-}3h} + 1.209$, $r^2 = 0.929$. The trough plasma concentration (C_{trough}) was defined as the sample taken immediately prior to intake of 400 mg raltegravir at time-point 0 hours. Interpretation of the pharmacokinetic parameters was done by a hospital pharmacist based on a Dutch TDM guideline for raltegravir. The following population reference values were used (unpublished data): 6.89 mg·h/L for $AUC_{0\text{-}12h}$, 4.30 mg·h/L for $AUC_{0\text{-}3h}$ with a peak plasma concentration at approximately 2 hours after intake of raltegravir on an empty stomach. From 2012 onwards the target trough plasma concentrations of rategravir has been defined as >0.020 mg/L.[26]

Case series

Case 1: HIV-associated Burkitt lymphoma

A 44-year-old HIV-infected man was referred to our institution in 2012 by his general practitioner. A summary of his clinical characteristics, his treatment and the pharmacokinetic parameters of raltegravir are given in Table 1. At presentation, he started a raltegravir-based antiretroviral regimen to obtain a rapid virological response and to reduce the risk of potential drug-drug interactions with chemotherapeutic agents. Induction chemotherapy was started immediately according to the acute lymphoblastic leukemia (ALL) 4 protocol.[27]

TDM of raltegravir was performed during cyclophosphamide infusion 750 mg/m^2 to evaluate the pharmacokinetics of raltegravir in this setting. We measured an adequate raltegravir C_{trough} (>0.020 mg/L) and an exposure to raltegravir ($AUC_{0\text{-}12h}$) that was 65% lower compared to our population reference value of 6.89 mg·h/L. However the maximum plasma concentration between the 0 to 3 hour time-window was measured at 3.0 hours

Table 1 Clinical characteristics and raltegravir pharmacokinetic parameters

Clinical characteristics	Case 1	Case 2	Case 3	
Year of presentation	2012	2011	2013	
Diagnosis	Burkitt lymphoma (c-Myc+)	Burkitt lymphoma (c-Myc+)	DLBCL (relapsed), initial diagnosis in 2009	
Ann-arbor stage	IV	IVB	IVB	
CNS involvement	Suspected	Yes	Yes	
HIV status	Confirmed HIV infection since 7 years, treatment-naive	Recent HIV diagnosis	Confirmed HIV infection since 8 years, cART since 2007 with good virological response	
CD4+ cell count (cells/ µL)	810	280	Unknown	
HIV RNA load (copies/mL)	20 000	100 000	80	
Antiretroviral therapy	raltegravir 400 mg BID tenofovir + emtricitabine QD Initiated before start chemotherapy	raltegravir 400 mg BID tenofovir + emtricitabine QD Initiated before start chemotherapy	raltegravir 400 mg BID (switch from boosted darunavir), tenofovir + emtricitabine QD	
Chemotherapy	ALL4 (cyclophosphamide, daunorubicine, vincristine and prednisolon) + intrathecal methotrexate + rituximab	HOVON 63 with R-CHOP + intrathecal methotrexate	R-DHAP + R-VIM , followed by BEAM + ASCT	
Pharmacokinetic assessment raltegravir				
Timing	Cycle 1, day 8 during cyclophosphamide infusion	Day before 2nd cycle R-CHOP	Before start BEAM	6 days after last dose BEAM
Intestinal mucositis	None	None	None	Severe, watery stools
Non-HIV concomitant medication	cyclophosphamide, ondansetron, sulphamethoxazole/trimethoprim, temazepam, colistine	omeprazol, bisacodyl, oxycodon, oxazepam, sulphamethoxazole/trimethoprim, fluconazol, acetylsalicylic acid, osmotic laxative	pantoprazol, sulphametoxazol/ trimethoprim	valaciclovir, pantoprazol, ceftazidim
$AUC_{0\text{-}12h}$ (mg·h/L)	2.46 (±65%↓)	3.79 (±50%↓)	9.37 (±40%↑)	4.20 (±65%↓ than baseline)
C_{trough}/C_0 (mg/L)	0.200	0.091	0.114	0.147
t_{max} (h)	≥ 3	0.5-1.0	1.7	≥ 3
Follow up				
NHL status	Complete remission after BEAM and ASCT	Clinical progression, died within one year after diagnosis of HIV/AIDS.	Good clinical response, additional radiotherapy for suspect lesion	
HIV clinical parameters	HIV RNA load <50 copies/mL after 7 weeks of cART	HIV RNA load <50 copies/mL after 4 weeks of cART	HIV RNA <50 copies/mL 2 weeks after BEAM	

Abbreviations: ALL, acute lymphoblastic leukemia; ASCT, autologous hematopoietic stem-cell transplantation; BEAM, high-dose chemotherapy with carmustine, etoposide, cytarabine and melphalan; BID, twice daily; cART, combination antiretroviral therapy; CNS, central nervous system; DBLC, diffuse large B-cell lymphoma; QD, once daily; R-CHOP, rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone; R-DHAP, rituximab, dexamethason, cytaribine, cisplatin; R-VIM, rituximab, etoposide, ifosfamide, methotrexate; t_{max}, time to reach the maximum plasma concentration (absorption peak).

and was relatively low (0.425 mg/L), suggesting that the peak plasma concentration had not been reached and the extrapolated $AUC_{0\text{-}12h}$ may be an underestimation. A possible explanation for the delay in absorption could be that raltegravir was taken with food as opposed to fasted. We were unable to recall the timing of breakfast. The patient had no complaints of vomiting and diarrhea that could have contributed to the observed decrease in absorption. Based on the metabolic profile of raltegravir a clinically significant drug-drug interaction with cyclophosphamide or the other co-medication used at the time of raltegravir blood sampling was not expected theoretically. Because the patient had an adequate raltegravir trough level no follow-up TDM was performed and the patient continued his antiretroviral regimen with success.

Case 2: HIV-associated Burkitt lymphoma

A 52-year-old man was referred to our institution in 2011 for treatment of a recently diagnosed HIV-infection and HIV-associated Burkitt lymphoma (Table 1). TDM of raltegravir was performed a day before the second cycle of R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone) was given. We intended to schedule it on the same day to evaluate concomitant use of raltegravir with the chemotherapeutic agents, but the second R-CHOP cycle was postponed at the very last minute due to leukopenia. We measured an adequate raltegravir C_{trough} well above 0.020 mg/L. Raltegravir $AUC_{0\text{-}12h}$ was approximately 50% lower compared to our reference values. Based on the concomitant use of omeprazol an increase of AUC would have been more likely. A drug-drug interaction study showed that omeprazol increased the AUC of raltegravir by 37% due to improved raltegravir solubility and oral absorption at higher gastric pH values. This drug-drug interaction is not clinically relevant according to the product labeling.[12] In our case we continued with a standard dosage of raltegravir as we considered the 50% decrease in AUC not clinically relevant. A rapid viral suppression was seen after one month of cART. Despite additional intensive chemotherapy no clinical improvement was achieved and the patient died of AIDS and Burkitt lymphoma within one year after clinical manifestation and diagnosis of HIV/AIDS.

Case 3: relapsed HIV-related DLBCL and intestinal mucositis

A 64-year-old HIV-infected man was admitted to our institution with disease progression of HIV-related high-grade DLBCL in 2013 (Table 1). In order to reduce the risk for potential drug-drug interactions between his antiretroviral regimen and chemotherapeutic agents, ritonavir-boosted darunavir was switched to raltegravir 400 mg twice daily prior to the start of chemotherapy.

TDM of raltegravir was performed to evaluate the potential influence of severe intestinal mucositis on the absorption of raltegravir. Blood sampling was scheduled at two different occasions: baseline pharmacokinetic assessment before start of BEAM chemotherapy

(high-dose carmustine, etoposide, cytarabine and melphalan) and a second evaluation 6 days after the last dose of BEAM chemotherapy. At the time of the first scheduled abbreviated $AUC_{0\text{-}3h}$ the patient was in a relatively good clinical condition with no complaints of abdominal discomfort and a normal defecation pattern. We measured a normal raltegravir trough plasma concentration and an exposure to raltegravir ($AUC_{0\text{-}12h}$) which is approximately 40% higher compared to our population reference values. The increase in AUC could be a result of the concomitant use of pantoprazol which increases gastric pH and could increase oral absorption of raltegravir. Although the combination of raltegravir and pantoprazol has not been studied, omeprazol, another proton pump inhibitor, has shown this effect as mentioned before. During the second assessment the patient suffered from severe intestinal mucositis with painfull abdominal cramps, nausea, vomiting, and diarrhea with frequent loose and watery stools. The plasma albumin level had decreased to 26 gr/L (normal range, 35-45 gr/L). The raltegravir trough concentration was slightly higher than at baseline. However, $AUC_{0\text{-}12h}$ was approximately 65% lower compared to baseline and 40% lower compared to our reference values. The peak plasma concentration measured at baseline was 4.55 mg/L at 1.7 hours after intake compared with 1.53 mg/L at 3.0 hours after intake at the second pharmacokinetic assessment. The limited sampling strategy that was used to extrapolate to 12 hours may underestimate the total exposure because the time to reach the absorption peak was ≥ 3 hours. The decrease in exposure could been have been (partly) a result of impaired and/or a delay in absorption due to intestinal mucositis. We advised to continue the normal dosage of 400 mg twice daily as the trough level remained normal and the decrease in $AUC_{0\text{-}12h}$ was not considered clinically relevant. HIV RNA load was measured regularly to monitor virological efficacy of his antiretroviral regimen.

General discussion

The optimal antiretroviral treatment for HIV-infected patients with malignancies and use of chemotherapy is complicated because of drug-drug interactions and overlapping toxicity.[12,13] To avoid potential drug-drug interactions via the CYP450 pathway we started or switched to a raltegravir-based antiretroviral regimen in three HIV-infected patients with advanced stage NHL and concomitant use of chemotherapy. We used a limited sampling strategy to estimate the exposure ($AUC_{0\text{-}12h}$) to raltegravir in this setting.[22,28] All three patients had adequate raltegravir trough levels (>0.020 mg/L) measured at various stages of their chemotherapy and a good virological response. The observed decrease in exposure (AUC) to raltegravir was not considered to be of clinical importance. The decrease in exposure in case 3 could be a result of reduced drug absorption caused by severe chemotherapy-induced intestinal toxicity.

Intestinal mucositis is tissue damage of the mucosal barrier and a common side effect of intense cytotoxic therapy and radiotherapy.[29] The impact of damage to intestinal villi on the pharmacokinetics of oral drugs remains poorly understood but may be associated with altered drug absorption due to accelerated or delayed gastric emptying, increased permeability of the mucosa, changes in intraluminal pH and decreased intestinal surface area. Whether this condition could have a clinically relevant impact on raltegravir absorption and efficacy is unknown. It is known that the solubility of raltegravir is highly dependent on gastric and intraluminal pH, as well as gastric transit time.[30,31] When patient 3 had complications of severe intestinal mucositis the exposure to raltegravir was reduced by approximately 65% compared to baseline measurements. The maximum plasma concentration of the absorption peak was lower and the time to reach this peak was delayed up to 3 hours or more after intake of raltegravir. Although this could have been a result of impaired absorption, we cannot rule out that this is partly due to intra-individual variation as we have not repeated the measurements. In addition to this we might underestimate the exposure because we did not sample beyond 3 hours and have missed (part of) the absorption peak. Despite potential impaired absorption, the magnitude of this effect was acceptable (40% reduction) compared to our population reference values. This is probably due to the concomitant use of intraveneous pantoprazol which has a positive effect on the extent of the absorption of raltegravir.

An important limitation in all three of the cases presented here and in the use of TDM of raltegravir in general is that the pharmacokinetic profile of raltegravir is known for its the extensive intraindividual variability.[32-34] Therefore the results of individual plasma concentrations of raltegravir should always be interpreted with caution as the plasma concentrations measured could have been subject to normal intrapatient variation.

The introduction of cART has reduced the risk of AIDS-defining malignancies and dramatically prolonged survival. As a result the HIV population is growing older and is increasingly more at risk for development of non-AIDS-defining malignancies that typically occur at older ages. Although the type of cancer that HIV-infected patients are diagnosed with is changing, the need for treatment with chemotherapy in combination with cART is increasingly common while the optimal antiretroviral treatment in this special patient population remains unknown. Some guidelines and articles on the management of cancer in HIV-infected patients recommend to use raltegravir as an alternative for NNRTIs and PIs to avoid potential pharmacokinetic drug-drug interactions via the CYP450 pathway. [12,13,17,18] Nonetheless, limited research has been performed on the efficacy, safety and pharmacokinetics of raltegravir in this setting in comparison with other antiretroviral agents and most publications are from recent years. The first publication was a case report in 2010 on the successful use of a raltegravir-based regimen in a treatment-naïve patient with DLBCL and chemotherapy with CHOP.[35] More recently Wong et al reported similar

toxicity and efficacy of CHOP when combined with a PI versus non-PI-based regimen in patients with DLBCL.[36] Unfortunately, in this retrospective pilot study the non-PI-based regimen included patients using raltegravir or efavirenz (a NNRTI). NNRTIs may induce doxorubicine and cyclophosphamide metabolism via CYP3A4 which could have influenced efficacy and toxicity rates of the chemotherapy in this cohort. Another retrospective study analyzed the efficacy, defined as the absence of virological failure, and safety of different cART regimens in HIV-infected patients with NHL or other types of cancer.[12] The authors conclude that cART including PIs were the least favorable in terms of virological efficacy and safety. NNRTIs and INSTIs (raltegravir) had comparable efficacy, but raltegravir appeared to be better tolerated. Raltegravir in combination with various chemotherapeutic agents in a prospective longitudinal study in 28 HIV-infected patients was found to be safe and effective, regardless of the type of tumor and type and duration of chemotherapy.[19] This study, which was presented on a conference, is to our knowledge the only study that has reported pharmacokinetics of raltegravir. They measured adequate trough levels (median 0.143 mg/L; n=8) which is comparable to our findings.

We have presented a series of three cases on the value of TDM of raltegravir in HIV-infected patients with typical HIV-associated hematological malignancies. Raltegravir trough plasma concentrations, which is the most important parameter with respect to virological efficacy, was adequate in all three patients when using the standard dosage of 400 mg twice daily. Severe chemotherapy-induced intestinal toxicity may negatively influence the absorption of raltegravir. If severe diarrhea and vomiting occurs, TDM could be used to monitor raltegravir treatment. The cases we described do not represent the general HIV population with cancer. However, they are illustrative for the complexity that clinicians face when managing drug-drug interactions in HIV-infected patients with chemotherapy. More research is needed on the safety and efficacy of raltegravir and other antiretroviral agents in combination with chemotherapeutic agents with special attention to pharmacokinetic drug-drug interactions and intestinal complications which could potentially influence the oral absorption or antiretroviral agents.

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Raltegravir pharmacokinetics in children: a case report and review of the literature

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Modified version of Clinical Infectious Diseases 2015; 61(2): 294-5

Abstract

Raltegravir is the first human immunodeficiency virus type 1 (HIV-1) integrase strand transfer inhibitor approved by the US Food and Drug Administration and the European Medicines Agency for the treatment of HIV infection in combination with other antiretroviral agents in children from ≥4 weeks to ≤18 years. The experience in the use of raltegravir in pediatric HIV care and information on the pharmacokinetics of raltegravir in children is still limited. We discuss our experience with therapeutic drug monitoring and dose optimization of raltegravir chewable tablets in a 4-year-old HIV-infected patient, as well as provide a review of the available literature and information on the pharmacokinetics of raltegravir in children. The presented case illustrates our concern of low raltegravir trough values when using chewable tablets in young HIV-infected patients.

Introduction

Worldwide 3.2 million children under 15 are living with human immunodeficiency virus (HIV), comprising 9.1% of the HIV-infected population.[1] The majority of children become infected through mother-to-child HIV transmission (MTCT) during pregnancy, delivery or breastfeeding. HIV-infected children need long-life antiretroviral treatment to improve survival, reduce opportunistic infections and other complications of HIV infection. Pediatric guidelines recommend an antiretroviral regimen including a minimum of three drugs from at least two different drug classes.[2] Antiretroviral drug-resistant virus can develop because of poor adherence, an ineffective regimen, or a combination of these factors. The availability of effective age-appropriate formulations of new antiretroviral drug classes is urgently needed for those children who fail on first-line antiretroviral therapy and have limited therapeutic options.

Raltegravir is the first HIV integrase strand transfer inhibitor (INSTI) approved by the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) for the treatment of HIV infection in combination with other antiretroviral agents in children and adolescents.[3,4] In December 2011, raltegravir was approved by the FDA for use in children between 2 and 18 years of age followed by an additional change of the label to include infants ≥ 4 weeks of age and ≥ 3 kilogram. Raltegravir is available as film-coated tablet and in two special formulations suitable for infants and young children who are unable to swallow whole tablets; chewable tablets and granules which are administered as oral suspension. Although published safety and efficacy data of raltegravir in children are promising, its use and experience in pediatric HIV care is still limited.[5-8] In particular, little is known on the pharmacokinetics and treatment outcomes of the recently marketed formulations in young children.

Therapeutic drug monitoring (TDM) of antiretroviral therapy in HIV infection by measuring plasma concentrations of antiretroviral agents may be used as a means to optimize and monitor treatment response.[9,10] TDM can be a useful tool in patient populations with a large interindividual variation in pharmacokinetics, including children, whose developmental changes occurring with age could alter the pharmacokinetics of drugs.[10-13]

We describe a 4-year-old HIV-infected patient in whom therapeutic drug monitoring and individualisation of therapy with raltegravir chewable tablets was performed based on measurement of raltegravir plasma concentrations, and give a review of the literature on the pharmacokinetics of raltegravir in children, with a focus on the age group ≥ 2 to < 6 years old.

Case

A 4-year-old HIV-infected boy presented with first-line virological failure (plasma HIV-1 RNA 59400 copies/mL) in December 2011. He had recently migrated from sub-Saharan Africa where he was diagnosed to be HIV-infected at the age of 3 years. Two nucleoside reverse transcriptase inhibitors (NRTIs) zidovudine and lamivudine, and the nonnucleoside reverse-transcriptase inhibitor (NNRTI) efavirenz was started as first-line antiretroviral therapy approximately a year prior to his arrival in the Netherlands. His clinical condition was good, CD4 T-cells were 394/mm^3 (22.4 %), and hepatitis B and C serology was negative.

Genotypic resistance testing was performed to collect information about patterns of HIV drug resistance that might explain treatment failure to his current regimen. Several drug-resistance mutations were found which had resulted in NNRTI resistance and reduced susceptibility to several NRTIs due to thymidine analogue mutations (TAMs). Because of the extensive drug-resistance pattern with an increased plasma HIV-1 RNA to 429000 copies/mL, the patient was switched to a regimen including a protease inhibitor (ritonavir-booster lopinavir), a dual NRTI backbone of tenofovir and lamivudine, and raltegravir (Isentress, Merck Sharp & Dohme Ltd, Hoddesdon, United Kingdom). At that time (February 2012) the raltegravir chewable tablets for oral use in children from the age of 2 years were not yet approved by the EMA and were not available on the European market. Fortunately our patient could receive raltegravir chewable tablets on a named-patient program by the manufacturer of raltegravir. He was administered raltegravir 100 mg chewable twice daily corresponding with the dosage guidelines for children in weight band 14 - 20 kg (Table 1).

Ten days after initiation of raltegravir TDM was performed by measuring raltegravir plasma concentrations. A validated liquid chromatography-tandem mass spectrometry method was used with a lower limit of quantification (LLOQ) of the assay of <0.014 mg/L. A pharmacokinetic sample 10 hours post dose after observed intake was below the LLOQ (<0.014 mg/L) and below the threshold for trough levels (C_{trough}) for adult patients, which is <0.020 mg/L. The following day pharmacokinetic sampling was performed predose and between 0-3.5 hours after intake. The predose C_{trough} value was <0.014 mg/L and the maximum plasma concentration was 1.6 mg/L. Since our patient repeatedly showed low trough levels despite good adherence, the dosing interval was shortened and the dosing regimen was switched from 100 mg twice daily to 100 mg three times daily. We realized that the three-times-daily dosing regimen was very unpractical, especially in a young child sleeping 10-12 hours a night. However, our main concern at that time was the high plasma HIV-1 RNA and repeated suboptimal plasma concentrations of raltegravir.

Table 1 Recommended dose for raltegravir granules for suspension, chewable tablets and film-coated tablets in children ≥ 4 weeks old and adolescents ≤ 18 years old according to body weight.[3,4]

Body weight and age	Raltegravir dose (mg) to be administered		
	Granules for suspension[a]	Chewable tablets[a]	Film-coated tablets
≥ 3 to < 4 kg and ≥ 4 wks old	20 mg twice daily		
≥ 4 to < 6 kg and ≥ 4 wks old	30 mg twice daily		
≥ 6 to < 8 kg and ≥ 4 wks old	40 mg twice daily		
≥ 8 to < 11 kg	60 mg twice daily		
≥ 11 to < 14 kg	80 mg twice daily	75 mg twice daily	
≥ 14 to < 20 kg	100 mg twice daily	100 mg twice daily	
≥ 20 to < 25 kg		150 mg twice daily	
≥ 25 to < 28 kg		150 mg twice daily	400 mg twice daily
≥ 28 to < 40 kg		200 mg twice daily	400 mg twice daily
≥ 40 kg		300 mg twice daily	400 mg twice daily

[a] The weight-based dosing recommendation is based on approximately 6 mg/kg/dose twice daily.

Follow-up with TDM on two occasions indicated adequate C_{trough} levels (0.10 mg/L and 0.17 mg/L). The clinical condition of the patient remained well, plasma HIV-1 RNA had dropped from 423000 copies/mL at the start of the new antiretroviral regimen to 64 copies/mL after 6 weeks of treatment and CD4 T-cells had increased to 637/mm^3 (23.4 %). Because of the limited safety experience in children there were some concerns regarding the total daily dose of raltegravir being 150% of that recommended in the prescribing information. Approximately 4 months after the start of the new regimen, the plasma HIV-1 RNA concentration had reached undetectable levels (<50 copies/mL) and raltegravir was temporarily switched back to 100 mg chewable tablets twice daily. The patient was admitted to the hospital for pharmacokinetic blood sampling. Plasma concentrations predose (C_{trough} morning) and respectively 4, 8 and 12 hours (C_{trough} evening) after observed intake on an empty stomach were 0.018 mg/L, 0.09 mg/L, 0.03 mg/L, and <0.014 mg/L. Because of persistent low plasma levels, the raltegravir dosage was changed to 75 mg three times daily which was assumed to deliver adequate C_{trough} levels due to shortening of the dosing interval. With this regimen, the total daily dose of 225 mg was closer to the daily dose of 200 mg as recommended by the prescribing information.

The patient continued with raltegravir 75 mg three times daily throughout his fifth year. Every three months TDM was performed and C_{trough} levels taken approximately 7 hours after intake were all adequate, ranging from 0.07 to 0.24 mg/L. Plasma HIV-1 RNA was low

but detectable during this period (50 to 200 copies/mL). When the boy turned 6 years old, the raltegravir dose was increased to 100 mg three times a day. C_{trough} levels remained adequate and plasma HIV-1 RNA was undetectable (<50 copies/mL) for over a year. The dosing schedule was then simplified to 150 mg twice daily following a weight gain to approximately 25 kg. TDM was performed and intensive pharmacokinetic sampling was scheduled. C_{trough} was 0.06 and 0.10 mg/L in respectively the morning and evening, C_{max} was 4.16 mg/L and area under the curve up to 12 hours after intake ($AUC_{0\text{-}12h}$) was 10 mg•h/L calculated by noncompartmental analysis by WinNonlin/Phoenix version 6.3 (Pharsight Corporation, St. Louis, MO, USA). These pharmacokinetic parameters were in line with geometric mean values observed in children in the age group of ≥6 to <12 years who used the chewable tablets in a mean dose of 6.47 mg/kg (cohort IIB, IMPAACT P1066 study).[8] The patient remained on raltegravir 150 mg chewable tablets twice daily, combined with tenofovir, lamivudine, and ritonavir-boosted lopinavir. His plasma HIV-1 RNA load is still undetectable.

Raltegravir pharmacokinetics in children

Raltegravir was the first INSTI approved by the FDA and EMA for treatment of HIV infection in the pediatric population in combination with other antiretroviral agents. In December 2011, raltegravir was approved by the FDA for children and adolescents (age group 2-18 years) followed by an additional change of the label to include infants ≥ 4 weeks of age and ≥ 3 kilogram in December 2013.[3] EMA approval for the age group 2-18 years and infants ≥ 4 weeks of age and ≥ 3 kilogram was obtained in December 2012 and June 2014 respectively.[4] The approved dose recommendations in the pediatric population are displayed in Table 1. The primary study to support its use in the pediatric population is a phase I/II open-label multicenter trial (protocol P1066) in treatment-experienced, integrase inhibitor-naive children and adolescents ranging from ≥ 4 weeks to ≤ 18 years of age.[3,6] The clinical trial was conducted by the International Maternal Pediatric Adolescents Aids Clinical Trials (IMPAACT) network and designed to evaluate pharmacokinetics, safety, tolerability ,and efficacy of 48 weeks of raltegravir. All subjects were enrolled in 6 cohorts with 5 different age groups and three formulations of raltegravir. Cohort I (≥12 years to <19 years of age), and cohort IIA (≥6 to <12 years of age) were assigned to receive film-coated poloxamer adult tablets. Cohort IIB (≥6 to <12 years of age), and cohort III (≥2 to <6 years of age) were assigned to receive ethylcellulose chewable tablets. Cohort IV (≥6 months to <2 years of age), and cohort V (≥4 weeks to <6 months of age) were assigned to receive granules for oral suspension. The study was conducted in two stages: (I) evaluation of intensive pharmacokinetics (PK) and short term safety data for dose selection and (II) assessment of long-term safety and efficacy data. Enrolment began with cohort I and progressed to the younger cohorts once raltegravir dose requirements were determined

for this age group and considered acceptable in terms of safety and tolerability. Cohorts IV and subsequent cohort V were opened for inclusion after the pharmacokinetic data in the older cohort was fully evaluated. Therefore, the application to add the younger age groups (≥4 weeks <2 years) to the product labeling of raltegravir was done in a later stage. The PK objective was to achieve a PK profile similar to the approved raltegravir dosage of 400 mg twice daily (film-coated tablet) in adults. The geometric mean of the specific PK target included the $AUC_{0\text{-}12h}$ between 6.2 and 11 mg·h/L and a geometric mean 12-hours post-dose concentration (C_{12h}) exceeding 0.015 mg/L.[3] The AUC target range was based on values observed in phase 2 trials (Merck protocol 004) in HIV-infected adults with an AUC geometric mean value of 6.3 mg·h/L for raltegravir 400 mg twice daily monotherapy and 11.2 mg·h/L for raltegravir in combination with tenofovir and lamivudine.[14-16] The threshold of 0.015 mg/L corresponds with the *in vitro* concentration at which 95% of virological replication is inhibited (IC_{95} value).

Table 2 Raltegravir steady-state pharmacokinetic parameters following final recommended doses of granules for oral suspension, chewable tablets, and film-coated tablets twice daily in HIV-infected children in different age groups from ≥ 4 weeks to ≤ 18 years and HIV-infected adults.[6, 14, 17]

Age group by formulation	n	Mean		Geometric mean (CV%)		
		Dose (mg)	Dose (mg/kg)	$AUC_{0\text{-}12h}$ (mg·h/L)	C_{max} [b] (mg/L)	C_{12h} (mg/L)
Granules for suspension						
≥ 4 wks to < 6 m (Cohort V)	11	31.4	5.70	9.9 (40)		0.0518 (68)
≥ 6 m to < 2 y (Cohort IV)	8	51.3	5.93	8.8 (34)		0.0481 (52)
Chewable tablet						
≥ 2 to < 6 y (Cohort III)	12	89.6	6.24	8.0 (59)	4.33 (57)	0.0316 (56)
≥ 6 to < 12 y (Cohort IIB)	10	230	6.47	10.0 (34)	4.66 (53)	0.0576 (88)
Film-coated tablet						
≥ 6 to < 12 y (Cohort IIA)	11	400	13.4	7.0 (120)	2.14 (130)	0.1094 (221)
≥ 12 to ≤ 18 y (Cohort I)	11	391	9.28	7.0 (98)	1.78 (95)	0.1478 (78)
Adults, monotherapy[a]	6	400	-	6.3	2.00	0.0629
Adults, TDF + 3TC[a]	6	400	-	11.2	3.82	0.1063

[a] CV% unknown.

[b] C_{max} values not reported for Cohort IV and V.

Micromolar values were converted to mg/L by multiplying by 0.4444 (molecular weight of raltegravir is 444.4 g/mol). Abbreviations: 3TC, lamivudine; CV%, percent coefficient of variation; $AUC_{0\text{-}12h}$, area under the plasma concentration-time curve up to 12 hours after intake; C_{max}, maximum plasma concentration; C_{12h}, plasma concentration 12 hours after intake; wks, weeks; m, months; y, years; TDF, tenofovir.

Intensive 12-hour PK sampling during stage I of the P1066 trial was performed predose and at 0.5, 1, 2, 3, 4, 6, 8, and 12 hours after observed intake of the raltegravir dose on an empty stomach. Additional sparse PK sampling was performed at intervals until week 48. Table 2 shows the results of the pharmacokinetic parameters of raltegravir for cohort I to V after intensive PK sampling at the final recommended dose that met the prespecified PK targets for $AUC_{0\text{-}12h}$ and C_{12h}.[6,15-17] The PK parameters of raltegravir in HIV-infected adults from the Phase 2 study (Merck protocol 004) on which the PK-objective for $AUC_{0\text{-}12h}$ in the P1066 trial is based, are added to Table 2 for comparison.[14] These small cohorts of HIV-infected patients (n=6) have used raltegravir 400 mg twice daily monotherapy or raltegravir with a background treatment with tenofovir and lamivudine.

The background antiretroviral treatment at the time of intensive PK sampling during stage I of the study for Cohorts I to III was not specifically reported by Nachman et al.[6] Overall the most common concomitant antiretroviral agents were tenofovir (47.9%), ritonavir (as booster, 49%), lamivudine (41.7%), lopinavir/ritonavir (40.6%), and darunavir (39.6%) with few differences across cohorts I to III.[6]

All cohorts had average C_{12h} values above the IC_{95} value of 0.015 mg/L. The lowest geometric mean C_{12h} value was 0.0316 mg/L and observed in cohort III with children (n=12) from ≥ 2 to < 6 years of age using the chewable tablets in a mean dose of 6.24 mg/kg. This C_{12h} value is approximately half of that observed in adults on monotherapy with raltegravir 400 mg twice daily and approximately one third of the values in adults who use raltegravir with an antiretroviral backbone of tenofovir and lamivudine. The $AUC_{0\text{-}12h}$ in cohort III was 8.0 mg·h/L which was well within the range of the PK target from 6.3-11.2 mg·h/L in adults (Table 2).

An important question is whether the downward trend in mean C_{12h} in the younger age groups is clinically relevant. Results from the QDMRK study in treatment-naive adults with 800 mg raltegravir once daily versus 400 mg raltegravir twice daily indicate that the C_{trough} values (which is C_{12h} for twice daily dosing) and not the AUC is best correlated with efficacy.[18] Although it was not possible to identify a robust threshold, the available PK data in the once-daily treatment arm in the QDMRK study suggest a threshold value of 0.020 mg/L, which is just above the IC_{95} value of 0.015 mg/L. In all cohorts in the IMPAACT P1066 trial mean C_{12h} values were above 0.015 mg/L, the PK objective for C_{12h}. However, no details on the number of individual patients in the intensive PK group with C_{12h} concentrations below 0.015 mg/L or 0.020 mg/L were reported by Nachman et al.[6] This information is available in the Assessment Reports from the EMA for the chewable tablets and the granules for suspension and displayed in Table 3.[3,6] Data from the QDMRK study with treatment arms of 400 mg twice daily versus 800 mg once daily are added to Table 3 for comparison.[3,6,18] The QDMRK study showed that failure to achieve an HIV-1 RNA <50

copies/mL appeared predominantly at high baseline HIV RNA load and was associated with lower values of geometric mean C_{trough} in the 800 mg once-daily arm. In cohort III 2/11 patients (17%) had a C_{12h} level <0.020 mg/L compared to 12/22 patients (55%) in the 800 mg once-daily treatment arm in adults. Although the patient group in cohort III (≥2 to <6 years of age, chewable tablet) seems to contain the highest percentage of patients with low C_{trough} levels, it is not possible to draw any conclusions based on the results as shown in Table 3. The number of patients in the intensive PK sampling group is limited and one of the two patients in cohort III <0.020 mg/L was not an actual measured value but extrapolated from 8 hours postdose which could have been an underestimated value.

Table 3 Geometric mean trough values of raltegravir and corresponding proportion of patients with a C_{trough} below 0.020 mg/L for each cohort (I-V) of the IMPAACT study 1066 and both treatment arms of Protocol 071 (QDMRK).[15, 17]

Cohort or study arm: (age group and formulation)	N	Dose	Geometric Mean C_{trough} in mg/L (%CV)	N (%) of patients <0.020 mg/L
Granules for suspension				
≥4 wks to <6 m (Cohort V)	11	5.70 mg/kg	0.0518 (68)	1 (9%)
≥6 m to <2 y (Cohort IV)	8	5.93 mg/kg	0.0481 (52)	0 (0%)
Chewable tablet				
≥ 2 to <6 y (Cohort III)	12	6.24 mg/kg	0.0316 (56)	2 (17%)
≥ 6 to <12 y (Cohort IIB)	10	6.47 mg/kg	0.0576 (88)	0 (0%)
Film-coated tablet				
≥6 to <12 y (Cohort IIA)	11	13.4 mg/kg	0.1094 (221)	1 (9%)
≥12 to ≤18 y (Cohort I)	11	9.28 mg/kg	0.1478 (78)	0 (0%)
Adults, twice daily QDMRK	20	400 mg BID	0.1142 (167)	1 (5%)
Adults, once daily QDMRK	22	800 mg QD	0.0178 (111)	12 (55%)

Abbreviations: BID, two times a day; QD, once a day; CV%, percent coefficient of variation; TDF, tenofovir; 3TC, lamivudine.

In the IMPAACT 1066 trial additional sparse PK sampling was performed at intervals until week 48 and compared with the sparse PK data in the QDMRK study in the EMA Assessment report of the chewable tablets.[6] The results of the sparse PK sampling in cohort I to III showed that the percentage of patients with C_{trough} levels <0.020 mg/L were 2% (n=50) in cohort I, 0% (n=2) in cohort IIA, 18% (n=11) in cohort II B and 11% (n=19) in cohort III compared to 27% (n=245) in the once daily 800 mg treatment arm in QDMRK. Cohort IIB (≥6 to <12 years of age, chewable tablets) had the highest percentage (18%) of patients with C_{trough} values <0.020 mg/L which was considerable lower (0%) in the

intensive PK group of cohort IIB (both included only 11 patients). In the intensive PK group the C_{12h} levels were measured 12 hours after observed intake of the raltegravir dose whereas the sparse PK samples are collected after unobserved intake. Therefore raltegravir plasma concentrations <0.010 mg/L, which is the LLOQ in the IMPAACT trial, were considered as missing values assuming these were the result of nonadherence rather than suboptimal dosing.[15] It is unclear whether this assumption is correct and also based on adherence data and pill count.

Discussion

We presented a case of a 4-year-old patient with first-line virological failure who switched to an antiretroviral regimen including raltegravir chewable tablets in the recommended dosage of 100 mg (approximately 6 mg/kg) twice daily. With TDM of raltegravir, inadequate raltegravir trough plasma concentrations (< 0.014 mg/L) were repeatedly measured. Shortening the dose interval is considered the most successful approach to increase trough levels to acceptable values. Therefore the raltegravir dose was adjusted from 100 mg twice daily to 100 mg three times daily. This intervention was successful as follow-up TDM showed adequate plasma levels and a good clinical response without adverse events. The dosage was further optimized to 75 mg three times daily with remaining adequate C_{trough} levels. At the age of 7 the patient was successfully switched back to a normal twice-daily regimen of 6 mg/kg raltegravir chewable tablets.

We do not have a good explanation why the raltegravir plasma concentrations were too low in this patient at the age of 4. Self-reported adherence was good and low C_{trough} levels were also measured after observed intake of raltegravir in the hospital when intensive PK sampling was planned. The patient did not use concomitant medication that is known to influence the pharmacokinetics of raltegravir other than tenofovir which gives a clinically not relevant increase in the exposure to raltegravir in adults.[14]

Information on the PK of raltegravir in children and subsequent dose selection is limited to the results of the IMPAACT P1066 study published by Nachman et al. with some additional data in the Clinical Pharmacology and Assessment reports by the FDA and EMA.[6,15-17] The results show that the lowest mean C_{12h} value (0.0316 mg/L) in this study was observed in children using the chewable tablets in the age group ≥2 to <6 years old, which is the age group of our patient. In this small cohort the number of individual patients with C_{12h} levels <0.020 mg/L after observed intake was the highest (2/12 patients, 17%). After sparse PK sampling this percentage was 11% (2/19 patients). This could be an underestimation as plasma concentrations below <0.010 mg/L were considered as missing values assuming these were the result of non-adherence rather than suboptimal

dosing. Although it is not possible to draw any definite conclusions due to the limited number of included patients, it seems that some young patients, including our patient, fail to reach adequate trough levels of raltegravir when using the recommended dosage of 6 mg/kg raltegravir chewable tablets twice daily.

The evaluation of intensive PK in children and dose selection in the IMPAACT P1066 study was based on two PK objectives: a geometric mean C_{12h} >0.015 mg/L (IC_{95}) and a geometric mean AUC based on PK data in adults (6.2-11 mg·h/L). Although in general it is an acceptable approach to evaluate the PK in children based on PK targets in adults, one could question whether the chosen AUC is an appropriate target for the pediatric formulations of raltegravir as these new formulations are not considered bioequivalent to the film-coated tablet and have a different PK profile. A bioequivalence single-dose study in healthy adults showed that both the chewable tablets and the granules for oral suspension demonstrate higher AUC and peak plasma concentrations (C_{max}) with similar C_{12h} values compared to the film-coated tablet.[19] The geometric mean ratios (GMRs) and 90% confidence intervals (CIs) of the chewable tablet 4x 100 mg versus the film-coated tablet 400 mg when taken in a fasted state were 3.22 (2.37-4.38) for C_{max}, 1.78 (1.47-2.15) for AUC_{0-12h} and 0.90 (0.70-1.18) for C_{12h}.

This may explain why the geometric mean C_{12h} level in cohort III (0.0316 mg/L) was lower compared with those in adults using the film-coated tablet while the observed AUC in cohort III was within the adult target range. The PK objective for C_{12h} was met because the geometric mean C_{12h} level in cohort III is above 0.015 mg/L (IC_{95}). However, large interindividual variability in PK was observed in all cohorts which is consistent with the known PK profile of raltegravir.[20-22] Therefore it is not surprising that some patients have C_{12h} values that we would consider too low (<0.020 mg/L) in clinical practice despite a mean value that is 2-fold higher than the IC_{95}.

The pharmacokinetics of the chewable tablets were also studied in children from ≥ 6 to <12 years who used a mean dosage of 6.47 mg/kg raltegravir twice daily. In this cohort the AUC_{0-12h} and C_{12h} values were approximately 1.3 and 1.8 times higher than in children from ≥2 to <6 years who used a similar dosage of 6.24 mg/kg raltegravir twice daily. In the case presented we repeatedly observed very low trough levels at the age of 4 when the patient used 6 mg/kg twice daily, while plasma concentrations returned to normal values when the patient used the same weight-based dosage at the age of 7. This could suggest that age-related differences in metabolism of raltegravir chewable tablets lead to distinctly lower C_{12h} values in children in the age group ≥2 to <6 years of age as compared with children ≥6 to <12 years of age. It is known that developmental changes can alter the pharmacokinetics of drugs.[11,12]

Raltegravir is primarily metabolized by glucuronidation via UDP-glucuronosyltransferase (UGT) 1A1 in the liver, with minor contributions from UGT1A3 and UGT1A9.[23] Considerable

differences occur in hepatic glucuronidation during development from fetal to adult liver. Currently, there is no evidence supporting the hypothesis that hepatic glucuronidation of raltegravir is increased in the age group 2 to 6 years old compared to older children and adults. The enzyme activity of UGT1A1, which is also responsible for bilirubin glucuronidation, is still immature after birth, especially in preterm neonates, but reaches adult levels within 3 to 6 months.[24,25] In contrast, results from physiology-based pharmacokinetic modelling suggest that hepatic clearance of UGT1A9 substrates could be higher at younger age and drops to adult levels during late adolescence. Although these results should be interpreted with caution, it may explain why propofol which is over 50% glucuronidated by UGT1A9, shows higher clearance at 5 years of age compared to adults.[26] UGT1A3 expression was not detected in fetal liver tissue but seems to be upregulated at approximately 6-12 months of age.[24] Developmental changes from one UGT-isoform cannot be extrapolated to others and there is still a lot to learn on the actual impact of these changes in hepatic glucuronidation on pediatric drug clearance *in vivo* in different age groups.[12]

We did question whether the oral bioavailability of raltegravir could be decreased if the chewable tablets are swallowed whole instead of chewed. The patient presented in our case had always swallowed the chewable tablets. The product labeling indicates that the chewable tablets can be either chewed or swallowed whole. This is not supported by *in vivo* pharmacokinetic data, apart from a single steady-state C_{12h} from a subject in cohort IIB of 0.0480 mg/L which was judged to be normal compared to the geometric mean C_{12h} value in cohort IIB of 0.0576 mg/L.[15] Given the product composition, characteristics of the active ingredient, and *in vitro* performance in multiple dissolution tests (including its rapid disintegration), *in vivo* drug release following ingestion would be expected to be similar chewed or swallowed (Biopharmaceutics Product Quality Review by the FDA).[16] Although there is no reason to believe that the oral bioavailability of the chewable ethylcellulose tablets is different when swallowed whole, one may expect raltegravir peak plasma concentrations to be lower and occur later compared to chewing. Therefore we cannot rule out that this has contributed to the lower raltegravir trough plasma concentrations measured.

Summary and conclusion

Raltegravir represents a new antiretroviral drug class in pediatric HIV care. Currently, clinical experience with raltegravir chewable tablets is limited. In this context, we described our experience with dose optimization of raltegravir chewable tablets in a 4-year-old HIV-infected patient based on TDM of raltegravir. Despite following the approved dose recommendations for raltegravir chewable tablets in children, we repeatedly measured low raltegravir trough plasma concentrations. To ensure adequate raltegravir trough levels

we changed successfully from a twice-daily dose regimen to an off-label three-times daily regimen for a period of 2.5 years. At the age of 7 the patient could switch back to a normal twice-daily dosage of raltegravir chewable tablets with remaining adequate raltegravir plasma concentrations.

We reviewed the available literature and information on the pharmacokinetics of raltegravir in children which has resulted in the approved dose recommendations for the use of raltegravir in children ≥4 week to <18 years.[6,15-17] In a small cohort of children from ≥2 to <6 years who used the chewable tablets, the mean raltegravir trough level was lower compared to the trough levels observed in other age groups or reference values in adults.[6,15,18] Based on these pharmacokinetic data we are concerned that some individual young patients who use chewable tablets in the recommended dose might experience suboptimal trough levels in clinical practice as illustrated by our case. This could especially be problematic in children who have a high viral load before starting a raltegravir-based regimen. In these patients TDM could be a useful tool to monitor and if necessary adjust the raltegravir dose regimen. Additional well-controlled post-marketing studies on the pharmacokinetics, safety, and efficacy of raltegravir in HIV-infected children between 2 and 6 years of age are needed to reassess optimal dosing in this age group.

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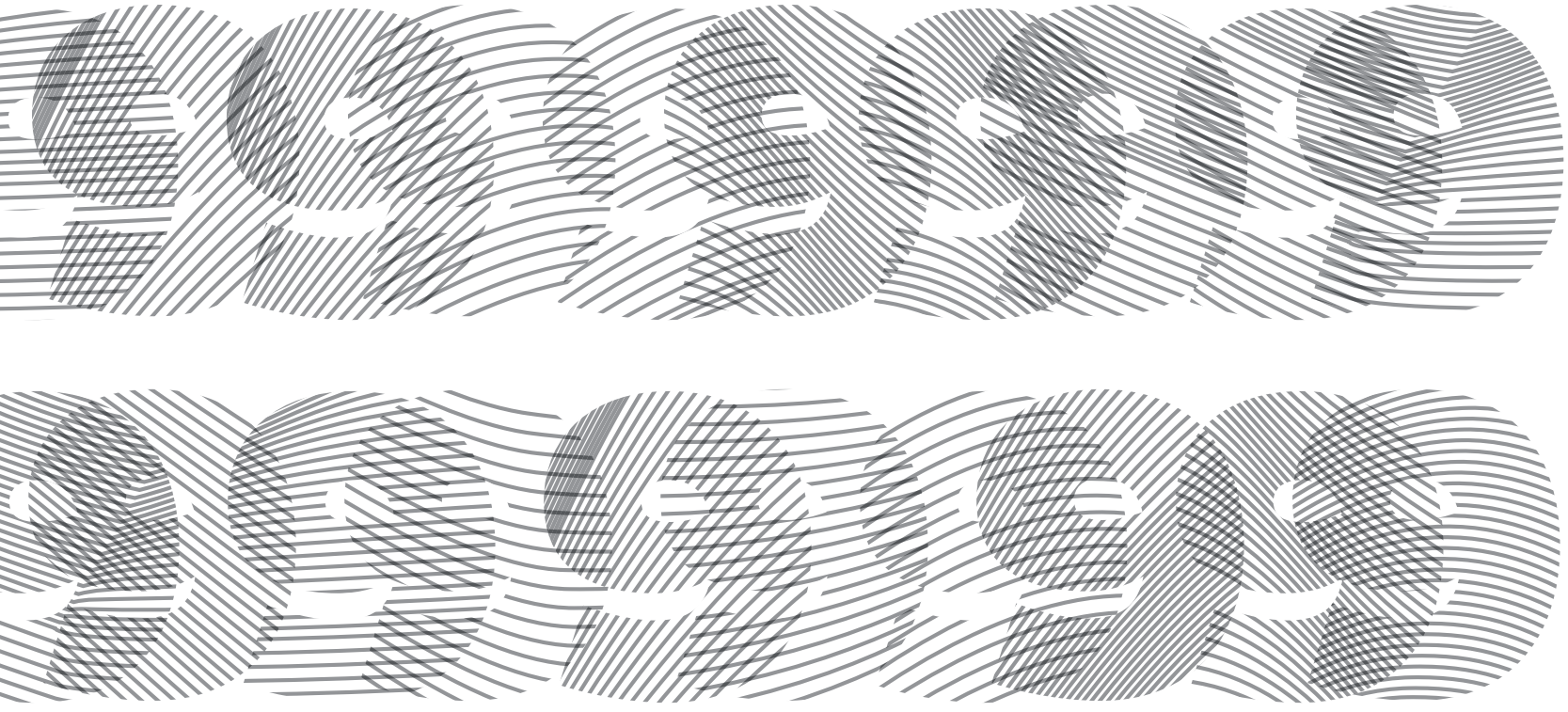

General discussion

Introduction

Knowledge of the clinical pharmacology of antiretroviral drugs is essential for their safe and effective use within the HIV-infected patient population. Drug-drug interactions, as well as intra- and interpatient variability in the pharmacokinetics of antiretroviral agents are some of the main causes for suboptimal drug exposure or toxicity. This could potentially lead to treatment failure and adherence issues in HIV-infected patients.

Raltegravir obtained accelerated approval in 2007 as the first of a new class of antiretroviral agents called HIV-1 integrase inhibitors. Immediately after the introduction of raltegravir with its new mechanism of action it played an important role in HIV management.

The general aim of this thesis was to study the clinical pharmacology of the HIV integrase inhibitor raltegravir to optimize its safe and effective use in HIV-infected patients in clinical practice. The first part of this thesis focused on pharmacokinetic drug-drug interactions between raltegravir and other frequently used concomitant medication. The pharmacokinetics of raltegravir in pregnant HIV-infected women and the application of therapeutic drug monitoring (TDM) of raltegravir in special patient populations were addressed in the second part of this thesis.

In the present chapter the results are discussed focussing on the following main topics: pharmacokinetic drug-drug interaction studies, the variability in the pharmacokinetics of raltegravir and potential influencing factors, the relationship between the pharmacokinetics and the pharmacodynamics of raltegravir, and the role of TDM in clinical practice. The general discussion will conclude with some closing remarks.

Pharmacokinetic research

The ultimate goal of drug development is to deliver the right drug in the right dose to the right patient. This requires detailed knowledge of the relationship between the dose and the clinical response in a variety of patients. Understanding what goes on between dose and response by use of pharmacokinetics gives insight on how to choose the dosages at which to evaluate a drug and on how best to use a drug in a (sub)population and in individual patients. Research on the pharmacokinetics of a new compound is an important part of drug development. Figure 1 gives a schematic overview of the application of pharmacokinetic research during the different phases of drug development.[1]

To characterize the pharmacokinetics of the investigational drug and its relationship with efficacy and safety, a diversity of studies are performed throughout drug development.

Intensive pharmacokinetic sampling schedules are used in single and multiple dose-ascending studies, in trials investigating the bioavailability of the drug, drug-drug or food-drug interactions, or the pharmacokinetics in special patient populations. In long-term phase II/III clinical trials in patients, extensive pharmacokinetic assessment is usually only feasible in a small subset of patients or over a limited period of time. Sparse pharmacokinetic sampling is obtained from as many patients at the time of clinical assessments and combined with rich pharmacokinetic data to characterize the relationship between drug exposure and efficacy in larger populations. Similar strategies apply for the exposure-safety relationship to evaluate the risk-benefit balance.[2]

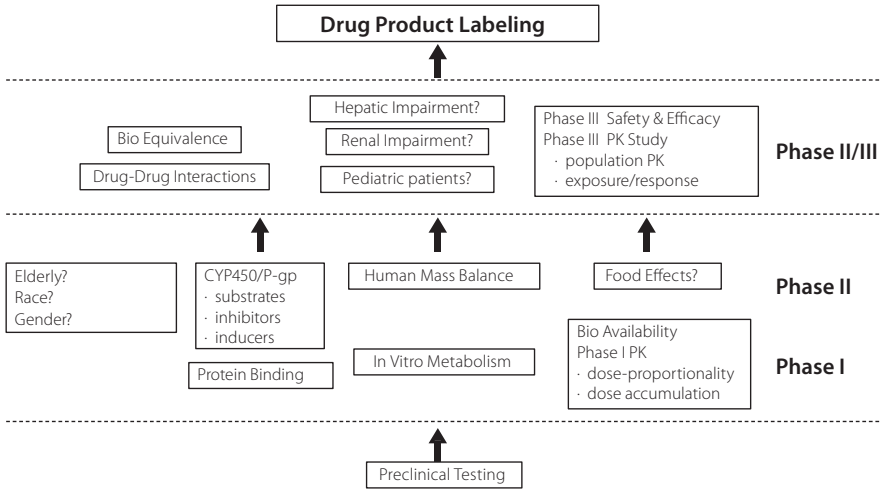

Figure 1 Application of pharmacokinetics (PK) in Phase I to III of drug development.

At the moment of drug approval there is still a degree of uncertainty about the safety and efficacy in daily clinical practice, including its value within the current treatment options. Especially when a drug has obtained accelerated approval, such as raltegravir. This is a well known and accepted consequence of drug evaluation before approval during which there is a relatively short follow-up with small study populations. Furthermore the setting of the clinical trial and characteristics of the study population are often seen as more idealistic than one would encounter in actual practice. After the investigational drug has been approved, pharmacokinetic research is continued in the post-approval phase to increase the knowledge on the pharmacokinetics of the drug needed to optimize or extend its use in clinical practice in a variety of patients.

Drug-drug interaction studies

General aspects

The overall objective of drug interaction studies is to determine whether any interactions occur that are sufficiently large to necessitate dose adjustment, the need for additional therapeutic monitoring, or whether the combination should be contraindicated. Knowledge about the interaction potential of a newly developed drug should be gained as early as possible to assure safety during clinical drug development and is generally required before the drug is being used in phase III studies. The drug interaction potential is usually investigated through *in vitro* studies followed by *in vivo* studies in healthy volunteers. The *in vivo* studies that are performed at this stage generally have a mechanistic approach and are performed with known strong or moderate inhibitors or inducers of enzymes that are involved in the metabolism of the particular drug. Other studies may be performed by the pharmaceutical industry with commonly used comedication that may be expected to interact with the developed new agent.

Phase II enzymes, which are enzymes that are involved in conjugation reactions, such as glucuronic acid, sulfonates, and glutathione, have historically gained less attention in drug-interaction evaluations than phase I metabolizing CYP enzymes. Nowadays there is deservedly so an increased interest in drug-drug interactions involving UDP-glucuronosyl-transferase (UGT)-enzymes because several UGT isoforms play an important role in the biotransformation of many drugs. If glucuronidation is the predominant pathway for drug elimination, as is the case for raltegravir, *in vitro* studies will be conducted at an early stage of drug development using recombinant human UGTs to identify which UGT isoforms are responsible for metabolism. However, determination of the contribution of each UGT isoform to the overall elimination is not as straightforward as that for CYP enzymes because of the absence of data on the abundance of these isoforms in drug eliminating organs and the lack of specific and selective inhibitors.

Although the evaluation of potential drug-drug interactions is part of the drug development and approval process, many questions remain unanswered or are still not discovered until after the introduction into clinical care. The importance of this issue is illustrated by the fact that half of the drugs withdrawn for safety reasons from the US market were associated with important drug-drug interactions. Therefore additional pharmacokinetic drug-drug interaction studies are often needed post-approval to optimize safe and effective use of drug-treatment combinations.[3-5]

Methodological aspects

The recommended design for pharmacokinetic drug-drug interaction studies is a crossover or sequential design in which the same subjects receive the investigational

drug alone (reference treatment) and with the potential interacting drug (test treatment). The advantage of a crossover or sequential design is that it allows a within-subject (or intrasubject) comparison between the reference and the test treatments, as each subject serves as its own control. In a crossover design, subjects are randomly assigned to receive a sequence of treatments, which contain all the treatments in the study. Therefore it is possible to correct for bias emerging from period effects. In a sequential design the treatment periods or sequences are not randomized but fixed.

Alternative strategies such as a parallel group design are usually chosen when a crossover or sequential design is not feasible. Although not recommended by the regulatory authorities it is possible to use pharmacokinetic data that is collected as part of a clinical trial that was not primarily designed to investigate a drug-drug interaction. In that case the collected pharmacokinetic data is usually compared with historical controls. The quality and evidence of those trials for the purpose to evaluate drug-drug interactions is low when compared with a crossover or sequential trial. It does, however, provide insight in potential interactions that may otherwise not have been investigated and it could be an incentive to perform a high-quality drug-drug interaction study.[4-6]

Drug-drug interaction studies are usually performed in healthy adults unless for specific tolerability and safety reasons patients are preferred. Historically the number of subjects in drug interaction studies have been small. The number of included subjects should be determined taking into account the intrasubject variability in pharmacokinetics, and the magnitude of the effect that is considered to be clinically relevant. It is recommended to analyze the pharmacokinetic data obtained in the drug-drug interaction studies according to an equivalence approach. Results should be reported as geometric mean ratio (GMR) and 90% confidence intervals (CIs) of the observed pharmacokinetic parameters of the investigational drug with the interacting agent versus without the interacting agent. The confidence intervals give an estimate of the distribution of the observed GMR as well as provide insight in the probability of the interaction. Tests of significance ($p < 0.05$) are not appropriate to use, as small but consistent differences in exposure can be statistically significant but not clinically relevant.

For the interpretation of the results specific boundaries or clinical equivalence intervals are used to indicate whether an observed interaction is clinically relevant. The standard equivalence range used for the interpretation of the GMR and the 90% CI is 0.80 to 1.25 (or 80-125%). When GMR and the 90% CI fall entirely within this range one can conclude that no clinically relevant interaction is present. This is, however, a conservative approach as in fact the relationship between the pharmacokinetic parameters and the pharmacodynamic response (efficacy and toxicity) defines whether a pharmacokinetic interaction is considered clinically relevant.[6] A wider equivalence range could be considered for highly variable

drugs (intrasubject coefficient of variation >30%), if this range in pharmacokinetic parameters is not considered to be clinically meaningful.[7]

Overview studies with raltegravir

The risk for drug-drug interactions in HIV-infected patients is likely to be reduced if an antiretroviral regimen is chosen with little propensity to interact with concomitant medication. This has become even more relevant as an increasing number of HIV-infected patients is being treated with multiple drugs. In contrast with many other antiretroviral agents raltegravir it is not known to inhibit or induce CYP450 enzymes which is a common hepatic metabolic route for many drugs. Therefore, raltegravir may be a good choice as antiretroviral agent if potential pharmacokinetic interactions are a concern. However, raltegravir could also be the victim drug, in other words the drug affected by the drug-drug interaction. Reduced exposure to raltegravir may have serious consequences for the development of resistance and the occurrence of virological failure. Furthermore, the metabolic profile of raltegravir or its transport via drug transporters, and therefore its role in potential drug interactions, may not be fully elucidated.

In Table 1 an overview is given of all pharmacokinetic drug-drug interaction studies in human, in which the effect of a drug on the pharmacokinetics of raltegravir has been investigated. In total 27 different potential interacting agents (or combinations) were studied in 35 drug-drug interaction studies in healthy volunteers and HIV-infected patients. The potential interacting agents represent 9 different therapeutic groups. Among the largest therapeutic groups are the antiretroviral agents, the antivirals for the treatment of hepatitis C virus (HCV), and the antimycobacterials. Approximately 60% of the drug-drug interaction trials were conducted by the pharmaceutical industry, mostly by Merck, the manufacturer of raltegravir. The remaining 40% of the trials fall into the category investigator-initiated academic research. Although investigator-initiated research is not industry-driven, the funding that is required to perform this type of pharmacokinetic research is often (partly) provided by the pharmaceutical industry.

Due to the lag time between the availability of the study results and the actual publication date in peer-reviewed journals, it is difficult to distinguish between drug-drug interaction studies performed as part of drug development and the studies that were conducted after the introduction of raltegravir onto the market at the end of 2007. Assuming that the publications in the year 2008 and 2009 by Merck were part of their drug development program, approximately two-thirds of the current drug-drug interaction studies can be labelled as post-approval studies.

Table 1 Overview of the pharmacokinetic drug-drug interaction studies with raltegravir.

Co-medication per therapeutic group	Design, Single Dose (SD)/ Multiple Dose (MD)[a]	Study population and number for PK evaluation	Effect (GMR and 90% CI)[b] of co-medication on raltegravir PK	Mechanism / Conclusion	Year, sponsor	Ref.
Analgesics						
buprenorphine/naloxone	Comparison of RAL MD + buprenorphine and naloxone with historical controls of RAL alone	12 opioid-dependent HIV-uninfected subjects	GM (95% CI): $AUC_{0\text{-}12h}$ 5.54 (3.60-8.54)mg·h/L C_{max} 1.07 (0.640-1.78) mg/L C_{12h} 0.196 (0.081-0.477) mg/L	No significant effect.	2013, II, $	64
Antidepressants						
citalopram (**Chapter 3**)	C/S, MD, citalopram 20 mg	22 healthy volunteers	$AUC_{0\text{-}12h}$ 0.77 (0.50-1.19) C_{max} 0.64 (0.38-1.09) C_{12h} 1.03 (0.71-1.50)	No clinically relevant decrease in RAL exposure.	II, $	65
Antimycobacterials						
rifabutin	C/S, MD, rifabutin 300 mg QD	19 healthy volunteers	$AUC_{0\text{-}12h}$ 1.19 (0.86-1.63) C_{max} 1.39 (0.87-2.21) C_{12h} 0.80 (0.68-0.94)	Minor UGT1A1 inhibition. No clinically relevant effect. Could be an alternative for rifampicin.	2011, Merck	66
rifampicin	Part I: C/S, RAL SD, rifampicin 600 mg QD	10 healthy volunteers	$AUC_{0\text{-}\infty}$ 0.60 (0.39-0.91) C_{max} 0.62 (0.37-1.04) C_{12h} 0.39 (0.30-0.51)	Significant reduction of C_{12h} due to strong induction of UGT1A1. Combination is not contraindicated but should be used with caution.	2009, Merck	67
rifampicin	Part II: C/S, MD, RAL 400 mg BID versus RAL 800 mg BID + rifampicin	18 healthy volunteers	$AUC_{0\text{-}12h}$ 1.27 (0.94-1.71) C_{max} 1.62 (1.12-2.33) C_{12h} 0.47 (0.36-0.61)	See Part I (above). Doubling the dose of RAL compensates for the effect of rifampicin on RAL exposure but not fully on the effect on $C_{12h.}$ Clinical studies are recommended.	2009, Merck	67
rifampicin	C/S, rifampicin intermittent dosing 3 times per week + respectively RAL MD 400 mg BID and 800 mg BID versus RAL 400 mg BID alone as reference	16 healthy volunteers	RAL 400 mg BID: $AUC_{0\text{-}12h}$ 1.08 (0.71-1.66) C_{max} 1.16 (0.69-1.93) C_{12h} 0.60 (0.44-0.82) RAL 800 mg BID: $AUC_{0\text{-}12h}$ 0.77 (0.50-1.19) C_{max} 0.64 (0.38-1.09) C_{12h} 1.03 (0.71-1.50) % subjects $C_{12h} \leq 0.021$ mg/L: 6% with RAL 400 mg BID alone, 25% with RAL 400 mg BID + rifampicin, 6% with RAL 800 mg BID + rifampicin	Clinically relevant induction of UGT1A1. C_{12h} is significantly reduced by intermittent rifampicin when RAL normally dosed in 400 mg BID. It is recommended to increase RAL dosage to 800 mg BID until clinical efficacy data suggest otherwise.	2015, II, $	68
rifapentine	C/S, MD, rifapentine two dosing strategies: 900 once weekly and 600 mg QD given 5 out of seven days	16 healthy volunteers	+ 900 mg rifapentine once weekly: $AUC_{0\text{-}12h}$ 1.71 (1.07-2.71) C_{max} 1.89 (1.04-3.44) C_{12h} 0.88 (0.62-1.25) + 600 mg rifapentine QD: $AUC_{0\text{-}12h}$ 0.95 (0.59-1.53) C_{max} 1.02 (0.60-1.72) C_{12h} 059 (0.34-1.02)	UGT1A1 is likely to be induced. Observed effect is dependent on dosing strategy. Preferred regimen is 900 mg QD because the increase in exposure is well tolerated and C_{12h} was not significantly affected.	2014, II, $	69

Table 1 Continued.

Co-medication per therapeutic group	Design, Single Dose (SD)/ Multiple Dose (MD)[a]	Study population and number for PK evaluation	Effect (GMR and 90% CI)[b] of co-medication on raltegravir PK	Mechanism / Conclusion	Year, sponsor	Ref.
Antivirals HCV						
boceprevir (**Chapter 4**)	C/S, RAL SD, boceprevir 800 mg TID	22 healthy volunteers	AUC_{0-12h} 1.04 (0.88-1.22) C_{max} 1.11 (0.91-1.36)	No clinically relevant effect.	2013, II, $	70
ABT-450 + ritonavir, ombitasvir, dasabuvir	C/S, MD, RAL with emtricitabine + tenofovir	12-24 healthy volunteers	≤ 134% increase of AUC_{0-12h}, C_{max}, C_{12h}	No dose adjustment recommended.	2014, Abbvie	71
faldaprevir	C/S, MD, faldaprevir 240 mg QD	23 healthy volunteers	AUC_{0-12h} 2.72 (1.997–3.707) C_{max} 2.457 (1.685-3.584)	Moderate increase RAL exposure, probably due to UGT1A1 inhibition. No unexpected safety concerns.	2015, Boehringer Ingelheim	72
grazoprevir	C/S, MD, grazoprevir 200 mg QD	12 healthy volunteers	AUC_{0-12h} 1.43 (0.89-2.3) C_{max} 1.46 (0.78-2.73) C_{12h} 1.47 (1.08-2.00)	Minor UGT1A1 inhibition. No clinically significant effect. No dose adjustments necessary.	2014, Merck	73
lersivirine	C/S, MD, lersivirine 1000 mg QD	16 healthy volunteers	AUC_{0-12h} 0.85 (0.64-1.11) C_{max} 0.71 (0.48-1.06) C_{12h} 1.25 (1.03-1.53)	Mechanism unknown. No clinically relevant effect. No dose adjustment necessary.	2012, Pfizer	74
ribavirin	C/S, RAL MD, ribavirin 800 mg SD	14 healthy volunteers	GMR (95% CI): AUC_{0-12h} 1.12 (0.74-1.70) C_{max} 1.16 (0.73-1.86) C_{12h} 0.82 (0.36-1.85)	No effect.	2011, II, $	75
Antivirals HIV						
atazanavir	RAL: C/S, SD 100 mg; atazanavir: MD 400 mg (Part I)	10 healthy volunteers	AUC 1.72 (1.47-2.02) C_{max} 1.53 (1.11-2.12) C_{12h} 1.95 (1.30-2.92)	Inhibition UGT1A1. No clinically important increase in exposure/ no dose adjustments. Needs to be confirmed in HIV+ patients.	2008, Merck	23
atazanavir + ritonavir	RAL: C/S, MD atazanavir/ritonavir: MD 300/100 mg (Part II)	10 healthy volunteers	AUC_{0-12h} 1.41 (1.12-1.78) C_{max} 1.24 (0.87-1.77) C_{12h} 1.77 (1.39-2.25)	See above (part I), including possible minor UGT1A1 induction by ritonavir.	2008, Merck	23
atazanavir	C/S, 1 period, MD, atazanavir 300 mg BID	22 healthy volunteers	AUC_{0-12h} 1.536 (1.135-2.081) C_{max} 1.394 (0.990-1.964) C_{12h} 1.479 (1.083-2.020)	Increased exposure due to UGT1A1 inhibition caused by atazanavir. Coadministration is safe and well tolerated.	2010, BMS + Merck	26
atazanavir	Designed to compare PK RAL 400 mg BID with RAL 400 QD + atazanavir 400 mg QD	22 healthy volunteers	AUC_{0-12h} 1.32 (0.62-2.81) C_{max} 1.37 (0.62-3.02) C_{12h} 1.49 (0.59-3.75)	Increased exposure RAL due to UGT1A1 inhibition. This effect did not result in similar trough values of RAL 400 QD with atazanavir compared to RAL 400 mg BID without atazanavir.	2010, II	24
atazanavir	Designed to compare PK RAL 400 mg BID with RAL 800 QD, both with atazanavir 600 mg QD	17 HIV-infected patients	GM (95%CI) RAL 400 mg with atazanavir: AUC_{0-12h} 7.5 (5.4-10.5) mg·h/L C_{max} 1.9 (1.4-2.6) mg/L C_{12h} 0.09 (0.05-0.16) mg/L	Inhibition UGT1A1. PK parameters were not compared with raltegravir alone in a C/S design	2013, II	25

Table 1 Continued.

Co-medication per therapeutic group	Design, Single Dose (SD)/ Multiple Dose (MD)[a]	Study population and number for PK evaluation	Effect (GMR and 90% CI)[b] of co-medication on raltegravir PK	Mechanism / Conclusion	Year, sponsor	Ref.
darunavir + ritonavir	Single-arm, MD, darunavir/ritonavir 800/100 mg QD	15 HIV-infected patients	GM (95% CI) $AUC_{0\text{-}12h}$ 3.05 (2.53-5.18) mg·h/L C_{max} 0.97 (0.84-2.27) mg/L C_{12h} 0.040 (0.030-0.080) mg/L	PK are lower than or equal to eference values, depending on the reference chosen. Conclusion: good PK profile.	2013, II, $	76
darunavir + ritonavir	C/S, MD, darunavir/ritonavir 600/100 mg BID	18 healthy volunteers included, 6 completed	$AUC_{0\text{-}12h}$ 0.71 (0.38-1.33) C_{max} 0.67 (0.33-1.37) C_{12h} 1.38 (0.16-12.12)	Many dropouts due to adverse events (rash).	2008, Merck	77
efavirenz	C/S, RAL: SD lactose formulation Efavirenz: MD	9 healthy volunteers	$AUC_{0\text{-}\infty}$ 0.64 (0.52-0.80) C_{max} 0.64 (0.41-0.98) C_{12h} 0.79 (0.49-1.28)	Minor induction UGT1A1. No clinically relevant effect.	2008, Merck	78
etravirine	C/S, MD, etravirine 200 mg BID	10 healthy volunteers	$AUC_{0\text{-}12h}$ 0.90 (0.68-1.18) C_{max} 0.89 (0.68-1.15) C_{12h} 0.66 (0.34-1.26)	Possibly induction UGT1A1. The effect is likely to be of no clinical importance.	2008, Merck and Tibotec	79
maraviroc	C/S, fixed sequence, MD, maraviroc 300 mg BID, range 0.80-1.25.	18 healthy volunteers	GMR (95%CI) $AUC_{0\text{-}12h}$ 0.633 (0.410-0.976) C_{max} 0.668 (0.371-1.20) C_{12h} 0.724 (0.551-0.952)	No clinically relevant decrease in RAL exposure. Mechanism unknown but might be related to changes in absorption/first-pass metabolism.	2010, Pfizer	80
ritonavir	C/S, RAL: SD lactose formulation Ritonavir: MD	10 healthy volunteers	AUC 0.84 (0.70-1.01) C_{max} 0.76 (0.55-1.04) C_{12h} 0.99 (0.70-1.40)	No clinically relevant effect.	2008, Merck	78
tenofovir	C/S, MD (Study A)	10 healthy volunteers	$AUC_{0\text{-}12h}$ 1.49 (1.15-1.94) C_{max} 1.90 (0.76-4.77) C_{12h} 1.69 (1.21-2.54)	Increase in RAL exposure, mechanism unknown. No dose adjustments necessary.	2008, Merck	30
tenofovir + lamivudine	C/S, MD (Study B)	6 HIV-infected patients received RAL 400 mg BID	$AUC_{0\text{-}12h}$ 1.78 (0.86-3.66) C_{max} 1.64 (1.16-2.32) C_{12h} 1.03 (1.21-2.54)	Increase in RAL exposure, mechanism unknown. No dose adjustments necessary.	2008, Merck	30
tipranavir + ritonavir	C/S, MD, tipranavir/ritonavir 500/200 mg BID	18 healthy volunteers	$AUC_{0\text{-}12h}$ 0.76 (0.49-1.19) C_{max} 0.82 (0.46-1.46) C_{12h} 0.45 (0.31-0.66)	Possible mechanism is UGT1A1 induction by tipranavir. Despite substantial decrease of C_{12h}, the combination may be administered without dose adjustments based on phase III efficacy data.	2009, Merck	81
Calcium channel blockers						
amlodipine	C/S, MD	17 healthy volunteers	$AUC_{0\text{-}12h}$ 1.39 (0.87-2.29) C_{max} 1.58 (0.84-3.09) C_{12h} 0.78 (0.57-1.04)	No clinically relevant effect.	2014, II, $	82

Table 1 Continued.

Co-medication per therapeutic group	Design, Single Dose (SD)/ Multiple Dose (MD)[a]	Study population and number for PK evaluation	Effect (GMR and 90% CI)[b] of co-medication on raltegravir PK	Mechanism / Conclusion	Year, sponsor	Ref.
Drugs for acid related disorders						
antacids (Maalox containing aluminium, magnesium)	C/S, SD	17 healthy volunteers included, 12 completed	AUC 1.08 (0.68-1.73) C_{max} 1.53 (0.9-2.60) C_{12h} 0.33 (0.26-0.42)	Combination of increased gastric pH (higher C_{max}) and divalent metal ion binding (lower C_{12h}), no conclusion.	2010, II	27
omeprazole	C/S, RAL SD, omeprazol 20 mg QD	14 healthy volunteers	AUC_{0-12h} 3.12 (2.13-4.56) C_{max} 4.15 (2.82-6.10) C_{12h} 1.46 (1.10-1.93)	Increased bioavailability due to increased gastric pH. Based on Phase III safety data in HIV-infected patients no dose adjustments are necessary.	2009, Merck	32
famotidine	Unknown	Unknown	AUC ↑ 44 % C_{12u} ↑ 6 % C_{max} ↑ 60 %	No dose adjustments required.	Merck	11
Lipid modifying agents						
atorvastatin (**Chapter 2**)	C/S, MD, atorvastatin 20 mg QD	23 healthy volunteers	AUC_{0-12h} 1.01 (0.68-1.51) C_{max} 1.14 (0.70-1.86) C_{12h} 0.96 (0.69-1.32)	No effect.	2015, II, $	83
ezetimibe	C/S, MD, ezetimibe 10 mg QD	20 healthy volunteers	AUC_{0-12h} 1.16 (0.89-1.51) C_{max} 1.13 (0.81-1.58) C_{12h} 1.12 (0.72-1.74)	No significant effect on RAL PK.	2011, II, $	84
pravastatin	C/S, MD, pravastatin 40 mg QD	24 healthy volunteers	AUC_{0-12h} 1.13 (0.77-1.65) C_{max} 1.31 (0.81-2.13) C_{12h} 0.59 (0.39-0.88)	No clinically relevant effect	2010, II, $	85
Other						
Ginkgo biloba (**Chapter 5**)	C/S, RAL SD, Ginkgo biloba MD	18 healthy volunteers	AUC_{0-12h} 1.24 (0.97-1.58) C_{max} 1.44 (1.03-2.02)	No clinically relevant effect.	2012, II, $	86

[a] Raltegravir dosage is 400 mg as a single dose or 400 mg twice daily (multiple dose) to reach steady-state plasma concentrations. If applicable, alternative dosages are mentioned in the table.
[b] Unless otherwise indicated.

Abbreviations and symbols: AUC, area under the plasma concentration-time curve; BID, twice daily; C/S, crossover or sequential design; C_{max}, maximum plasma concentration; C_{12h}, plasma concentration 12 hours after intake of raltegravir; CI, confidence interval; GM, geometric mean; GMR, geometric mean ratio; II, Investigator-Initiated Research, $ Financial support by Merck; SD, single dose; MD, multiple dose; PK, pharmacokinetics; RAL, raltegravir; TID, three times daily; QD, once daily.

Table 1 shows that, apart from a few exceptions, all drug-drug interactions were conducted with healthy volunteers by means of a crossover or sequential design. The average number of subjects that are included in the pharmacokinetic analysis is 16 subjects, with a minimum of 6 subjects and a maximum of 24 subjects. The sample size should be based on the intrasubject variability in the exposure to raltegravir and the effect that is considered to be clinically relevant. Therefore it is remarkable that in trials that are so similar in design, and research question, there is a 4-fold difference in the number of included subjects. The effect of therapeutic agents on the main pharmacokinetic parameters of raltegravir is in the majority of the trials presented as the GMR with 90% confidence intervals. Information on the sample size calculation and which clinical equivalence intervals were chosen is often missing in the published data.

Summary of results

The main findings of our pharmacokinetic drug interaction studies in healthy volunteers in which we evaluated the effect of atorvastatin (**Chapter 2**, AVIATOR study), citalopram (**Chapter 3**, RECITAL study), boceprevir (**Chapter 4**, OPAL study) and Ginkgo Biloba (**Chapter 5**, GINGER study) on the pharmacokinetics of raltegravir are summarized in Figure 2. The GMR and 90% confidence interval is given of the main pharmacokinetic parameters of raltegravir with concomitant use versus the intake of raltegravir alone. The main pharmacokinetic parameter to evaluate with respect to pharmacokinetic drug-drug interactions is the exposure to raltegravir expressed as the area under the plasma concentration-time curve (AUC). Another important pharmacokinetic parameter is the maximum plasma concentration (C_{max}). From a clinical perspective it is also important to know whether the trough plasma concentration (C_{12h}) is influenced by the coadministered drug, as C_{12h} is currently seen as the most important parameter to evaluate with regard to virological efficacy.

The AUC of raltegravir is not significantly influenced by atorvastatin or boceprevir. The same applies to the other pharmacokinetic parameters of raltegravir when combined with atorvastatin or boceprevir. In combination with citalopram or Ginkgo biloba we observed, respectively a 23% decrease, and 24% increase in raltegravir exposure (AUC). This change in exposure is primarily caused by a change in the peak plasma concentrations (36% decrease with citalopram, and 44% increase with Ginkgo biloba) because the $t_{1/2}$ of raltegravir did not change significantly. This would suggest that citalopram and Ginkgo biloba might change the oral bioavailability of raltegravir rather than influence its glucuronidation.

Characterization of potential drug transporters involved in the transcellular transport of raltegravir has shown that raltegravir is a substrate of permeability glycoprotein (P-gp).[8,9] Active efflux mechanisms, such as P-gp, are responsible for transporting a broad range of

compounds out of the intestinal epithelial cells back into the intestinal lumen and play an important role in oral drug absorption. An interesting hypothesis, which is discussed in more detail in **Chapter 5**, is that Ginkgo biloba may increase the oral bioavailability and subsequent C_{max} values by inhibition of P-gp. However, this hypothesis does not explain the decrease in C_{max} of raltegravir combined with citalopram, as *in vitro* research showed that citalopram might be a weak P-gp inhibitor and therefore is more likely to increase oral bioavailability instead of decrease.[10] Perhaps a more plausible explanation for the difference we found in the C_{max} and AUC of raltegravir alone versus raltegravir with citalopram or Ginkgo biloba within subjects, is that this is a result of normal intrasubject variability in raltegravir pharmacokinetics instead of an effect caused by an interacting agent. This is supported by our findings that the mean change (increase with Ginkgo biloba, decrease with citalopram) we observed in raltegravir C_{max} was not a uniform effect in

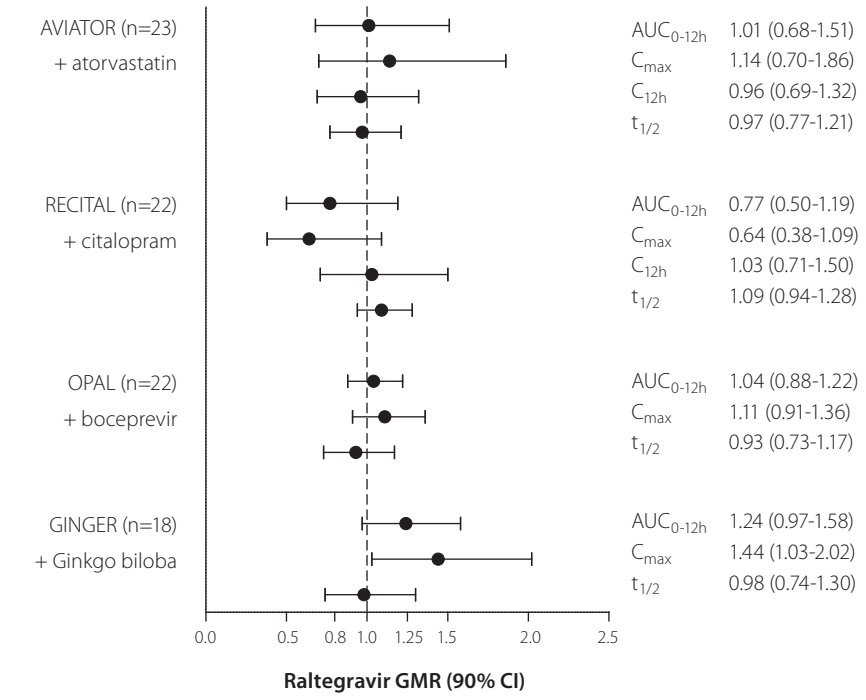

Figure 2 The effect of respectively atorvastatin, citalopram, boceprevir, and Ginkgo biloba on the pharmacokinetic parameters of raltegravir in healthy volunteers.

Abbreviations: AUC, area under the curve; CI, confidence interval; C_{max}, maximum plasma concentration; C_{12h}, plasma concentration 12 h after intake; GMR, Geometric Mean Ratio; $t_{1/2}$, apparent elimination half-life.
[a] $t_{1/2}$ could not be determined in all subjects, the number of included subjects are: AVIATOR n=17; RECITAL n=16; OPAL n=10; GINGER n=16.

all subjects. In approximately one third of the included subjects an opposite effect was seen. Individual changes in C_{max} and AUC were characterized by large differences in the magnitude of the effect as well as the direction of the effect. This is reflected by the large 90% confidence intervals displayed in Figure 2 and also graphically in Figure 2 in **Chapter 3** and **5**.

An important consideration when interpreting these results is whether the changes in pharmacokinetic parameters are clinically relevant. The magnitude of the observed effect on the C_{max} and the AUC of raltegravir is not regarded to be of clinical importance. Similar effects on the pharmacokinetics of raltegravir are described with drug-interacting agents in the product information leaflet without special recommendations to adjust the dosage of raltegravir.[11] Raltegravir C_{12h}, which is an important parameter from a clinical perspective, did not change with concomitant use of citalopram. Overall, we concluded that atorvastatin, citalopram, boceprevir and Ginkgo biloba do not have a clinically relevant effect on the pharmacokinetics of raltegravir.

The AVIATOR and RECITAL study (**Chapter 2** and **3**) were designed as two-way pharmacokinetic drug-drug interaction studies, meaning that also the potential effect of raltegravir on respectively atorvastatin, and citalopram was investigated. Although the OPAL study (**Chapter 4**) was not designed to evaluate boceprevir plasma concentrations with and without raltegravir use by intrasubject comparison, we did compare the boceprevir plasma concentrations when coadministered with raltegravir with historical reference values of boceprevir in plasma. In all three studies we found no effect of raltegravir on the pharmacokinetics of atorvastatin, citalopram and boceprevir, including their metabolites.

Pharmacokinetic variability

The pharmacokinetic profile of raltegravir is characterized by extensive variability, which was observed in our studies as well.[12-15] In fact, the variability in raltegravir pharmacokinetics has been a recurrent point of discussion, not only in the controlled settings of our trials but also in the application of TDM of raltegravir in the clinical setting. A distinction can be made between the variability that occurs within subjects or patients (intrasubject variability) and the variability between subjects or patients (intersubject variability). Intrasubject variability is defined as the variation that is seen when a measurement (for example sampling of a pharmacokinetic sample or curve) is repeated a number of times under the same conditions. The source of intrasubject variability could be multifold, but one important source is biological variability in absorption, distribution, metabolism and clearance. Intersubject variability is caused by a combination of genetic, demographic, physiological and environmental factors. It should be pointed out that in practice when measuring the variability between subjects under the same experimental conditions this always includes a certain amount of intrasubject variability (for example due to biological variation in clearance). Therefore intersubject variability in studies usually refers to the total (intersubject) variability. The intrasubject variability in the pharmacokinetics of a particular drug is generally much smaller compared with the total intersubject variability.

The drug-drug interaction studies and the pharmacokinetic study of raltegravir in pregnant HIV-infected women presented in this thesis have a similar crossover or sequential design in which each subject serves as its own control. This approach allows us to compare the effect of the reference and the test treatment or test situation within a subject (intrasubject comparison) which minimizes the potential contribution of genetic differences and the variability between subjects. Of course, in order to evaluate the true influence of the drug-drug interaction or pregnancy on the pharmacokinetics of raltegravir, other factors that might hamper the study results will be controlled or avoided as much as possible by carefully choosing the in- and exclusion criteria and specify standard procedures.

The schematic examples in Figure 3 A, B and C illustrate the change in the exposure (AUC) of a particular drug with the reference versus the test treatment and how to interpret these results with respect to the variability. The variability between subjects does not have a negative impact on the outcome as the change in exposure is evaluated by intrasubject comparison. In examples A and B the exposure is not influenced (A) or consistently increased (B) by the interacting agent. The GMR of the test AUC versus reference AUC will be (close to) 1.0 in all subjects in example A and above 1 in example B with narrow or normal confidence intervals. This is not the case for example C, which

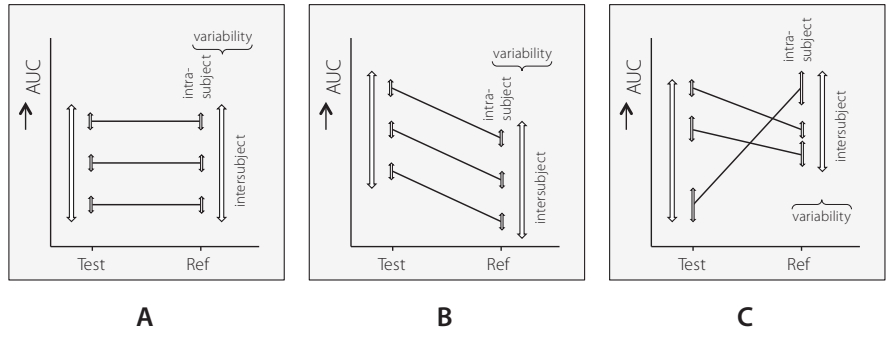

Figure 3 Individual changes (n=3) in exposure (AUC, y-axis) to the investigational drug when administered with an interacting agent (test treatment; Test) or alone (reference treatment; Ref): A, no drug interaction; B, increased exposure with test treatment; C, no uniform effect of test treatment.

shows large variability, as the potential interacting agent increased the AUC in some subjects and decreased the AUC in other subjects. Example C could represent the pharmacokinetics of a drug with extensive intrasubject variability, unless a plausible explanation can be given for the observed changes in some individuals (for instance vomiting). In that case it might be difficult to distinguish a potential effect of an interacting agent, especially when the effect is relatively small, from the variation in AUC due to intrasubject variability. In example C the GMR could be either 1.0 or below/above 1.0 with large confidence intervals given the variation in the individual ratios. The advantage of within subject comparison in crossover or sequential studies is diminished when investigating the pharmacokinetics of a highly variable drug, such as raltegravir. We have encountered this problem in our pharmacokinetic studies with raltegravir in which we used a within-subject comparison. The Figures are displayed in **Chapter 2, 3, 5** and **6**, and show that the individual changes in exposure to raltegravir with and without concomitant medication or postpartum versus third trimester pregnancy look similar to Figure 3C. The mean change in the $AUC_{0\text{-}12h}$ of raltegravir when coadministered with citalopram and Ginkgo biloba (**Chapter 3** and **5**) could (partly) be attributed to this intrasubject variability and at least gives some uncertainty in the interpretation of the results.

To give some insight in the variability in the pharmacokinetics of raltegravir as observed in our drug-drug interaction studies, a summary of the pharmacokinetic parameters of raltegravir when administered without concomitant medication, are given in Table 2 and 3. Table 2 presents the steady-state pharmacokinetic parameters of the AVIATOR study

Table 2 Raltegravir steady-state pharmacokinetic parameters and pooled data of raltegravir administered alone as 400 mg twice daily in the AVIATOR and RECITAL study.

Pharmacokinetic parameter	AVIATOR (n=23)		RECITAL (n=23)		Pooled (n=46)	
	GM (95% CI)	CV(%)	GM (95% CI)	CV(%)	GM (95% CI)	CV(%)
$AUC_{0\text{-}12h}$ (mg·h/L)	4.13 (2.91-5.87)	97	6.82 (4.64-10.0)	110	5.31 (4.09-6.90)	108
C_{max} (mg/L)	1.29 (0.83-1.99)	132	2.45 (1.60-3.76)	128	1.78 (1.31-2.42)	139
C_{12h} (mg/L)	0.052 (0.035-0.077)	114	0.054 (0.037-0.080)	110	0.053 (0.041-0.069)	110
t_{max} (h)[a]	1.6 (0-12)	119	2.0 (0.5-12)	95	2.0 (0-12)	105
$t_{1/2}$ (h)[b]	3.28 (2.64-4.09)	48	2.65 (2.28-3.09)	33	2.94 (2.58-3.35)	42

Abbreviations: AUC, area under the curve; CI, confidence interval; C_{max}, maximum plasma concentration; C_{12h}, plasma concentration 12 h after intake; CV, geometric coefficient of variation; GM, Geometric Mean; $t_{1/2}$, apparent elimination half-life; t_{max}, time to reach C_{max}.
[a] For t_{max}, median (min-max) is reported.
[b] $t_{1/2}$ could not be determined in all subjects, the number of included subjects are: AVIATOR 19 subjects; RECITAL 20 subjects; pooled data 39 subjects.

Table 3 Raltegravir pharmacokinetic parameters and pooled data of raltegravir administered as 400 mg single dose in the OPAL and GINGER study.

Pharmacokinetic parameter	OPAL (n=24)		GINGER (n=18)		Pooled (n=42)	
	GM (95% CI)	CV (%)	GM (95% CI)	CV(%)	GM (95% CI)	CV(%)
$AUC_{0\text{-}12h}$ (mg·h/L)	4.04 (3.09-5.28)	70	5.93 (4.21-8.34)	77	4.74 (3.85-5.84)	75
C_{max} (mg/L)	0.93 (0.70-1.23)	75	2.08 (1.39-3.12)	96	1.30 (1.00-1.68)	99
C_{12h} (mg/L)	0.093 (0.047-0.186)	368	0.036 (0.022-0.058)	121	0.062 (0.039-0.097	268
t_{max} (h)[a]	4.0 (1.0-12.00)	96	2.0 (0.5-12)	105	3.0 (0.5-12)	83
$t_{1/2}$ (h)[b]	1.80 (1.31-2.47)	55	3.17 (2.61-3.86)	29	2.52 (2.12-2.99)	48

Abbreviations: AUC, area under the curve; CI, confidence interval; C_{max}, maximum plasma concentration; C_{12h}, plasma concentration 12 h after intake; CV, geometric coefficient of variation; GM, Geometric Mean; $t_{1/2}$, apparent elimination half-life; t_{max}, time to reach C_{max}.
[a] For t_{max}, median (min-max) is reported.
[b] $t_{1/2}$ could not be determined in all subjects, the number of included subjects are: OPAL 13 subjects; GINGER 16 subjects; pooled data 29 subjects.

(**Chapter 2**), and RECITAL study (**Chapter 3**), as well as the pooled data of both studies (n=46). The pharmacokinetic data from the OPAL study (**Chapter 4**), and GINGER study (**Chapter 5**) after a single dose of 400 mg raltegravir is presented in Table 3, including the pooled data (n=42).
The geometric mean $AUC_{0\text{-}12h}$ in the AVIATOR study is lower than the observed value in the RECITAL study (respectively 4.13 mg·h/L versus 6.82 mg·h/L). This is somewhat surprising as the study procedures are similar with respect to the number of healthy volunteers, the intake of raltegravir on an empty stomach, the standardization of breakfast and lunch, the steady-state conditions, and the collection and handling of pharmacokinetic sampling. The most likely explanation for the difference in exposure is the variability, which is supported by the fact that the width of the confidence intervals around the geometric mean is large. This example illustrates well that it is difficult to speak of a 'true' reference value in the context of comparing raltegravir plasma concentrations collected for research purposes or regular patient care to historical control values. When interpreting raltegravir pharmacokinetic values it is important to always take into account the variability and the width of the confidence interval.

The OPAL and GINGER study are similar in design and the raltegravir dosage used, except for the conditions around the intake of raltegravir. In the OPAL study raltegravir was taken with breakfast as opposed to the fasted state in the GINGER study. It is known that food influences the pharmacokinetics of raltegravir and increases pharmacokinetic variability relative to fasting.[16,17] The effect of food explains (at least partly) the differences in pharmacokinetics in both studies and could be an argument against pooling of the data.

To describe the amount of variability in the geometric mean, the geometric coefficient of variation in percentage (CV%) is reported in Table 2 and 3. Because the CV% has no unit of measurement such as the standard deviation, it is often used to compare the variability. However one should pay attention to which CV% is reported. For example, the geometric mean CV of the pooled data of $AUC_{0\text{-}12h}$ in Table 2 is 108%, whereas the arithmetic mean CV for $AUC_{0\text{-}12h}$ is 81% (not shown in Table 2), which is considerably lower. Unlike the arithmetic CV which is dependent on the arithmetic mean, the geometric CV is calculated differently. The CV% of the $AUC_{0\text{-}12h}$ and the C_{max} in the multiple-dose studies (respectively 108% and 139%, pooled data Table 2) is higher than the variability observed in the single-dose studies (75% and 99%, pooled data Table 3). The variability in C_{max} is larger than in $AUC_{0\text{-}12h}$. This is logical as the AUC is based on multiple sampling points and the C_{max} is a single plasma sample which could have been taken before or after the true absorption peak concentration.

In phase III studies in HIV-infected patients the coefficient of variation (geometric CV %) of the inter- and intrasubject variability in C_{12h} levels obtained by sparse sampling was respectively, 211% and 122%, as reported by Merck.[15,18] The CV% of the intersubject variability was approximately two-fold higher than observed in our multiple-dose drug-drug interaction studies in healthy volunteers (Table 2). The higher CV% can be explained by the nature of phase III clinical trials in HIV-infected patients and the method of sparse pharmacokinetic sampling. Compared with the controlled setting of a study in healthy volunteers, phase III clinical trials are associated with considerably more factors that could contribute to the variability in the pharmacokinetics between subjects. Fortuna et al described an arithmetic mean CV% of raltegravir C_{trough} in the clinical setting between 100% and 125% for the total intersubject variability, and between 80 and 86% for the intrasubject variability.[14] The CV% was dependent on the degree of adherence to the protocol with a marginally lower CV% in the fully adherent subgroup. Overall, the authors conclude that the intrasubject variability was about 30% lower than the total intersubject variability.[14]

This is not consistent with the findings of Siccardi et al who described the inter- and intrapatient variability of raltegravir trough plasma levels (C_{trough}) in the clinical setting in 86 HIV-infected patients (type of CV% not reported). The intrasubject variability was higher than the intersubject variability.[13] In their study the median intrasubject CV% was 128% (64-265%) in 13 patients with 3 or more C_{trough} samples available per patient, and 245% in 7 patients with ≥ 10 C_{trough} samples available per patient. The observed intersubject CV% was 110%, which is similar to the results of Fortuna et al, and the CV% of the pooled data (n=46) in our multiple-dose drug interaction studies (Table 2).

Although even the amount of variability in raltegravir pharmacokinetics varies between studies, one can conclude that the total intersubject variability is very large with a CV% of approximately 100% or more. Unfortunately there is some conflicting data on the CV% of the intrasubject variability. We did not collect repeated pharmacokinetic measurements per subject for the purpose of calculating the intrasubject variability. If we assume that the intrasubject variability of raltegravir is approximately one-third lower than the intersubject variability, it is considerably higher than the lower threshold of 30% that is being used to identify highly variable drugs.[7]

Variability and influencing factors

To better understand the pharmacokinetics of raltegravir it is important to discuss which potential sources might contribute to the high pharmacokinetic variability.[15] The variability in raltegravir pharmacokinetics in our studies and research described in this thesis could be due to a combination of the following factors which will be discussed into more detail in this section:

- pharmacogenetics
- drug-drug interactions
- pH dependent solubility and formulation
- the effect of food

Pharmacogenetics

As raltegravir is primarily metabolized by UGT1A1, the hepatic UGT1A1 expression levels may alter the metabolism of raltegravir. Approximately 7-19% of the Caucasian population is homozygous for UGT1A1*28 which is associated with a decreased UGT1A1 expression.[19] Individuals with a decreased UGT1A1 expression caused by UGT1A1*28 polymorphism (e.g. subjects with Gilbert's syndrome) were compared with individuals with wild-type UGT1A1 (UGT1A1*1/*1) who have a normal UGT1A1 activity.[20] Subjects with a decreased UGT1A1 expression have moderately elevated plasma levels of raltegravir (GMR (90% CI); 1.41 (0.96-2.09) for AUC). This increase in plasma levels is not considered to be of clinical importance.[20] However, there is quite some variability in the phenotypic expression of individuals who are homozygous for UGT1A1*28 making a correlation between UGT1A1 genotype and UGT1A1 activity less strong.

The UGT1A1*28 did not exert any relevant effect on raltegravir C_{trough} values in a study by Siccardi et al investigating the variability of raltegravir in the clinical setting.[13] In the drug interaction studies with raltegravir and atorvastatin, citalopram, and boceprevir, we performed UGT1A1*28 genotyping in all included subjects (n=72) (**Chapter 2, 3** and **4**, data not presented). The individuals found to be homozygous for UGT1A1*28 (n=3; 4%),

were not excluded from pharmacokinetic analysis. The percentage of subjects homozygous for UGT1A1*28 was lower as would be expected in a predominant Caucasian study population. Based on our screening evaluation and in- and exclusion criteria we have excluded two subjects with total and direct bilirubin levels above the upper limit of normal (respectively >17 and >5 µmol/L). These individuals could have been homozygous for UGT1A1*28 because they are known to have episodes of mild hyperbilirubinemia.[20] Genetic variances between subjects were considered to be of minor importance in our drug interaction studies because we have used a crossover design instead of a parallel group design. However, in case there would have been a clear effect of the interacting agent on UGT1A1, it would have been interesting to know if any of the subjects were homozygous for UGT1A1*28. The degree of inhibition or induction of UGT1A1 by an interacting agent will probably be dependent on the UGT1A1 activity a priori.

In our drug interaction studies we were not able to observe any differences in pharmacokinetics depending on UGT1A1*28 polymorphism because of the small number of subjects homozygous for UGT1A1*28. Nonetheless it is likely that UGT1A1 polymorphism has contributed to some extent to the intersubject variability observed in our studies. Genotyping of UGT1A1 is not recommend in HIV-infected patients on treatment with raltegravir because it is not an important determinant for raltegravir pharmacokinetics and has no clinically relevant consequences. Therefore UGT1A1 genotyping was not performed in the patients described in **Chapter 6, 7** and **8** of this thesis.

Raltegravir is a substrate of P-gp which plays an important role in oral drug absorption.[8,9] The expression and transport activities of P-gp may differ between individuals due to genetic variation in the highly polymorphic ABCB1 gene.[21,22] Therefore, the extent of P-gp-mediated efflux of raltegravir may vary accordingly between subjects and contribute to the intersubject variability in raltegravir pharmacokinetics.

Drug-drug interactions

It is needless to say that concomitant use of raltegravir with an interacting agent, such as an UGT1A1 inhibitor or inducer, is going to influence the metabolism and the pharmacokinetics of raltegravir. Although drug-drug interactions are among the most frequently reported contributing factors when interpreting pharmacokinetic data in clinical trials and its variability between HIV-infected patients, it is a contributing factor that is easily avoided in drug-drug interaction trials in healthy volunteers. In our drug-drug interaction studies in healthy volunteers the intake of other therapeutic agents other than the study medication was prohibited during the whole conduct of the study including two weeks prior to the start of the study. This included herbal medicines, multivitamins and other dietary supplements. This is a common study procedure and one of the main advantages of conducting these type of studies in healthy volunteers.

However, in our study investigating the pharmacokinetics, efficacy and safety of raltegravir in HIV-infected pregnant women (**Chapter 6**), there were no restrictions on the use of concomitant HIV and nonHIV medication which could influence raltegravir exposure. Although it was not part of our objective and presentation of the study results in **Chapter 6**, it would be interesting to know whether the use of interacting agents has contributed to the variability in raltegravir pharmacokinetics in this study population. The HIV-infected pregnant women (n=22) can be divided in three subgroups according to the occurrence of drug-drug interactions and their effect on raltegravir exposure: neutral (no effect, n=15), favourable (increased exposure, n=4), and deleterious (decreased exposure, n=3). The respective interacting agents used were ritonavir boosted atazanavir (n=3) and ranitidine (n=1) in the favorable subgroup, and calcium carbonate (n=2) and magnesium (n=1) supplement in the deleterious subgroup.[23-29] One patient used both ritonavir-boosted atazanavir and magnesium and was assigned to the neutral subgroup. We did not include the potential influence of tenofovir, which was used by the majority of the included HIV-infected patients, on the pharmacokinetics of raltegravir. The mechanism by which tenofovir might increase raltegravir exposure (although only investigated in a small population) is unknown and not considered clinically relevant.[30] To illustrate the variability in raltegravir exposure ($AUC_{0\text{-}12h}$) per subgroup and change in the third trimester of pregnancy versus postpartum a scatter-dot plot was made (Figure 4).

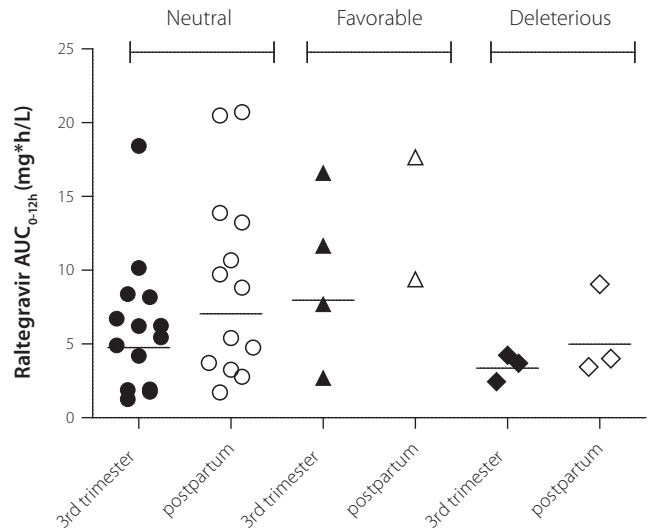

Figure 4 Individual and geometric mean exposure ($AUC_{0\text{-}12h}$) to raltegravir in HIV-infected women per drug-drug interaction subgroup in the third trimester compared with postpartum.

Table 4 Geometric mean AUC_{0-12h} of raltegravir in HIV-infected pregnant women in the third trimester of pregnancy compared with postpartum for all patients and per drug-drug interaction subgroup.

Group	3rd trimester raltegravir AUC_{0-12h} (mg·h/L)		postpartum raltegravir AUC_{0-12h} (mg·h/L)	
	n	GM (95% CI)	n	GM (95% CI)
All patients	21	5.00 (3.56-7.01)	18	7.11 (4.91-10.3)
Neutral	14	4.76 (3.05-7.43)	13	7.04 (4.36-11.4)
Favorable	4	7.96 (2.28-27.9)	2	12.9
Deleterious	3	3.37 (1.66-6.86)	3	4.99 (1.37-18.2)

Abbreviations: AUC, area under the curve; CI, confidence interval; GM, Geometric Mean.

The geometric mean AUC is higher in the favorable subgroup en lower in the deleterious subgroup compared with the neutral subgroup (Table 4). However due to the large variability in AUC_{0-12h} within each subgroup this difference is not significant. An important limitation of presenting the data like this, is the limited number of patients in the subgroup favorable and deleterious (2 to 4 subjects). Another limitation worth mentioning is that the patients were assigned to a certain subgroup in a rather straightforward way. We were not able to take into account for example the timing of the intake of the interacting agents on the day of pharmacokinetic assessment.

Nonetheless, we can conclude that drug-drug interactions have contributed to the variation seen between the HIV-infected women but the relative contribution is unknown. More importantly the mean exposure is lower in third trimester compared to postpartum in all subgroups resulting in a ratio < 1.0 of the exposure in third trimester versus postpartum. All potential drug-interacting agents were used during both pharmacokinetic assessments, limiting its influence on the comparison between the exposure in the third trimester versus postpartum. These data however do suggest that HIV-infected women during the third trimester could be more at risk for suboptimal exposure when interacting agents (such as magnesium) are used that could potentially further decrease raltegravir exposure.

pH-dependent solubility and formulation

Among the numerous plasma concentration-time profiles of raltegravir collected in our studies we have encountered a number of unusual pharmacokinetic profiles that lacked a sharp absorption peak or had a delayed (up to 12 hours after intake) sometimes blunted absorption peak which was not seen in the second pharmacokinetic profiles of these subjects, nor related to a certain treatment period. Incidental poor or altered absorption

of raltegravir in this extent is reported by others as well and evidently contributes to the high variability between and within subjects.[12] Although these were infrequent occurrences of an extreme delayed or altered absorption profile, this experience has led to our hypothesis that variation in the extent of absorption of raltegravir may be one of the key factors that contributes to the high variability in raltegravir pharmacokinetics.

The extent of absorption from the gastrointestinal tract can be influenced by the dissolution rate of the tablet as well as the solubility and permeability of the drug. Important physiological factors that influence absorption are gastrointestinal pH and transit time in the gastrointestinal tract.[31] Both factors are capable of causing intrasubject variability in pharmacokinetics *in vivo*.[28] It is known that the pharmacokinetics of raltegravir is affected by acid-reducing agents such as omeprazole and famotidine because raltegravir solubility (and as a result its oral bioavailability) is improved at higher gastrointestinal pH.[11,32] High-fat food has been shown to increase raltegravir exposure as well, which may be attributable to improved solubility, but could as well be a result of longer transit time and hence more time for absorption.[17,28] Raltegravir pharmacokinetic profiles occasionally contain multiple peaks. It has been suggested that raltegravir is able to undergo enterohepatic recirculation via conversion of raltegravir glucuronide metabolite back to the parent form in the intestine which could lead to reabsorption. However, a study in healthy volunteers using radiolabelled raltegravir could not confirm this theory.[28,33,34] Magnesium and other divalent ion metals negatively influence raltegravir absorption by binding to raltegravir and reducing its permeability.[27,29]

Moss et al developed a population-based *in vitro-in vivo* extrapolation model (IVEVE) to investigate the effects of gastrointestinal pH on raltegravir pharmacokinetics.[28] IVEVE is the process of using *in vitro* physiochemical, permeability, and metabolic parameters to predict *in vivo* drug characteristics using pharmacokinetic simulation modelling. In simulated subjects, increased gastrointestinal pH and transit time in the small intestin were both associated with improved raltegravir exposure (increased AUC, C_{max} and C_{12h}). Whereas the presence of magnesium in the lumen significantly decreases raltegravir AUC, C_{max} and C_{12h}. Furthermore the model demonstrates preferential absorption of raltegravir from particular sections of the gastrointestinal tract due to local pH differences. This together with tablet disintegration rate, which has been shown to increase in higher pH solutions *in vitro*, may help explain variability and unusual raltegravir pharmacokinetic profiles. The used model has its limitations and its predictability can be improved for example by including the impact of drug transporters.

The solubility and permeability of a drug is determined by physicochemical characteristics, such as pKa and aqueous/lipid solubility. The biopharmaceutics classification system (BCS) has four categories depending on its aqueous solubility and intestinal permeability:

1, high solubility-high permeability drugs; 2, low solubility-high permeability drugs; 3, high solubility-low permeability drugs; and 4, low solubility-low permeability drugs.[35] Raltegravir poloxamer tablets 400 mg are categorized as BCS 4 which means that raltegravir as an active pharmaceutical ingredient in this formulation is poorly soluble and poorly permeable.[36] Raltegravir ethylcellulose chewable tablet, which is one of the pediatric formulations (**Chapter 8**) is categorized as BCS 2: low solubility-high permeability drugs.[36]

Theoretically, it is expected that the influence of the formulation on pharmacokinetics will be largest for drugs with low solubility.[31] The formulation of the 400 mg tablet is standard and based on a matrix of microcrystalline cellulose, lactose monohydrate, calcium phosphate dibasic, hypromellose and poloxamer 407. The excipients were selected to provide a tablet that would erode rather than disintegrate.[11] Poloxamer enhances solubilisation of poorly water-soluble drugs. Both pediatric formulations (raltegravir chewable tablet and granules for suspension) exhibited lower variability than the adult tablet and give a different pharmacokinetic profile compared to the poloxamer tablets. The pediatric formulations are not bioequivalent to the adult tablets.[37,38] This shows that a different formulation of raltegravir may positively improve the variability in drug absorption.

Cattaneo et al investigated whether the observed wide inter- and intrapatient distribution of raltegravir plasma concentrations is related to the release of the drug from its pharmaceutical formulation.[39] HIV-infected patients receiving raltegravir by chewing the poloxamer tablet showed higher drug absorption and reduced pharmacokinetic variability compared with patients swallowing the intact tablet. The authors conclude that this difference in absorption and variability is related to tablet disintegration and drug absorption. Improving the pharmaceutical formulation could improve raltegravir pharmacokinetics. However, new conflicting data on the pharmacokinetics chewed versus swallowing of raltegravir (n=1) suggest otherwise.[40]

The possibility that the large intrasubject variability in raltegravir pharmacokinetics may be related to the pharmaceutical formulation and to poor solubility and permeability is an interesting hypothesis that deserves further investigation. In a recent publication by Merck the discovery of the compound MK-8970 is described. This is an optimized acetal carbonate prodrug of raltegravir with enhanced colonic absorption and suitable physiochemical properties to support the development of a special formulation for once-daily dosing.[41]

One of the physiological changes that can occur during pregnancy and could theoretically alter raltegravir drug absorption is an increase in gastric pH, as well as a reduction in gastrointestinal motility. However there is little data documenting that any of these changes significantly alter drug absorption in general.[42] Raltegravir solubility and oral

absorption is improved at higher gastrointestinal pH. Therefore the absorption and exposure of raltegravir may be increased during pregnancy. We did not observe this as an overall effect in our study in HIV-infected pregnant women as we measured a mean decrease in exposure instead of an increase (**Chapter 6**). Nonetheless, because gastrointestinal pH is one of the factors that contributes to the variability in raltegravir pharmacokinetics in nonpregnant HIV-infected patients, it is likely to have contributed to the variability seen within our pregnant population as well.

The effect of food

Although it is recommended in the product label that raltegravir can be taken with or without food, it is known that food influences the pharmacokinetics of raltegravir and increases pharmacokinetic variability relative to fasting.[16,17] In particular the variability in C_{12h} is increased with reported CV% up to 4-fold higher when taken with a meal compared with the fasted state.[17] The fat content of meals is believed to create variability in absorption of raltegravir with a higher time to reach the maximum plasma concentration. Nonetheless, it is difficult to make solid recommendations regarding dosing with a specific type of food as different meal types seems to have varying effects on the pharmacokinetics of raltegravir. It is not known whether this effect is related to the content of the meal, or the change in transit time or gastric pH, or a combination of these factors. Because none of these effects appear to be clinically relevant, raltegravir can be taken without regard to food in the clinical setting, which is more convenient in daily practice and generally improves adherence. However when evaluating and interpreting raltegravir pharmacokinetic data for research purposes, it is important to take into account the effect of food.

To minimize the variability in pharmacokinetics caused by food, raltegravir was taken on an empty stomach on the pharmacokinetic sampling days in our drug-drug interaction trials. Due to practical reasons an exception was made for the study with boceprevir (OPAL, **Chapter 4**) as boceprevir is to be taken with food to increase its absorption and oral bioavailability.[43] In Table 3 is shown that the absorption peak occurs later (t_{max} is 4 hours) in the subjects from the OPAL study who took raltegravir as a single dose with breakfast compared with the subjects in the GINGER study who took raltegravir on an empty stomach (t_{max} is 2 hours). The geometric mean C_{12h} level in the OPAL study was higher compared with the C_{12h} in the GINGER study with a considerable higher CV%: 0.093 mg/L and 368% in OPAL, 0.036 mg/L and 121% in GINGER.

In these crossover pharmacokinetic studies with raltegravir the standardization of the breakfast is far more important than the actual content of the breakfast. Standardization implies that all the subjects must eat the same type of breakfast at exactly the same time during or after intake of the study medication on each pharmacokinetic sampling day. However the variability in absorption profile in healthy subjects under the same

standardized feeding conditions (as illustrated in Table 2), indicates that factors other than food intake are equally or even more important contributors to the pharmacokinetic variability.

Pharmacokinetics/pharmacodynamics

The patient's exposure to drug is a crucial determinant of the drug's actions, and therefore its efficacy and safety. Knowledge on the relationship between pharmacokinetics and pharmacodynamics (PK/PD relationship) is important to be able to interpret (changes in) the pharmacokinetics of raltegravir in terms of clinical relevance.

Raltegravir *in vitro* antiretroviral activity, defined as a 95% inhibitory concentration (IC_{95}) is 33 nM (= 0.015 mg/L) in the presence of 50% human serum. A proof of concept 10-day placebo-controlled monotherapy study (protocol 004, part I) plus dose-finding study was performed in a small group of treatment-naive patients using 100 mg (n=7), 200 mg (n=7), 400 mg (n=6), or 600 mg (n=8) raltegravir twice daily or placebo (n=7).[44] The results showed superior virological response rates in all raltegravir treatment arms compared with placebo, regardless of the raltegravir dosage used and the fact that more patients in the lower dose groups (100 and 200 mg) had minimum plasma concentrations below the IC_{95} compared with the higher dose groups (400 and 600 mg). This observation was important in the discussion whether C_{12h} was the most likely pharmacokinetic driver to predict viral response as seen with other antiretroviral agents. The exposure of the lower limit of clinical experience with 100 mg twice daily was approximately 60% lower (GMR of 0.4) than the C_{12h} observed in the recommended dose of 400 mg twice daily. Because they considered 100 mg twice daily an efficacious dose a decrease in plasma exposure of up to 60% was considered unlikely to affect efficacy. Note that this assumption was based on very small subsets of subjects (6 to 8) which is not uncommon for proof-of-concept studies. Given the high variability in raltegravir pharmacokinetics these conclusions had to be interpreted with extreme caution.[15,18]

In the PK/PD analysis of two phase III studies in treatment-experienced patients with 48-week efficacy data, the geometric mean of all sparse raltegravir plasma concentrations (C_{all}) for a given subject regardless of the time of collection showed a weak but consistent significant positive correlation with efficacy measurements.[45] This association was less with C_{min} (the lowest measured plasma concentration) and not observed with C_{12h} (geometric mean of raltegravir plasma concentrations collected 11-13 hours post dose). Baseline HIV RNA load and the presence of other active antiretroviral agents in the regimen were the most important predictors for antiretroviral response. The interpretation of C_{all} is not clear but is probably best defined as the overall exposure within the dose interval (0 to12 hours).[34] This was supported by the results of an *in vitro* hollow-fiber model showing that raltegravir AUC was best linked with virological response.[46] These data has lead to the hypothesis that in contrast with other antiretroviral agents not raltegravir C_{12h} is the pharmacokinetic parameter best related to the efficacy but the overall exposure to raltegravir ($AUC_{0\text{-}12h}$), although a threshold for AUC was not determined.[45]

The pharmacokinetic parameter AUC is compared with C_{12h} a very impractical parameter to monitor in HIV-infected patients in the clinical setting because it requires 12-hour multiple blood sampling. To limit patient burden and practical issues an alternative limited sampling strategy was developed by our research group in which an abbreviated AUC from 0 to 3 hours ($AUC_{0\text{-}3h}$) is calculated and extrapolated to $AUC_{0\text{-}12h}$.[47] A similar model and strategy for raltegravir is well described by Cattaneo et al.[48] We used this limited plasma sampling method to estimate the exposure to raltegravir in HIV-infected patients with advanced stage non-Hodgkin lymphoma as described in **Chapter 7**. Its value for TDM purposes of raltegravir will be discussed in the section on TDM.

Raltegravir was well tolerated with no reported dose-related toxicity and no acute safety findings related to peak plasma concentrations. For many drugs the overall exposure (AUC) and C_{max} are the pharmacokinetic parameters most likely to be associated with toxicity. When Merck applied raltegravir as new drug, the upper bound of broad clinical experience was used to define the highest value of AUC that was achieved without being associated with an increased risk of clinically relevant changes in safety and tolerability. The highest value of AUC achieved in phase II studies was in patients (n=51) taking 600 mg twice daily in combination with atazanavir and/or tenofovir leading to approximately a 2-fold increase in AUC compared to the registered 400 mg twice daily dosing. These data supported that a two-fold increase in exposure is not likely to be clinically relevant.[15,18]

When raltegravir was introduced to the market, the results from the PK/PD analyses of phase III studies, as well as the lack of a clear PK/PD relationship in the phase II dose-ranging studies as described above, suggested that C_{12h} might not be related to the clinical outcome. The lack of an observed relationship between C_{12h} and efficacy had to be interpreted with caution because it could have been due to high intrapatient variability leading to a poor prediction of the true exposure. Another explanation could be the high potency of the approved dosage regimen of raltegravir 400 mg twice daily. The C_{12h} levels in the approved dosage might already be well above the IC_{95} and on top of the C_{12h}-virological success curve. These issues were addressed by the European Medicines Agency (EMA) and the Food and Drug Administration (FDA) in the assessment reports with the recommendation to further explore the importance of raltegravir exposure to virological outcome measurements post-approval.[15,49]

It wasn't until the pharmacokinetic data of the QDMRK study were published in 2012 that the trough level is considered the most important parameter to evaluate with respect to raltegravir's virological efficacy.[50] Although no clear relationship between pharmacokinetic parameters and the efficacy of raltegravir could be established for the registered twice daily dose regimen, failure to achieve an HIV RNA load <50 copies/mL appeared predominantly at high baseline HIV RNA load and was associated with lower values of trough levels in the 800 mg once-daily arm with a suggested threshold of 0.020 mg/L which is just above the IC_{95} (0.015 mg/L).[50]

The variability in raltegravir pharmacokinetics has complicated the search for the best pharmacokinetic driver for clinical response. And even today the PK/PD relationship of raltegravir is not fully elucidated. Generally one can conclude that the therapeutic range of raltegravir when dosed twice daily is considered to be large. The initial assumption that up to a two-fold increase and up to a 60% decrease in exposure (AUC) of raltegravir was not likely to be clinically relevant might have been a bit premature. Nonetheless, these have remained roughly the extreme boundaries of clinical relevance although the focus in the interpretation lies predominantly on a potential decrease in AUC and C_{12h} and its potential effect on virological efficacy instead of a concern for safety issues with an increase in exposure. This is also seen in the interpretation of the drug-drug interactions with raltegravir in the overview in Table 1 with a shift towards a focus on C_{12h} in the more recent studies.

Therapeutic drug monitoring

General and practical aspects

Therapeutic drug monitoring (TDM) of antiretroviral therapy in HIV infection by measuring plasma concentrations of antiretroviral agents may be used as a means to optimize and individualize antiretroviral treatment response.[51-55] Especially certain patient groups who are at increased risk for pharmacokinetic variability resulting in potential low or elevated plasma concentrations could benefit from TDM.

TDM may also be a useful tool to manage drug-drug interactions of antiretroviral agents as well as monitor adherence issues, both of which are important issues for optimal treatment response.

The following criteria are generally used to evaluate the value of routine TDM to optimize treatment response[56]:

- correlation between drug concentration and clinical response
- correlation between drug concentration and toxicity
- the drug must have a narrow therapeutic index
- there is large interpatient variation in pharmacokinetics
- low intrapatient variation at steady-state concentrations
- the effect of the drug must be difficult to assess clinically
- dose adjustment is possible in the case of a too low or high drug concentration
- an accurate bioanalytical assay to analyse drug concentrations

The product labelling of raltegravir and treatment guidelines do not recommend to perform routine TDM of raltegravir. When using the criteria to evaluate the value of TDM of raltegravir, one can conclude that raltegravir is indeed not an ideal candidate for TDM. Because of high intrasubject variability, TDM might not be considered useful in the general HIV-infected population on a raltegravir-based regimen as significant changes in raltegravir plasma concentrations among different sampling times may not reflect, for example, poor adherence or changes in pharmacokinetics due to drug-drug interactions.

The absence of a clear relationship between pharmacokinetic parameters and the efficacy of raltegravir in the standard 400 mg twice daily regimen has further complicated the use of TDM. However, the QDMRK study revealed in 2012 a greater risk of treatment failure observed with a once-daily raltegravir regimen compared with a twice-daily regimen. This suggests that drug exposure remains a critical determinant of the efficacy of raltegravir.[50] Although a robust threshold for virological efficacy could not be found, patients with raltegravir C_{12h} levels <0.020 mg/L might be more at risk for treatment failure. Nowadays, when interpreting the plasma concentrations of raltegravir for TDM purposes the 0.020 mg/L threshold is being used. No association between exposure to raltegravir and toxicity has been found when used in the recommended dosage. Therefore the main reason to perform TDM of raltegravir is when there is a concern for suboptimal plasma exposure. In that case we advise to collect a trough sample which is ideally taken just before the next dose but should be taken at least 8 hours post dose.

Due to practical reasons blood sampling for raltegravir TDM is often performed before the morning dose. In our experience with the pharmacokinetic drug-drug interaction studies with raltegravir, we have observed that the mean predose plasma concentration in the morning is higher than the C_{12h} level taken in the evening 12 hours after intake. This difference (2- to 5-fold), first reported by Neely et al, would suggest a circadian rhythm to raltegravir pharmacokinetics and is most likely explained by a difference in absorption profiles due to the intake of raltegravir with or without a (high-fat) meal in the morning versus the evening.[24,57] When interpreting C_{trough} levels for TDM we take into account the 0.020 mg/L threshold regardless of the sampling time. However, it is important to realize that the reference mean C_{trough} values obtained from the literature might vary because of differences in the time of collection of the blood samples (morning versus evening) and whether that particular dose was taken with or without food.

Special patient populations

Despite the fact that use of TDM is not believed to be useful in the general HIV-infected population, here a few examples are given when TDM can be of value in special patient populations in clinical practice.

In pregnant HIV-infected women, in whom physiological changes may lead to altered pharmacokinetics, it is difficult to predict whether this might lead to suboptimal drug exposure and the need for dose adjustment. The variability in pharmacokinetics as a result of pregnancy support the use of TDM in this special patient population.[58] However the research we have performed on the pharmacokinetics, efficacy, and safety of raltegravir in pregnant HIV-infected women (**Chapter 6**) is reassuring with respect to the changes in exposure to raltegravir in the third trimester. We have concluded that dose adjustment is not necessary during pregnancy which also argues against routine TDM of raltegravir. However, we did illustrate that additional factors, such as the use of interacting agents, may contribute to a further decrease and potentially suboptimal exposure to raltegravir during the third trimester. In these individual pregnant HIV-infected patients who are more at risk for suboptimal drug exposure, TDM of raltegravir could be of value.

In **Chapter 7** a small case series is presented of three HIV-infected patients with non-Hodgkin lymphoma at risk for drug-drug interactions and impaired absorption of raltegravir, in whom we performed TDM by means of a limited sampling strategy. Pharmacokinetic sampling was performed at steady-state conditions from predose up to 3 hours after intake of raltegravir 400 mg on an empty stomach. The calculated abbreviated $AUC_{0\text{-}3h}$ was subsequently extrapolated to $AUC_{0\text{-}12h}$ which is the pharmacokinetic parameter that best represents the overall absorption and systemic exposure to raltegravir. The results suggest that chemotherapy-induced intestinal mucositis may influence the absorption and total exposure to raltegravir. Although in our case it did not seem to have clinically relevant consequences.

We have encountered delayed absorption (no absorption peak <3 hours after intake of raltegravir) which, given the limited sampling time, probably has led to an underestimation of the true exposure when extrapolated to 12 hours. Unfortunately it is not possible to distinguish whether this is due to intake with breakfast (as opposed to fasted), normal variability in drug absorption or indeed impaired absorption due to severe diarrhoea. Although this strategy provides more insight into the pharmacokinetics of raltegravir than a single trough level, the patient burden and costs probably do not outweigh the benefits in most patients.

HIV treatment of children, especially in young children, often lags behind that of adults with limited availability of age-appropriate formulations suitable for a wide dosage range.

Even if a pediatric formulation is available, developmental changes occurring with age could alter the pharmacokinetics of antiretroviral agents in children with large interindividual variation in pharmacokinetics as a result. [51,59-61] In **Chapter 8** we described the successful application of TDM in a 4-year-old HIV-infected patient who used raltegravir chewable tablets conform prescription guidelines and repeatedly had trough levels below the IC_{95} of raltegravir (0.015 mg/L). To ensure adequate raltegravir trough levels the patient switched from a twice-daily dosage to an off-label three-times daily dosage. Although this case illustrates the value of TDM to optimize individual raltegravir treatment, it does not advocate the application of routine TDM of raltegravir in all pediatric patients. First of all, we have reported only one case and do not know whether our dose adjustment has had a relevant effect on the treatment response. Furthermore, the raltegravir drug assay is not widely available which limits its application in routine pediatric HIV care. In fact, TDM of antiretroviral agents is probably nowhere as accessible as currently in the Netherlands. Nonetheless, we do believe that TDM (if available) is a useful tool in the pediatric HIV-infected population to monitor raltegravir exposure.

Conclusions and closing remarks

The best strategy for long-term control of HIV infection is the use of combinations of potent antiretroviral agents from different drug classes that target different steps of the viral life cycle. The accelerated approval of raltegravir in 2007 by the FDA and EMA was highly anticipated as it introduced us to the first of a new class of antiretroviral agents called the HIV-1 integrase inhibitors. From 2007 and onwards the knowledge of the clinical pharmacology of raltegravir has greatly improved. The research in this thesis has contributed to that knowledge and to a better understanding of its drug interaction potential and the pharmacokinetics of raltegravir in (special) HIV-infected patients.

Despite the extensive pharmacokinetic research that has been done with raltegravir, the variability in the pharmacokinetics has never been truly unravelled. The large intra- and intersubject variability in raltegravir pharmacokinetics has complicated the investigation to establish a dose-response relationship and the development of a population PK model. It also contributed to difficulties in assessing the relevance of pharmacokinetic data obtained by sparse sampling at single or minimal times.

The lack of knowledge on the PK/PD relationship of raltegravir in the drug development phase, as well as post-approval, has clearly made the prediction of the pharmacokinetics in dose-finding studies and the interpretation in terms of response and clinical relevance more difficult. The disappointing results of the QDMRK study, which evaluated 800 mg raltegravir once daily, might have been prevented if the pharmacokinetics of raltegravir

would have been better predicted and interpreted. We believe that the pharmaceutical formulation of raltegravir poloxamer tablets and its poor solubility and permeability is an important contributor to the inter- and intrasubject variability in raltegravir pharmacokinetics. The development of a pharmaceutical formulation with raltegravir with a more predictable absorption profile should have deserved more attention in the biopharmaceutical stage of drug development. The question is whether this would have severely delayed the development of raltegravir as a new compound. From a clinical perspective, raltegravir has been and still is a very potent and safe antiretroviral drug despite its variable and unpredictable absorption.

Effective study design in early drug development that incorporates both pharmacokinetics and pharmacodynamic properties can help to elucidate the PK/PD relationship and better understand the mechanism of drug action and pharmacokinetic characteristics of the compound for further improvement. Optimal use of PK/PD modelling relies on the continuous integration of new relevant data throughout the different stages of drug development and enables crucial decisions to be reached earlier.[62,63] This has led to a new strategy within drug development that has a more PK/PD-guided approach. An important condition would be the development of a pharmaceutical formulation that delivers predictable and consistent pharmacokinetic profiles.

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APPENDIX

Summary

Infection with human immunodeficiency virus (HIV) is one of the world's most significant public health challenges. Currently, 35 million people are living with HIV worldwide and the HIV population is still growing. HIV destroys and impairs the function of immune cells in the body. The most advanced stage of HIV infection is Acquired Immunodeficiency Syndrome (AIDS). AIDS is one of the top causes of infectious disease-related mortality worldwide.

During the past 30 years there has been a remarkable progress in the treatment of HIV infection. Antiretroviral therapy prevents HIV from multiplying and reduces the level of HIV in the body. Suppressing and controlling viral replication protects the immune system, prevents HIV infection from advancing to AIDS, and reduces the risk of HIV transmission. The best strategy for long-term control of HIV infection is the use of combinations of potent antiretroviral agents from different drug classes that target different steps of the viral life cycle. HIV treatment nowadays consists of at least three antiretroviral agents from two different drug classes.

Raltegravir was the first of a new class of antiretroviral agents called HIV integrase inhibitors. Immediately after the accelerated approval of raltegravir in 2007 it played an important role in HIV management. Initially raltegravir was licensed in combination with other antiretroviral agents for treatment-experienced patients with evidence of HIV replication despite ongoing antiretroviral therapy. The good safety profile and its potent and rapid antiretroviral activity has quickly extended the use of raltegravir to include first-line treatment of HIV-infected patients. Raltegravir is to be administered orally in a dosage of 400 mg twice daily.

Clinical pharmacology is the scientific discipline that studies the relationship between drugs and humans in order to improve the efficacy and safety of drugs in patients. It includes two closely associated pharmacological principles: pharmacokinetics and pharmacodynamics. Pharmacokinetics is what the body does to the drug. The pharmacokinetic characteristics of a drug comprises the absorption, distribution, metabolism, and elimination of the drug. Pharmacodynamics is what the drug does to the body, in other words the drug's actions in terms of efficacy and toxicity/safety. The relationship between pharmacokinetics and pharmacodynamics is important because the patient's exposure to the drug is a crucial determinant of the drug's actions.

It is widely acknowledged that better understanding of the clinical pharmacology of antiretroviral drugs is essential for their safe and effective use in HIV-infected patients. HIV-infected patients take at least three antiretroviral agents but may also take a variety of

medication for other medical illnesses. This significantly enhances the risk of drug-drug interactions. Drug-drug interactions occur when a drug interacts, or interferes, with another drug. This can alter the pharmacokinetics of one or both of the drugs, and the way they act in the body. Drug-drug interactions, but also other factors that could change the pharmacokinetics of antiretroviral agents in patients, are an important cause for suboptimal drug exposure or increased drug levels. This could potentially lead to treatment failure or toxicity problems in HIV-infected patients.

Pharmacokinetic research of raltegravir is performed by measuring the plasma concentration of the drug in time under certain circumstances or in certain patient populations. The plasma concentration of antiretroviral agents can also be measured to optimize and individualize antiretroviral treatment as part of regular HIV patient care. This is called therapeutic drug monitoring (TDM). Especially patients who are at risk for potential low or elevated plasma concentrations could benefit from TDM.

The main pharmacokinetic parameter to evaluate in pharmacokinetic studies is the exposure to raltegravir expressed as the area under the plasma concentration versus time curve (AUC). From a clinical perspective it is also important to know the trough level of raltegravir as this is currently the most important pharmacokinetic parameter of raltegravir to evaluate with regard to virological efficacy. The trough level can be measured by taking a blood sample just prior to the next dose, in other words 12 hours after the last dose.

The general aim of this thesis was to study the clinical pharmacology of the HIV integrase inhibitor raltegravir to optimize its safe and effective use in HIV-infected patients in clinical practice. The first part of this thesis focuses on pharmacokinetic drug-drug interactions between raltegravir and other frequently used concomitant medication. The pharmacokinetics of raltegravir in pregnant HIV-infected women and the application of TDM of raltegravir in special patient populations were addressed in the second part of this thesis.

Part 1: Drug-drug interactions

HIV-infected patients are at increased risk of cardiovascular disease. Dyslipidemia is highly prevalent among patients with HIV infection and contributes to this increased cardiovascular risk. Statins are frequently being used as lipid-lowering therapy in this patient population by reducing plasma low-density lipoprotein (LDL) cholesterol levels. The concomitant use of statins and certain antiretroviral agents have led to clinically relevant pharmacokinetic drug-drug interactions with potentially severe toxicity as a result. **Chapter 2** describes the results of a pharmacokinetic drug-drug interaction study between raltegravir and atorvastatin in 24 healthy volunteers. The study was designed to investigate the effect of raltegravir 400 mg twice daily on the pharmacokinetics of atorvastatin 20 mg once daily and vice versa by intrasubject comparison. Geometric mean

ratios (GMRs) of the test treatment (combination raltegravir + atorvastatin) versus the reference treatment (raltegravir or atorvastatin alone) and 90% confidence intervals (CI) were calculated for the AUC. The GMR (90% CI) was 1.01 (0.68-1.51) for raltegravir $AUC_{0\text{-}12h}$. In other words the mean exposure to raltegravir was similar regardless of concomitant atorvastatin use, suggesting that atorvastatin 20 mg has no clinically relevant effect on the pharmacokinetics of raltegravir. However, the large confidence intervals indicate that there was quite some variability between subjects. The GMR (90%CI) was 1.00 (0.90-1.11) for atorvastatin $AUC_{0\text{-}24h}$. Fasting lipid profiles were obtained to assess the short-term lipid-lowering effect of atorvastatin when combined with raltegravir versus the use of atorvastatin alone. Raltegravir does not influence the pharmacokinetics of atorvastatin and has no effect on its short-term lipid-lowering effect. We have concluded that the combination of raltegravir and atorvastatin can be used without dose adjustments.

Another drug treatment that is frequently being used within the HIV population is antidepressive therapy. Depression is the most common mental health disorder among HIV-patients with a lifetime prevalence that is approximately 2-fold higher than among HIV-uninfected individuals. Depression is associated with an increased risk of treatment failure and viral resistance of antiretroviral agents due to adherence problems. Therefore treating depression with antidepressant therapy is important to improve health outcomes in those living with HIV. **Chapter 3** evaluates the two-way pharmacokinetic drug-drug interaction and tolerability of concomitant administration of citalopram, a selective serotonin reuptake inhibitor (SSRI) for the treatment of depression, and raltegravir in 24 healthy volunteers. Citalopram was given in a dosage of 20 mg once daily and raltegravir in a regular dosage of 400 mg twice daily. Raltegravir does not influence the pharmacokinetics of citalopram and its main metabolite desmethylcitalopram. The GMR (90% CI) was 1.00 (0.98-1.03) for citalopram $AUC_{0\text{-}24h}$ and 0.99 (0.88-1.12) for desmethylcitalopram $AUC_{0\text{-}24h}$. The citalopram metabolite-to-parent ratio, which is a measure for metabolic activity, did not appear to be affected by concomitant raltegravir use. The GMR of raltegravir $AUC_{0\text{-}12h}$ was 0.77 (0.50-1.19). The 23% decrease in raltegravir exposure (AUC) in combination with citalopram is not considered to be clinically important. Raltegravir C_{12h} did not change with concomitant use of citalopram with a GMR (90% CI) of 1.03 (0.71-1.50). This study shows that in HIV-infected patients with depression citalopram and raltegravir can be used without dose adjustment.

Of the 35 million people living with HIV worldwide, approximately 4 to 5 million are co-infected with hepatitis C virus (HCV). As a result many HIV-infected patients need to be treated for their HCV infection as well. Boceprevir was introduced in 2011 as a newly developed NS3 serine protease inhibitor for the treatment of chronic HCV genotype 1 infection. Unfortunately several drug combinations with boceprevir and commonly used antiretroviral agents for the treatment of HIV should be avoided or used with great caution

due to pharmacokinetic interactions. In order to recommend raltegravir as a preferred agent for combined HIV/HCV treatment with boceprevir, we performed a pharmacokinetic drug-drug interaction study between raltegravir and boceprevir (800 mg three times daily) in healthy volunteers as presented in **Chapter 4**. The GMR (90%CI) of raltegravir $AUC_{0\text{-}12h}$ for raltegravir with boceprevir versus raltegravir alone was 1.04 (0.88-1.22). Boceprevir plasma concentrations when coadministered with raltegravir were comparable with historical reference values of boceprevir in plasma. Due to the absence of a clinically significant drug-drug interaction, raltegravir can be recommended for combined HIV/HCV treatment including boceprevir.

Besides conventional medication, approximately 60% of the HIV-infected patients use alternative medicines. A popular herbal product used worldwide by HIV-infected patients is Ginkgo biloba extract. Ginkgo biloba is used for its claimed beneficial effects on concentration, memory and depressive disorders. Ginkgo biloba may influence the P-glycoprotein membrane transporter and UDP-glucuronosyltransferase (UGT) liver enzymes that play a role in the farmacokinetics of raltegravir. However, these studies show conflicting results and were mainly performed *in vitro*. We performed a herb-drug pharmacokinetic interaction study between Ginkgo biloba and raltegravir in healthy volunteers **(Chapter 5).** GMRs (90% CI) of the exposure (AUC) and maximum plasma concentration (C_{max}) of raltegravir with Ginkgo biloba versus raltegravir alone were, respectively, 1.21 (0.93-1.58) and 1.44 (1.03-2.02). Ginkgo biloba did not reduce the exposure to raltegravir. The potential increase in C_{max} of raltegravir is probably of minor importance given the large variability of raltegravir pharmacokinetics between subjects and its reported safety profile. There is no need to discourage the use of Ginkgo biloba in HIV-infected patients who take raltegravir.

Part 2: Pharmacokinetics in special patient populations

In **Chapter 6** we evaluated the effect of pregnancy on the pharmacokinetics of raltegravir and its safety, and efficacy in HIV-infected pregnant women. This study was performed within the European PANNA research network. HIV can be transmitted from the HIV-infected woman to her child during pregnancy, childbirth, or breastfeeding. Mother-to-child transmission of HIV is the most common route of HIV-infection among infants and children. To reduce the risk of HIV transmission to the (unborn) child it is important that the mother takes antiretroviral agents to reduce the amount of HIV particles in her body (HIV load).

Pregnancy may influence the pharmacokinetic profile of antiretroviral agents and lead to decreased drug exposure. Suboptimal drug exposure can result in treatment failure and an increased risk of HIV transmission to the child. Antiretroviral regimens including raltegravir were initially only recommended during pregnancy in special circumstances because information on the pharmacokinetics and the safety of raltegravir in pregnancy was limited.

We included 22 HIV-infected pregnant women on a raltegravir-based regimen in 10 different European hospitals. Pharmacokinetic assessment was performed in the third trimester of pregnancy and repeated approximately 4-6 weeks postpartum which we considered to be the normal nonpregnant reference situation. Our findings show an average decrease of 29% in the exposure (AUC) to raltegravir during the third trimester of pregnancy compared with postpartum. A similar effect of pregnancy on trough levels was observed leading to an average decrease of 36%. Approaching delivery 86% of the patients had an undetectable HIV load (<50 copies/mL). None of the children were HIV-infected and no birth defects were reported. In nine patients we were able to take blood samples together with umbilical cord blood samples after delivery. The ratio of the amount of raltegravir in the cord blood in relation to the mother blood gives information on the placental transfer of raltegravir. The median ratio was 1.21 (interquartile range 1.02-2.17) meaning that raltegravir readily crosses the placenta.

Raltegravir was well tolerated during pregnancy. Exposure to raltegravir was highly variable. The observed mean decrease in exposure is not considered to be of clinical importance. Our results support the use of raltegravir in standard dosages in HIV-infected pregnant women for HIV treatment and prevention of mother-to-child HIV transmission. The results of our study have been incorporated into the international guideline of the American Department of Health and Human Services (DHHS) for the use of antiretroviral drugs in pregnant HIV-infected patients. In the latest update of this guideline in August 2015 raltegravir has been promoted to one of the preferred agents for initial therapy during pregnancy.

The optimal antiretroviral treatment for HIV-infected patients with cancer and use of chemotherapy is complicated because of drug-drug interactions and overlapping toxicity. Although raltegravir is increasingly being recommended and used in clinical practice in HIV-infected patients with cancer, surprisingly little is known on the pharmacokinetics of raltegravir in this setting. In **Chapter 7** we describe a short case series of three HIV-infected patients with advanced stage non-Hodgkin lymphoma who used chemotherapy in combination with a raltegravir-based antiretroviral regimen. We used a limited blood sampling strategy to estimate the total exposure ($AUC_{0\text{-}12h}$) to raltegravir. The estimated total exposure to raltegravir in these patients was on average lower compared to population reference values. However the extent of this reduction was not considered to be of clinical importance. Raltegravir trough plasma concentrations, which is the most important parameter with respect to virological efficacy, was adequate (>0.020 mg/L) in all three patients when using the standard dosage of 400 mg raltegravir twice daily. Severe diarrhoea caused by intestinal chemotherapy-induced mucositis may negatively influence the extent of absorption of raltegravir. In one patient we observed a decrease in absorption of raltegravir compared to baseline levels after developing severe diarrhea. In these circumstances TDM could be used to monitor the treatment of raltegravir.

Raltegravir is the first HIV integrase inhibitor approved by the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) for treatment of HIV infection in the pediatric population. Raltegravir is available as film-coated tablet (400 mg raltegravir) and recently also in two special formulations suitable for infants and young children who are unable to swallow whole tablets; chewable tablets (25 and 100 mg) and granules which are administered as oral suspension (20 mg/mL). There is little experience in the use of raltegravir in children and the knowledge of the pharmacokinetics of raltegravir in particularly young children is still limited. The pediatric HIV population is at risk for variability in antiretroviral drug exposure due a number of developmental changes in children that could alter the pharmacokinetics of drugs. To optimize antiretroviral treatment in this special patient population TDM could be a useful tool. In this context, we describe in **Chapter 8** our experience with dose optimization of raltegravir chewable tablets in a 4-year-old HIV-infected patient based on TDM of raltegravir. Despite following the approved weight-based dose recommendations for raltegravir chewable tablets, we repeatedly measured very low raltegravir trough levels (<0.015 mg/L). The patient switched from a twice daily dose regimen to an off-label three times daily regimen. By shortening the dose interval we could successfully increase the raltegravir trough levels in this young patient to acceptable values. At the age of 7 he was able to switch back to a normal twice daily dosage of raltegravir chewable tablets.

In **Chapter 8** we reviewed the available literature and information on the pharmacokinetics of raltegravir in children which has resulted in the approved dose recommendations for the use of raltegravir in children ≥4 week to <18 years. The lowest mean raltegravir trough level compared to other age groups and reference values in adults was found in a small cohort of children from ≥2 to <6 years who used the chewable tablets. We are concerned that some individual young patients who use chewable tablets might experience suboptimal trough levels in clinical practice as illustrated by our case. This could especially be problematic in children who have a high HIV load before starting a raltegravir-based regimen. In these patients TDM could be a useful tool to monitor and if necessary adjust the raltegravir dose regimen.

General discussion

The best strategy for long-term control of HIV infection is the use of combinations of potent antiretroviral agents that target different steps of the viral life cycle. The accelerated approval of raltegravir in 2007 by the FDA and EMA was highly anticipated as it introduced us to the first of a new class of antiretroviral agents called the HIV integrase inhibitors.

Since raltegravir was introduced to the market the knowledge about the clinical pharmacology of raltegravir has greatly improved. The research in this thesis has contributed to that knowledge and to a better understanding of its drug interaction

potential and the pharmacokinetics of raltegravir in (special) HIV-infected patients. In the general discussion the results of this thesis are discussed focussing on the following main topics: pharmacokinetic drug-drug interaction studies, the variability in the pharmacokinetics of raltegravir and potential influencing factors, the relationship between the pharmacokinetics and pharmacodynamics of raltegravir, and the role of TDM in clinical practice.

Despite the extensive pharmacokinetic research that has been done with raltegravir, the variability in the pharmacokinetics has never been truly unravelled. This has complicated the investigation to establish a clear dose-response relationship and the development of a population pharmacokinetic model. We believe that the pharmaceutical formulation of raltegravir poloxamer tablets and its poor solubility and permeability is an important contributor to the inter- and intrasubject variability in raltegravir pharmacokinetics.

Samenvatting

Infectie met het humaan immunodeficiëntie virus (hiv) is één van de grootste uitdagingen voor de wereldgezondheid. Momenteel zijn er 35 miljoen mensen wereldwijd geïnfecteerd met hiv en dit aantal groeit nog steeds. Hiv vernietigt en schaadt de functie van immuuncellen in het lichaam. Het meest vergevorderde stadium van hiv-infectie is acquired immunodeficiency syndrome (aids). Aids behoort tot de belangrijkste oorzaken van infectieuze ziektegerelateerde sterfte wereldwijd.

De afgelopen 30 jaar is er een enorme vooruitgang geboekt bij de behandeling van hiv. Antiretrovirale therapie voorkomt dat hiv zich vermenigvuldigt waardoor het aantal hiv-deeltjes in het lichaam vermindert. Het onderdrukken van deze virale replicatie beschermt het immuunsysteem, voorkomt de progressie naar aids en vermindert de kans op overdracht van hiv. De beste strategie voor langdurige onderdrukking van hiv is het combineren van meerdere hiv-remmers die op verschillende wijze aangrijpen op de levenscyclus van het virus. Tegenwoordig bestaat de behandeling voor hiv uit een combinatie van drie hiv-remmers uit twee verschillende geneesmiddelgroepen.

Raltegravir is de eerste hiv-remmer van een nieuwe groep geneesmiddelen genaamd integraseremmers. Direct nadat raltegravir versneld werd goedgekeurd door de registratie-autoriteiten eind 2007, nam raltegravir een belangrijke positie in binnen de behandeling van hiv. Aanvankelijk was raltegravir geregistreerd voor de behandeling van hiv bij volwassen patiënten bij wie hiv, ondanks uitgebreide behandeling, niet onder controle was. Omdat raltegravir een snelle en krachtige hiv-remmer bleek te zijn met een gunstig bijwerkingenprofiel, werd de toepassing van raltegravir vervolgens uitgebreid naar een eerstelijns behandeling. Raltegravir tabletten worden oraal ingenomen in een dosering van tweemaal daags 400 mg.

Klinische farmacologie is de wetenschappelijke discipline die de relatie tussen geneesmiddelen en mensen bestudeert om de werkzaamheid en veiligheid van geneesmiddelen bij patiënten te verbeteren. Het omvat twee aan elkaar gerelateerde farmacologische principes: farmacokinetiek en farmacodynamiek. Farmacokinetiek kan worden gedefinieerd als wat het lichaam doet met het geneesmiddel. De farmacokinetische eigenschappen van een geneesmiddel omvatten de absorptie, distributie, metabolisme en eliminatie van het geneesmiddel. Farmacodynamiek kan worden gezien als wat het geneesmiddel doet met het lichaam, dat wil zeggen het effect van het geneesmiddel in termen van werkzaamheid, toxiciteit en veiligheid. De relatie tussen farmacokinetiek en farmacodynamiek is belangrijk omdat de blootstelling aan een geneesmiddel in het lichaam (farmacokinetiek) een bepalende factor is voor het effect van het geneesmiddel (farmacodynamiek).

Het is algemeen geaccepteerd dat kennis van de klinische farmacologie van antiretrovirale geneesmiddelen essentieel is voor een veilig en effectief gebruik van deze middelen bij hiv-geïnfecteerde patiënten. Hiv-patiënten gebruiken minimaal drie verschillende hiv-remmers maar moeten vaak ook geneesmiddelen gebruiken voor andere aandoeningen. Dit vergroot aanzienlijk de kans op geneesmiddelinteracties. Geneesmiddelinteracties treden op wanneer een geneesmiddel een wisselwerking heeft, of interfereert met een ander geneesmiddel. Dit kan invloed hebben op de farmacokinetiek van één of beide geneesmiddelen en het effect van het geneesmiddel. Interacties met geneesmiddelen, maar ook andere factoren die van invloed zijn op de farmacokinetiek van hiv-remmers zijn een belangrijke oorzaak van suboptimale blootstelling aan deze geneesmiddelen of verhoogde bloedspiegels van deze middelen. Dit kan vervolgens leiden tot het falen van de therapie of problemen met bijwerkingen bij hiv-patiënten.

Door op verschillende tijdstippen na inname van raltegravir en onder verschillende omstandigheden de concentratie van raltegravir in het bloedplasma te meten kun je meer te weten komen over de farmacokinetiek. De concentratie van raltegravir in het bloed kan ook worden gemeten in het kader van reguliere patiëntenzorg met als doel om de hiv-behandeling te optimaliseren van de betreffende patiënt. Het op deze wijze monitoren van de therapie wordt 'therapeutic drug monitoring' (TDM) genoemd. Vooral patiënten met een verhoogd risico op te lage of juist verhoogde bloedspiegels kunnen baat hebben bij TDM.

De belangrijkste parameter in het onderzoek naar de farmacokinetiek van raltegravir is de blootstelling aan raltegravir. De blootstelling wordt berekend door het oppervlak onder de plasmaconcentratie versus tijd curve te berekenen en wordt uitgedrukt als 'area under the curve' (AUC). Vanuit een klinisch perspectief is het ook belangrijk om de dalspiegel van raltegravir te weten aangezien dit de belangrijkste parameter blijkt te zijn in relatie tot de effectiviteit. De dalspiegel kan worden gemeten door het nemen van een bloedmonster net voor de volgende inname, dat wil zeggen 12 uur na de laatste inname.

Het belangrijkste doel van dit proefschrift is het bestuderen van de klinische farmacologie van de hiv integraseremmer raltegravir om de effectiviteit en de veiligheid van dit middel te verbeteren bij hiv-patiënten in de klinische praktijk. Het eerste deel van dit proefschrift beschrijft een viertal onderzoeken naar geneesmiddelinteracties tussen raltegravir en andere veelgebruikte geneesmiddelen. Het tweede deel van dit proefschrift gaat over de farmacokinetiek van raltegravir bij zwangere hiv-geïnfecteerde vrouwen en de toepassing van TDM van raltegravir bij speciale patiëntengroepen.

Deel 1: Geneesmiddelinteracties

Hiv-geïnfecteerde patiënten hebben een verhoogd risico op het krijgen van hart- en vaatziekten. Dyslipidemie, oftewel een verstoorde vetstofwisseling, komt veel voor bij hiv-patiënten en draagt in belangrijke mate bij aan dit verhoogde risico. Daarom gebruiken veel hiv-patienten lipidenverlagende geneesmiddelen zoals de groep geneesmiddelen genaamd statines. Statines verlagen het plasma low-density lipoprotein (LDL) cholesterol en kunnen de kans op hart- en vaatziekten verminderen. Er zijn echter klinisch relevante farmacokinetische interacties beschreven tussen statines en bepaalde hiv-remmers met ernstige bijwerkingen als gevolg. **Hoofdstuk 2** beschrijft de resultaten van ons onderzoek naar de wisselwerking tussen raltegravir en atorvastatine in 24 gezonde proefpersonen. We hebben het effect onderzocht van het gebruik van tweemaal daags 400 mg raltegravir op de farmacokinetiek van atorvastatine in een dosering van 20 mg eenmaal daags en andersom. Voor iedere deelnemer werden de plasmaconcentraties van de geneesmiddelen tijdens de test behandeling (combinatie raltegravir + atorvastatine) vergeleken met de waarden van de referentie behandeling (raltegravir of atorvastatine alleen). Het gemiddelde van deze verhouding en de spreiding hierin wordt weergegeven als Geometric Mean Ratio (GMR) met een 90% betrouwbaarheidsinterval (BI). De GMR (90% BI) was 1,01 (0,68-1,51) voor raltegravir $AUC_{0\text{-}12h}$. Dit betekent dat de gemiddelde blootstelling aan raltegravir niet werd beïnvloed door het gelijktijdig gebruik van atorvastatine. Het ruime betrouwbaarheidsinterval geeft weer dat er veel variatie is tussen de waarden binnen onze onderzoekspopulatie. De GMR (90% BI) was 1,00 (0,90-1,11) voor atorvastatine $AUC_{0\text{-}24h}$. We hebben tevens het lipidenprofiel gemeten van de deelnemers om te kijken of het lipidenverlagende effect van atorvastatine beïnvloed wordt door het gebruik van raltegravir. Dit bleek niet het geval te zijn. We concluderen dat de combinatie atorvastatine en raltegravir veilig gebruikt kan worden zonder dosisaanpassing.

Andere veel gebruikte geneesmiddelen bij hiv-positieve patiënten zijn geneesmiddelen tegen depressieve klachten. Depressie is namelijk de meest voorkomende psychische aandoening bij hiv-patiënten en komt ongeveer twee keer vaker voor dan bij mensen zonder hiv. Depressie wordt geassocieerd met een verhoogd risico op het falen van de hiv-behandeling en het ontwikkelen van resistentie tegen het virus omdat de hiv-remmers niet regelmatig genoeg worden ingenomen. Dit maakt dat een goede behandeling met antidepressiva de algehele gezondheidssituatie van mensen met hiv en een depressie sterk kan verbeteren. In **hoofdstuk 3** is het onderzoek beschreven naar de farmacokinetische interactie en de veiligheid van het gelijktijdige gebruik van citalopram, een selectieve serotonine heropname inhibitor (SSRI) voor de behandeling van depressie, en raltegravir bij 24 gezonde proefpersonen. Citalopram werd gegeven in een dosering van eenmaal daags 20 mg en raltegravir in een dosering van tweemaal daags 400 mg. Raltegravir beïnvloedt niet de farmacokinetiek van citalopram en zijn belangrijkste metaboliet desmethylcitalopram. De GMR (90% BI) was 1,00 (0,98-1,03) voor citalopram $AUC_{0\text{-}24h}$ en

0,99 (0,88-1,12) voor desmethylcitalopram AUC_{0-24h}. De gemiddelde verhouding tussen de metaboliet desmethylcitalopram ten opzichte van de moederstof citalopram is een maat voor de metabole activiteit en deze bleek niet te worden beïnvloed door raltegravir. De GMR (90% BI) was 0,77 (0,50-1,19) voor raltegravir AUC_{0-12h}. De gemiddelde daling van 23% in de blootstelling (AUC) aan raltegravir in combinatie met citalopram is klinisch niet relevant. De dalspiegel van raltegravir veranderde niet bij gelijktijdig gebruik van citalopram met een GMR (90% BI) van 1,03 (0,71-1,50). Dit onderzoek laat zien dat bij hiv-patiënten met een depressie gelijktijdig de geneesmiddelen raltegravir en citalopram gebruikt kunnen worden zonder dosisaanpassing.

Van de 35 miljoen mensen met hiv wereldwijd zijn ongeveer 4 tot 5 miljoen mensen ook geïnfecteerd met het hepatitis C virus (HCV). In de praktijk komt het dus regelmatig voor dat hiv-patiënten zowel hiv-remmers moeten gebruiken als antivirale geneesmiddelen tegen chronische HCV. In 2011 werd het direct werkende antivirale geneesmiddel boceprevir op de markt gebracht voor de behandeling van HCV. Helaas laat boceprevir zich niet goed combineren met verschillende hiv-remmers vanwege farmacokinetische interacties. Om bij een gecombineerde behandeling van hiv en HCV raltegravir te kunnen aanbevelen met boceprevir, hebben we een onderzoek uitgevoerd naar de wisselwerking tussen raltegravir en boceprevir (inname van driemaal daags 800 mg) in gezonde proefpersonen (**hoofdstuk 4)**. De GMR (90% BI) van raltegravir AUC_{0-12h} voor raltegravir met boceprevir versus raltegravir alleen was 1,04 (0,88-1,22). De gemeten plasmaconcentraties van boceprevir in combinatie met raltegravir waren vergelijkbaar met referentiewaarden van boceprevir in bloedplasma. We concluderen dat er geen farmacokinetische geneesmiddelinteractie is tussen raltegravir en boceprevir. Raltegravir is een goede keuze om te gebruiken als hiv-remmer wanneer patiënten tevens behandeld worden met boceprevir vanwege een chronische HCV infectie.

Naast reguliere medicatie gebruikt ongeveer 60% van de hiv-patiënten ook alternatieve medicatie. Een populair kruidenpreparaat dat wereldwijd door hiv-positieve patiënten wordt gebruikt is Ginkgo biloba extract. Ginkgo biloba wordt gebruikt omdat het gunstige effecten zou hebben op het concentratievermogen, het geheugen en depressiviteit. Uit een aantal onderzoeken blijkt dat Ginkgo biloba mogelijk de membraantransporter en leverenzymen beïnvloedt die een rol spelen bij de opname en afbraak van raltegravir. Maar de resultaten uit deze voornamelijk *in vitro* onderzoeken zijn tegenstrijdig en er zijn geen of nauwelijks onderzoeken gedaan naar dit effect in mensen. In **hoofdstuk 5** onderzochten we het effect van het gebruik van Ginkgo biloba extract gedurende 2 weken op de farmacokinetiek van een eenmalige dosering van 400 mg raltegravir bij 18 gezonde proefpersonen. De GMR (90% BI) van de blootstelling (AUC) en de maximale plasmaconcentratie van raltegravir met Ginkgo biloba versus raltegravir alleen waren respectievelijk 1,21 (0,93-1,58) en 1,44 (1,03-2,02). Ginkgo biloba zorgt in ieder geval niet

voor een te lage blootstelling aan raltegravir. De gemiddelde toename van de maximale plasmaconcentratie van raltegravir is klinisch niet relevant gezien de grote variabiliteit in de gemeten waarden en het goede veiligheidsprofiel van raltegravir. Het gebruik van Ginkgo biloba hoeft niet te worden ontraden bij hiv-patiënten die raltegravir gebruiken.

Deel 2: Farmacokinetiek bij speciale patiëntengroepen

In **hoofdstuk 6** wordt het effect van de zwangerschap op de farmacokinetiek van raltegravir en de veiligheid en werkzaamheid bij hiv-geïnfecteerde zwangere vrouwen beschreven. Dit onderzoek is tot stand gekomen door het Europese PANNA onderzoeksnetwerk. Een hiv-geïnfecteerde vrouw kan hiv overdragen aan haar kind tijdens de zwangerschap, bevalling of borstvoeding. Moeder-op-kind overdracht van hiv is verreweg de meest voorkomende oorzaak van hiv-infectie bij kinderen. Om het risico van overdracht van hiv aan het (ongeboren) kind te verminderen is het belangrijk dat de moeder hiv-remmers gebruikt om het aantal virusdeeltjes in haar lichaam te verminderen.

De fysiologische veranderingen tijdens de zwangerschap kunnen van invloed zijn op de farmacokinetiek van antiretrovirale middelen met als mogelijk gevolg een verminderde blootstelling aan het geneesmiddel. Suboptimale blootstelling kan vervolgens leiden tot therapiefalen en een verhoogd risico van moeder-op-kind transmissie van hiv. Omdat er weinig bekend was over de farmacokinetiek en veiligheid van raltegravir tijdens de zwangerschap werd in de richtlijnen het gebruik van raltegravir tijdens de zwangerschap aanvankelijk alleen aanbevolen onder speciale omstandigheden.

We hebben 22 hiv-geïnfecteerde zwangere vrouwen geïncludeerd die raltegravir gebruikten in 10 verschillende Europese ziekenhuizen. De hoeveelheid raltegravir in het bloed werd gemeten in het derde trimester van de zwangerschap en dit werd herhaald ongeveer 4-6 weken na de bevalling wat we beschouwden als de normale niet-zwangere situatie. Onze resultaten laten zien dat de bloostelling (AUC) aan raltegravir in het derde trimester van de zwangerschap gemiddeld 29% lager is dan de blootstelling aan raltegravir na de bevalling. We zagen een vergelijkbare daling van gemiddeld 36% in de dalspiegels van raltegravir tijdens de zwangerschap. Vlak voor de bevalling had 86% van de patiënten een niet detecteerbare virale lading (<50 kopieën/ml). De kinderen waren allemaal hiv-negatief en er zijn geen geboorteafwijkingen gerapporteerd. Bij negen patiënten konden we direct na de bevalling zowel bloed afnemen bij de moeder als uit de navelstreng. De verhouding van de hoeveelheid raltegravir in het navelstrengbloed ten opzichte van het bloed van de moeder geeft informatie over de placentapassage van raltegravir. De gemiddelde (mediane) verhouding was 1,21 wat betekent dat raltegravir gemakkelijk de placenta passeert.

Raltegravir werd goed verdragen tijdens de zwangerschap. Wel viel op dat de blootstelling aan raltegravir zeer variabel was. De waargenomen gemiddelde daling van de blootstelling wordt niet als klinisch relevant beschouwd. Onze gegevens ondersteunen het gebruik van raltegravir in standaard doseringen bij hiv-geïnfecteerde zwangere vrouwen voor de behandeling van hiv en de preventie van moeder-op-kind transmissie. De resultaten van ons onderzoek zijn opgenomen in de internationale richtlijn van de Amerikaanse Department of Health and Human Services (DHHS) die gaat over het gebruik van antiretrovirale geneesmiddelen bij zwangere hiv-geïnfecteerde patiënten. In de versie van augustus 2015 is de plaatsbepaling van raltegravir tijdens de zwangerschap gewijzigd naar één van de voorkeursmiddelen voor de behandeling van hiv tijdens de zwangerschap.

De optimale antiretrovirale behandeling voor hiv-geïnfecteerde patiënten met kanker en het gebruik van chemotherapie is ingewikkeld vanwege geneesmiddelinteracties en overlappende toxiciteit. Hoewel raltegravir steeds vaker wordt aanbevolen in de klinische praktijk bij hiv-geïnfecteerde patiënten met kanker, is er verrassend weinig bekend over de farmacokinetiek van raltegravir in deze speciale patiëntengroep. In **hoofdstuk 7** beschrijven we drie hiv-geïnfecteerde patiënten met een vergevorderd stadium van non-Hodgkin lymfoom die chemotherapie gebruiken in combinatie met een raltegravir-bevattend regime. We namen tot 3 uur na inname van raltegravir meerdere keren bloed af om een schatting te kunnen doen van de totale blootstelling aan raltegravir in 12 uur ($AUC_{0\text{-}12h}$). De geschatte totale blootstelling aan raltegravir bij deze patiënten was gemiddeld lager dan onze referentiewaarden, maar de mate van deze verminderde blootstelling vonden we klinisch niet relevant. De dalspiegels van raltegravir, de belangrijkste farmacokinetische parameter met betrekking tot effectiviteit, bleek voldoende te zijn (>0,020 mg/L) bij alle drie de patiënten bij gebruik van de standaard dosering. Wel zagen we bij één patiënt een verminderde absorptiecurve van raltegravir na het volgen van een chemokuur ten opzichte van de referentiecurve voor het starten van deze hoge dosis chemotherapie. Deze patiënt had last gekregen van ernstige diarree en braken wat een bijwerking is van hoge doseringen chemotherapie. In deze situaties kan TDM ingezet worden om bijvoorbeeld een hogere dosis raltegravir voor te schrijven als de blootstelling te laag wordt.

Hiv-positieve kinderen zijn bij uitstek een patiëntengroep waarbij het monitoren van bloedspiegels van antiretrovirale middelen zinvol kan zijn omdat er veel variatie is in de farmacokinetiek vanwege de lichamelijke ontwikkeling die ze doormaken. Voor de behandeling van hiv bij kinderen is het bovendien belangrijk dat er geschikte kinder-formuleringen zijn van hiv-remmers die gebruikt kunnen worden voor een breed doseringsgebied. Raltegravir is de eerste integraseremmer die is goedgekeurd door de registratieautoriteiten in de Verenigde Staten en Europa voor de behandeling van hiv infectie bij kinderen. Raltegravir is verkrijgbaar als tablet en sinds kort ook in twee speciale

vormen (formuleringen) die geschikt zijn voor zuigelingen en jonge kinderen die niet in staat zijn om hele tabletten te slikken; kauwtabletten (25 en 100 mg) en kleine korreltjes die kunnen worden opgelost in een vloeistof. Het gebruik en de ervaring met betrekking tot de farmacokinetiek van raltegravir bij met name jonge kinderen is nog heel beperkt. In deze context, beschrijven we in **hoofdstuk 8** onze ervaring met dosisoptimalisatie van raltegravir kauwtabletten bij een 4-jarige hiv-positieve patiënt op basis van TDM van raltegravir. Bij deze patiënt werden bij herhaling zeer lage raltegravir dalspiegels gemeten (<0,015 mg/L). Door raltegravir vaker te geven op een dag, namelijk 3 keer per dag in plaats van 2 keer per dag, konden we met succes de raltegravir dalspiegels verhogen tot acceptabele waarden. Toen de patiënt 7 jaar oud was is hij succesvol terug gegaan naar een normale dosering van tweemaal daags raltegravir kauwtabletten.

In **hoofdstuk 8** worden tevens de onderzoeken over de farmacokinetiek van raltegravir bij kinderen besproken die aan de basis liggen van de doseringsadviezen van raltegravir bij kinderen vanaf 4 weken tot 18 jaar oud. Opvallend was dat de laagste raltegravir plasmaconcentraties werden gemeten in het cohort van kinderen vanaf 2 tot 6 jaar die de kauwtabletten gebruiken. Wij zijn bezorgd dat sommige jonge patiënten die raltegravir kauwtabletten gebruiken in de praktijk suboptimale dalspiegels hebben zoals beschreven in onze casus. Bij jonge hiv-patiënten kan het daarom zinvol zijn om bij gebruik van raltegravir kauwtabletten de bloedspiegels van raltegravir te meten en indien nodig de dosering aan te passen.

Algemene discussie

De beste strategie voor het langdurig onderdrukken van hiv is het combineren van krachtige antiretrovirale middelen met verschillende werkingsmechanismen. Raltegravir werd eind 2007 door de registratieautoriteiten versneld goedgekeurd omdat er op dat moment grote behoefte was aan nieuwe hiv-remmers. Raltegravir was het eerste geneesmiddel van een nieuwe klasse van antiretrovirale middelen, namelijk de integraseremmers.

Sinds raltegravir in gebruik is genomen zijn we veel meer te weten gekomen over de klinische farmacologie van raltegravir. Het onderzoek in dit proefschrift heeft hier aan bijgedragen, met name op het gebied van geneesmiddelinteracties en de farmacokinetiek van raltegravir in bijzondere (groepen) hiv-geïnfecteerde patiënten. In de algemene discussie van dit proefschrift worden de resultaten besproken met een focus op de volgende hoofdthema's: farmacokinetisch interactieonderzoek met raltegravir, de variabiliteit in de farmacokinetiek van raltegravir en de mogelijke factoren die hierin een rol spelen, de relatie tussen de farmacokinetiek en farmacodynamiek van raltegravir, en de rol van TDM in de klinische praktijk.

Ondanks al het onderzoek dat is gedaan naar de farmacokinetiek van raltegravir, is de zo kenmerkende variabiliteit in de farmacokinetiek nooit echt goed opgehelderd. Deze variabiliteit heeft er ook voor gezorgd dat het aanvankelijk moeilijk was om een duidelijke relatie te vinden tussen de concentratie van raltegravir in het bloed (farmacokinetiek) en het effect (farmacodynamiek). Wij denken dat een groot deel van de variabiliteit in de farmacokinetiek van raltegravir veroorzaakt wordt door de farmaceutische formulering van de raltegravir tabletten en de slechte oplosbaarheid en permeabiliteit.

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Dankwoord

Met heel veel plezier heb ik de afgelopen jaren aan mijn promotieonderzoek gewerkt. Een promotietraject zou je kunnen vergelijken met een mooie reis, een soort ontdekkingsreis. Onbevangen ging ik eind 2010 het avontuur tegemoet. Op onderzoek uit….

Met veel ervaring rijker en een paar illusies armer heb ik mijn eindbestemming bereikt. Dat heb ik gelukkig niet alleen gedaan. Laat ik daarom beginnen met een algemeen maar oprecht '**Bedankt!**' voor iedereen die een bijdrage heeft geleverd aan dit proefschrift. Want of het nu de hulp was bij het meten en analyseren, of de interesse en steun van collega's, vrienden en familie…ik heb het allemaal zeer gewaardeerd. Toch wil ik graag een aantal mensen in het bijzonder noemen.

Allereerst wil ik de patiënten en gezonde proefpersonen bedanken die hebben deelgenomen aan de onderzoeken. Dit alles was nooit mogelijk geweest zonder jullie bijdrage en vertrouwen.

Prof. dr. Burger, beste David, bedankt voor de kans om onderzoek te gaan doen bij jouw onderzoeksgroep. Het is een vakgroep om trots op te zijn en dat is voor een groot deel jouw verdienste. Als je een promotietraject vergelijkt met een mooie reis dan was jij mijn reisleider. De inhoud van dit proefschrift (onze eindbestemming) lag van te voren niet vast maar dat we er zouden komen was zeker. David, enorm veel dank voor de fijne (en relaxte) manier waarop je mij begeleid hebt. Ik heb veel van je geleerd.

Angela Colbers kan ik dan het beste omschrijven als assistent reisleider en tevens reisgenoot. Lieve Angela, ik ben heel blij met jou als paranimf aan mijn zijde. Je bent mijn grote steun en toeverlaat geweest bij al mijn onderzoek. Het is heel fijn samenwerken met jou en ik kijk met veel plezier terug op onze gezamenlijke congresbezoeken en gezellige uitstapjes. Aan mijn andere zijde staat mijn paranimf Bregje Ruijs-Witjes. Lieve Bregje, van collega ziekenhuisapotheker in opleiding in het Erasmus MC werd je al snel een hele goede vriendin. Bedankt hiervoor en voor je interesse en steun. Ik vind het bijzonder dat we dit moment samen kunnen delen.

De leden van de manuscriptcommissie, prof. dr. André van der Ven, prof. dr. Peter Reiss en dr. Matthijs van Luin, wil ik hartelijk bedanken voor het bestuderen en het positief beoordelen van dit proefschrift. Matthijs, wat leuk dat jij deze rol hebt vervuld.

Remco de Jong, afdelingshoofd van de Apotheek van het Radboudumc, wil ik bedanken voor het mogelijk maken van dit promotietraject binnen de afdeling.

Ik heb veel tijd doorgebracht op het Clinical Research Centre Nijmegen (CRCN) van het Radboudumc waar de interactieonderzoeken zijn uitgevoerd. Alle (oud-)medewerkers van het CRCN wil ik bedanken. In het bijzonder bedank ik Christel, Joyce, Wendy, Jackie, Adrianne, Simone, Inge, Karin, Corina, Marielle, Willeke, Lanny, Anja, Michel, Mike en Luuk voor hun hulp bij de uitvoering. Bas Schouwenberg, bedankt voor je inzet en al je (medische) input. Op de drukste momenten van het onderzoek konden we gelukkig ook een beroep doen op collega artsen, die ik ook hartelijk wil bedanken: Michiel van Beek, David Jansen, Femke Besemer, Helle-Brit Fiebrich, Douwe Dekker en Petra Spies.

Ik kan de analisten van het TDM/Research lab niet genoeg bedanken voor het meten van de vele bloedspiegels en alles wat daarbij kwam kijken. Khalid, Erik, Marga, Corrien, Noor, Kirsten, Martijn, Guus, Michel en Karin (Maastricht), het was fijn samenwerken met jullie.

Anne Poirters heeft als stagiaire Biomedische Wetenschappen fantastisch meegeholpen aan het GINGER (Ginkgo biloba) onderzoek, waaronder veel labwerk. Charlotte Langemeijer deed dat voor het RECITAL (citalopram) onderzoek. Dames, bedankt.

Ron van Schaik (Erasmus MC) wil ik bedanken voor het farmacogenetisch onderzoek en de bijdrage aan het RECITAL onderzoek.

Ik ben heel trots op ons onderzoek naar de farmacokinetiek van raltegravir bij zwangere vrouwen dat is voortgekomen uit een groot internationaal samenwerkingsverband met Angela als projectmanager. I would like to thank all PANNA investigators (Chapter 6) for making a difference in the treatment of HIV-infected pregnant women and the prevention of mother-to-child HIV transmission.

Onderzoek doen kost geld. We zijn dan ook dankbaar voor de financiële steun die we hebben gekregen voor onze interactieonderzoeken van de firma Merck in het kader van hun Investigator-Initiated Study Program. Het PANNA onderzoek is financieel mogelijk gemaakt door NEAT/Penta, Merck, BMS, Janssen Research BV en ViiV Healthcare.

Door het bijwonen van de wekelijkse hiv-patiëntenbespreking heb ik veel geleerd over de behandeling van hiv-patiënten. Dat heeft mijn onderzoek medische diepgang en context gegeven. Graag wil ik de infectiologen (i.o.) en verpleegkundig consulenten hiv van het Radboudumc hiervoor bedanken. Daarnaast heb ik ten behoeve van dit proefschrift samengewerkt met een aantal hele goede medisch specialisten en collega's. Walter van der Velden en prof. dr. Nicole Blijlevens (Hematologie), Reinout van Crevel (Infectieziekten) en mijn collega Roger Brüggemann bedank ik voor ieders bijdrage aan de korte case series over de hiv-patienten met non-Hodgkin lymfoom. Erik van Maarseveen (Klinische Farmacie, UMCU) en Sibyl Geelen (Infectieziekten, Wilhelmina Kinderziekenhuis) wil ik bedanken voor de fijne en enthousiaste samenwerking bij het hoofdstuk over de farmacokinetiek van raltegravir bij kinderen, waarin een casus is beschreven van een jonge en dappere hiv-patiënt.

Ik heb me vanaf het begin op mijn plek gevoeld binnen de vakgroep. Het is een leuke en inspirerende club mensen bij elkaar. Mede door de gezellige sfeer ging ik iedere dag met plezier naar de Apotheek. Bedankt allemaal! Mijn kamergenoten zijn me dierbaar vanwege het delen van het dagelijks lief en leed: Quirine, Marieke, Klaartje, Diane, Monique, Robert, Eline, Jackie en Vincent. Klaartje, ook bedankt voor de energie die je hebt gestopt in het OPAL (boceprevir) onderzoek.

Mijn lieve familie en vrienden wil ik bedanken voor hun belangstelling, gezelligheid en warmte. Een speciale plek in dit dankwoord verdienen mijn ouders omdat zij mij altijd met veel liefde gesteund hebben. Chris, lieve mama, ik vind het heel bijzonder hoe je altijd voor ons klaar staat. Ik heb dit proefschrift opgedragen aan mijn vader, Arthur, die er in mijn gedachten bij is en zonder twijfel het meest trots is van iedereen.

Ik ben aangekomen op de eindbestemming: het proefschrift ligt voor u! Het was een mooie ontdekkingsreis. Gelukkig is de allerfijnste plek op deze wereldbol bij mijn lieve Sander en onze meisjes Zoë & Milou. Jullie zijn mijn thuis.

Curriculum Vitae

Maren Blonk was born on the 10th of August 1977, in Groningen, The Netherlands. She completed her secondary school education at the 'Scholengemeenschap Stad & Esch' in Meppel in 1995. After spending a year abroad in the United Kingdom, she started the study Pharmacy at the University of Groningen in 1996. During her studies she performed a research project at the Department of Clinical Chemistry at the University Medical Center Groningen. She obtained her Master's degree and Pharmacy degree in 2001, and in 2003, respectively. Maren worked temporarily as a pharmacist for a volunteer project of the International Pharmaceutical Students' Federation in Tanzania. In 2004 she started her professional career at the department of Hospital Pharmacy at Erasmus Medical Centre in Rotterdam, where she began her residency in hospital pharmacy in 2005 under supervision of prof. dr. Arnold Vulto. During her residency she was involved in two research projects: drug-related falls in elderly people, and the pharmacokinetics of levetiracetam in neonates. In 2009 she registered as a hospital pharmacist. After a round-the-world trip she returned to The Netherlands and worked as a hospital pharmacist at the Hospital St Jansdal in Harderwijk. She received an award for the best review article on the use of oral ketamine in chronic pain management by the Dutch Society of Hospital Pharmacists (NVZA). In November 2010 Maren continued her career at the department of Pharmacy at the Radboud university medical center where she began her PhD research project which is described in this thesis. The PhD research project was supervised by prof. dr. David Burger and focussed on the clinical pharmacology of the HIV integrase inhibitor raltegravir. As of November 2015 Maren holds a position as a hospital pharmacist at the department of Clinical Pharmacy at the Canisius-Wilhelmina Ziekenhuis in Nijmegen. She lives together with Sander Koppen de Neve and their two daughters Zoë and Milou.