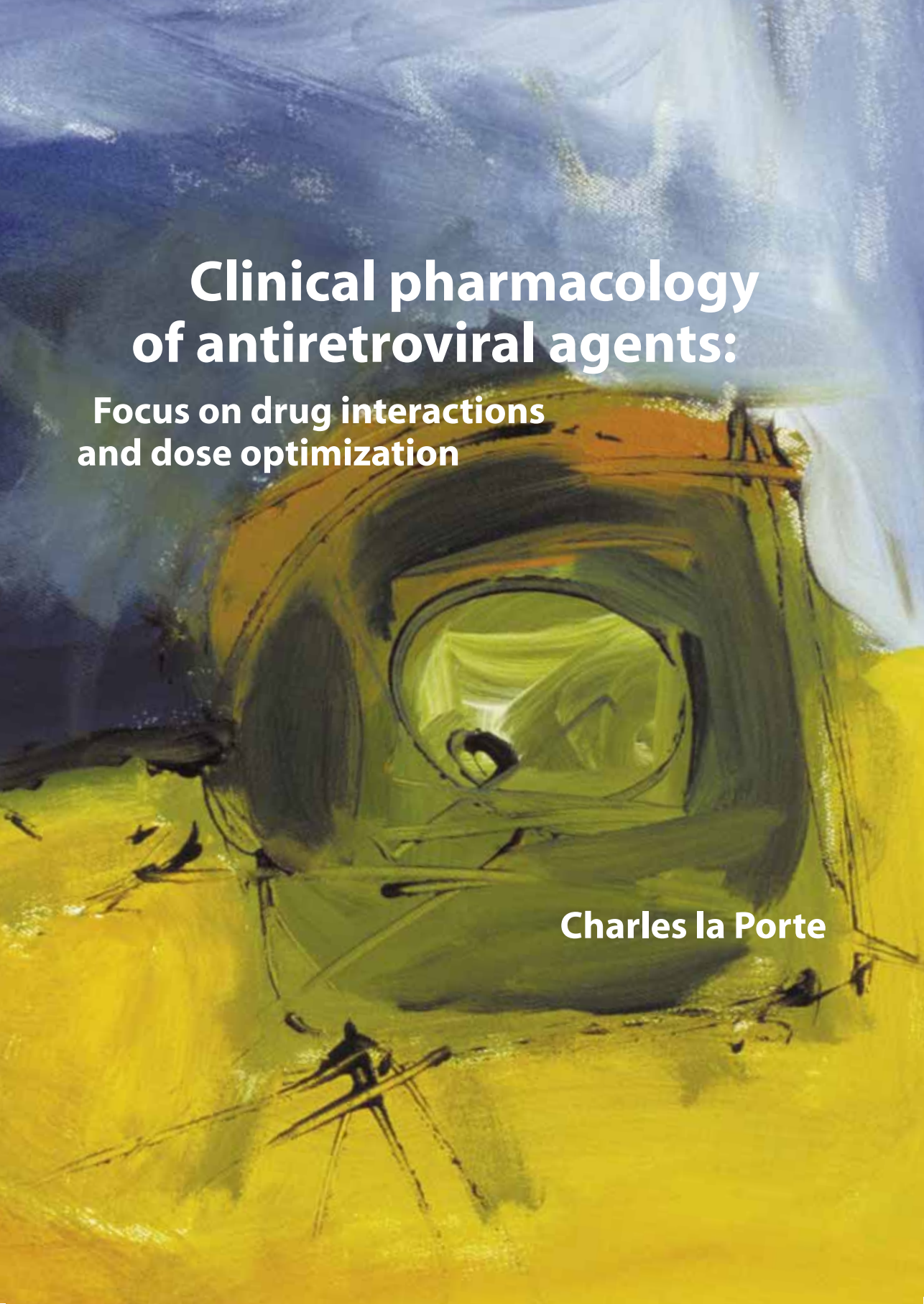

Clinical pharmacology of antiretroviral agents:
Focus on drug interactions and dose optimization
Charles la Porte

Clinical pharmacology of antiretroviral agents:

Focus on drug interactions and dose optimization

Publication of this thesis was financially supported by
Stichting Klinisch Farmaceutische Dienstverlening, Nijmegen

Design: Rozemarijn Oudejans
Artwork cover, based on the themes interaction and optimization:
Madelon Surminski-la Porte
Printing: PrintPartners Ipskamp, Enschede

ISBN 90–9019051–1

Clinical pharmacology of antiretroviral agents:

Focus on drug interactions and dose optimization

een wetenschappelijke proeve
op het gebied van de Medische Wetenschappen

Proefschrift

ter verkrijging van de graad van doctor
aan de Radboud Universiteit Nijmegen
op gezag van de Rector Magnificus Prof. Dr. C.W.P.M. Blom,
volgens het besluit van het College van Decanen
in het openbaar te verdedigen op donderdag 14 april 2005
des namiddags om 1.30 uur precies
door

Charles Jean Louis la Porte

geboren op 3 mei 1975
te Halsteren

Promotor
Prof. Dr. Y.A. Hekster

Co-promotores
Dr. D.M. Burger
Dr. P.P. Koopmans

Manuscriptcommissie
Prof. Dr. F.G.M. Russel
Prof. Dr. J.W.M. van der Meer
Prof. Dr. J.M.D. Galama
Prof. Dr. H.-J. Guchelaar, Leids Universitair Medisch Centrum
Dr. C. Richter, Ziekenhuis Rijnstate Arnhem

On ne voit bien qu'avec le cœur.
L'essentiel est invisible pour les yeux.

Le Petit Prince

Contents

Introduction

Human immunodeficiency virus

Patients living with the acquired immunodefienciency syndrome (AIDS) are infected with the human immunodeficiency virus (HIV). HIV belongs to the class of retroviruses and destructs the patients' immune response. The virus depletes T lymphocytes, expressing the CD4 phenotypic marker. This destruction takes place gradually and in the late stage of the disease, with CD4 cells dropping below 200 per µl, there is an increasing chance for opportunistic infections, if no medical treatment is given[1]. Several pathways transmit HIV, such as unprotected sexual intercourse, sharing of contaminated injection needles, receipt of contaminated blood products and mother-to-child transmission. Mother-to-child transmission can occur in uterus, which is rare, during delivery, or post partum when the newborn is breastfed. In the fight against the spread of HIV, consciousness of these pathways is very important.
Worldwide around 40 million people are infected with HIV-1, this number includes around 4 million children living with HIV infection[2]. A distinction is made between HIV-1 and HIV-2, the latter being less virulent and pathogenic. HIV-2 has a lower prevalence than HIV-1 and is seen primarily in West-Africa[3,4]. The treatment of HIV as studied in this thesis concerns HIV-1 infection. Although the treatment of children with HIV remains challenging[5], this thesis deals with HIV-1 infection in adults.

Antiretroviral agents

Antiretroviral agents play a key-role in the treatment of HIV-1-infected persons. In contrast to the early days of HIV treatment where single agents were used, nowadays combinations of several agents have high success rates[6-8]. The success rate of highly active antiretroviral therapy (HAART) however is not 100%. HAART can inhibit replication of the virus, but cannot eradicate it. For this reason patients with HIV infection need to continue their medication life-long to suppress the virus. To monitor the effect of therapy the viral load is measured. The viral load is the number of viral RNA copies per ml, in the blood of the patient. Therapy is considered successful if the viral load is undetectable on an ongoing basis. With current techniques the lower limit of detection for the viral load is 50 copies/ml. A detectable viral load is a sign for virological failure or treatment failure[9]. Reasons for treatment failure can be very divers and involve the trias of patient, virus and drug[10].
Nowadays, more than 20 years after the start of the pandemic of HIV there are 18 different antiretroviral agents available. These agents are divided over five different groups. In Table 1 an overview of the available antiretrovirals is provided.

Table 1. Overview of available antiretroviral agents by group.

NRTIs	NtRTI	NNRTIs	PIs	Fusion inhibitors
zidovudine	tenofovir	nevirapine	amprenavir	enfuvirtide
stavudine		efavirenz	fosamprenavir	
lamivudine		delarvidine	indinavir	
abacavir			nelfinavir	
emtricitabine			saquinavir	
didanosine			lopinavir	
zalcitabine			ritonavir	
			atazanavir	

NRTIs : Nucleoside reverse transcriptase inhibitors, NtRTI : Nucleotide reverse transcriptase inhibitor, NNRTIs : Non nucleoside reverse transcriptase inhibitors, PIs : Protease inhibitors.

Clinical pharmacology

The popular medical science search engine pub med (www.pubmed.com) reported more than 30,000 hits, at the time that this thesis was written, when the keywords "pharmacology" and "HIV" were used. The oldest hits were from the first days of the HIV epidemic in the beginning of the 80's. This finding represents an impressive number of articles published with regard to the studies of drugs (pharmacology[11]) in the era of HIV. This number also represents the complexity of clinical pharmacology (studies of drugs in men[12]) of antiretroviral drugs. Apparently, after all these years of hard work there is still need for more knowledge of the ever-changing HAART regimens.

Clinical pharmacology can be divided in a pharmacokinetic and a pharmacodynamic part. Pharmacokinetics is the knowledge about the effects of the human body on the drug, whereas pharmacodynamics describes what the drug is doing to the body. In clinical pharmacology of antiretroviral agents there is a central role for the relationship between concentration of the drugs in blood and their efficacy and toxicity.

With the development of new drugs new questions arise with regard to pharmacokinetics and pharmacodynamics. Fields of interest with regard to clinical pharmacology of antiretroviral agents are: drug interactions, dose optimization and additional parameters like gender, race and pharmacogenetics.

Drug interactions

Drug interactions are common in HAART regimens. The protease inhibitors as well as the NNRTIs are susceptible to pharmacokinetic drug interactions. Metabolism of these agents greatly depends on Cytochrome P450 (CYP) isoenzymes. CYP isoenzymes are known to be vulnerable to induction or inhibition by other drugs. Two well known examples are rifampin, a tuberculostatic agent, for its inducing effect on CYP3A4[13,14] and ritonavir, a protease inhibitor, for its inhibiting effect on the same isoenzyme[15]. In practice co-administration of rifampin will greatly accelerate CYP3A4 metabolism of several drugs such as lopinavir[16] and saquinavir[17], whereas ritonavir will slow down the metabolism of the same drugs. Drug interactions were studied in **Chapter 1** for lopinavir/ritonavir combined with rifampin and in **Chapter 6** for the combination of nelfinavir/ritonavir and efavirenz.

Interactions can also occur between food and drugs. The protease inhibitors lopinavir and nelfinavir have to be taken with a meal to ensure a good absorption from the gastrointestinal tract[18,19]. Other drugs such as didanosine are susceptible for acid from the stomach and are therefore preferably not combined with food[20]. Drug interactions and food effects were studied in a combination of indinavir/ritonavir with didanosine in **Chapter 4**.

Drug interactions can have a pharmacodynamic background as well. Combining drugs that exhibit synergism can be useful in the treatment of patients, as the combined antiviral activity of the two drugs is higher than the sum of the activity of the two independent drugs. Synergism of antiretroviral activity has been reported for lopinavir and saquinavir[21]. Other combinations of drugs can antagonize each other's activity, leading to less activity than the sum of the activity of each drug. In **Chapter 2** the pharmacokinetics, tolerability and efficacy of lopinavir combined with saquinavir were studied.

Dose optimization

In the development of new antiretroviral agents, as in the development of any other drug, studies are undertaken to find a dose at which patients experience an optimal effect of the medication, without suffering from adverse events. In clinical practice however, situations might occur in which the recommended dose does not have the desired effect. This might be the case when drug interactions are expected, or when dose frequency is changed, or if unexpected toxicity or inactivity occurs.

Knowledge of drug interactions can give reasons for dose optimizations to ensure equal exposure to the drug affected by the interacting drug. For example rifampin is known to induce lopinavir metabolism, leading to subtherapeutic blood concentrations of lopinavir[16]. In this particular situation an adjusted dose of lopinavir might compensate for the accelerated metabolism. Dose adjustments of lopinavir/ritonavir to compensate

for the inducing effect of rifampin were studied in **Chapter 1**.
Dose frequency is an important factor in the development of HAART regimens. Currently most HAART regimens allow for twice daily dosing. For the patient it can be more convenient and probably more easily to adhere to if dose frequency is less frequent[22,23]. For some agents it seems reasonable to give the daily dose in one dose rather than divided over two doses[24]. Nevertheless this should be tested with pharmacokinetic profiles to study the exposure to the drug. If necessary, dose adjustments can be made to find the right dose in each patient. **Chapter 3** is the reflection of a study in which dose adjustments were made subsequently to reducing dose frequency from twice daily to once daily for lopinavir/ritonavir.
The occurrence of adverse events sometimes compels to a more balanced optimum between efficacy and toxicity. The protease inhibitor indinavir is normally given with ritonavir dosed as 800/100 mg twice daily[25]. It was found that patients receiving this combination stopped their medication for reasons of toxicity more often than for a lack of efficacy[26]. A dose reduction of indinavir to 600 or 400 mg might lead to less adverse events, without losing antiviral activity. **Chapter 5** presents a study in healthy subjects exploring the pharmacokinetic profile of these reduced doses in order to predict indinavir exposure in patients.
Therapeutic drug monitoring in individual patients can detect drug concentrations that are too low to be antiviral active. In such cases an intervention is necessary. In some cases a dose increase will result in the desired effect, nevertheless there are very few data that support such measures. In **Chapter 7** the effect of dose increases in patients with low plasma nelfinavir concentrations was evaluated.

Other parameters in clinical pharmacology

Gender, race and pharmacogenetics are three other parameters that can have their influence on the clinical pharmacology of antiretroviral agents.
Several studies have shown pharmacokinetic differences between males and females[27-29], and between patients of different ethnicity[30-33]. The observed differences are not always large and considerable numbers of patients should be studied to observe these differences. In general, drug exposure, with same dosage for males and females, seems to be higher in females. This might put females at higher risk for toxicity. In **Chapter 8** gender and race differences for nevirapine pharmacokinetics were studied.
Pharmacogenetic studies evaluate the effect of genetic polymorphism on clinical pharmacology. Single nucleotide polymorphisms (SNPs) leading to genetic heterogeneity can cause variability in disposition of P450 isoenzymes[34-36]. Different genotypes of P450 isoenzymes, due to SNPs, could result in an altered drug metabolism in individual patients[37]. Currently SNPs and their effects on drug disposition are subject of research and the knowledge of SNPs is therefore expanding. The antiretrovirals of the NNRTI and PI groups are supposed to be vulnerable for this source of variability,

as they greatly depend on P450 isoenzyme mediated metabolism. A pharmacogenetic study for efavirenz is described in **Chapter 9**.

Objectives of this thesis

This thesis is a presentation of studies that were undertaken to shed more light on the clinical pharmacology of antiretroviral agents. In particular drug interactions and dose optimizations were studied. Additionally, studies were performed to learn about the role of gender, race and pharmacogenetics in the clinical pharmacology of antiretroviral agents. Multiple objectives were studied in some of the chapters in this thesis, whereas single objectives were studied in others. Studies with the protease inhibitors lopinavir, indinavir and nelfinavir are presented in **Parts I, II and III,** respectively. **Part IV** focuses on the group of non-nucleoside reverse transcriptase inhibitors. Finally, a general discussion is presented.

References

1. **Fauci, A. S.** 1988. The human immunodeficiency virus: infectivity and mechanisms of pathogenesis. Science **239**:617-622.
2. Anonymous. http://www.unaids.org/en/resources/epidemiology.asp. 2003.
3. **Kanki, P. J., K. U. Travers, S. Mboup, C. C. Hsieh, R. G. Marlink, A. Gueye-NDiaye, T. Siby, I. Thior, M. Hernandez-Avila, J. L. Sankale, and**. 1994. Slower heterosexual spread of HIV-2 than HIV-1. Lancet **343**:943-946.
4. **Marlink, R., P. Kanki, I. Thior, K. Travers, G. Eisen, T. Siby, I. Traore, C. C. Hsieh, M. C. Dia, E. H. Gueye, and**. 1994. Reduced rate of disease development after HIV-2 infection as compared to HIV-1. Science **265**:1587-1590.
5. **Bergshoeff, A. S.** Exploring the Clinical Pharmacology of Antiretroviral Agents in HIV-1 Infected Children. 2004.
6. **Egger, M., M. May, G. Chene, A. N. Phillips, B. Ledergerber, F. Dabis, D. Costagliola, M. A. D'Arminio, F. de Wolf, P. Reiss, J. D. Lundgren, A. C. Justice, S. Staszewski, C. Leport, R. S. Hogg, C. A. Sabin, M. J. Gill, B. Salzberger, and J. A. Sterne.** 2002. Prognosis of HIV-1-infected patients starting highly active antiretroviral therapy: a collaborative analysis of prospective studies. Lancet **360**:119-129.
7. **Hogg, R. S., K. V. Heath, B. Yip, K. J. Craib, M. V. O'Shaughnessy, M. T. Schechter, and J. S. Montaner.** 1998. Improved survival among HIV-infected individuals following initiation of antiretroviral therapy.JAMA **279**:450-454.
8. **Palella, F. J. J., K. M. Delaney, A. C. Moorman, M. O. Loveless, J. Fuhrer, J. E. Satten, D. E. Aschman, and S. D. Holmberg.** 1998. Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. New England Journal of Medicine **338**:853-860.
9. **Mellors, J. W., A. Munoz, J. V. Giorgi, J. B. Margolick, C. J. Tassoni, P. Gupta, L. A. Kingsley, J. A. Todd, A. J. Saah, R. Detels, J. P. Phair, and C. R. Rinaldo, Jr.** 1997. Plasma viral load and CD4+ lymphocytes as prognostic markers of HIV-1 infection. Ann.Intern.Med. **126**:946-954.

10. **Back, D. J. and S. H. Khoo.** 2003. The role of clinical pharmacology in optimizing antiretroviral therapy. Br.J.Clin.Pharmacol. **55**:473-476.
11. **Reidenberg, M. M.** 1999. Clinical pharmacology: the scientific basis of therapeutics. Clin. Pharmacol.Ther. **66**:2-8.
12. **C. J. Van Boxtel, B. Santoso, and I. R. Edwards (eds.).** Preface. 2003. Drug benefits and risks. International textbook of clinical pharmacology. John Wiley & Sons Ltd, Chichester, U.K.
13. **Burman, W. J., K. Gallicano, and C. Peloquin.** 2001. Comparative pharmacokinetics and pharmacodynamics of the rifamycin antibacterials. Clin.Pharmacokinet. **40**:327-341.
14. **Piscitelli, S. C. and K. D. Gallicano.** 2001. Interactions among drugs for HIV and opportunistic infections. N.Engl.J.Med. **344**:984-996.
15. **Hsu, A., G. R. Granneman, and R. J. Bertz.** 1998. Ritonavir. Clinical pharmacokinetics and interactions with other anti-HIV agents. Clinical Pharmacokinetics **35**:275-291.
16. **Bertz, R., A. Hsu, W. Lam, L. Williams, C. Renz, M. D. Karol, S. Dutta, A. Carr, Y. Zhang, Q. Wang, S. Schweitzer, C. Foit, A. Andre, B. Bernstein, G. Granneman, and E. Sun.** 2000. Pharmacokinetic interactions between lopinavir/ritonavir (ABT-378/r) and other non-HIV drugs. 5th International Congress on Drug Therapy in HIV Infection.Glasgow, UK, October 2000. Abstract P291.
17. **Veldkamp, A., R. M. W. Hoetelmans, J. H. Beijnen, J. W. Mulder, and P. L. Meenhorst.** 1999. Ritonavir enables combined therapy with rifampin and saquinavir. Clinical Infectious Diseases **29**:1586.
18. Kaletra® Summary of Product Characteristics. 2000. Abbott, Chicago, IL, USA.
19. Viracept® Summary of Product Characteristics. 2001. Roche Registration Limited, Hertfordshire, United Kingdom.
20. Videx EC® Summary of Product Characteristics. 2003. Bristol-Myers Squibb B.V., Woerden, The Netherlands.
21. **Molla, A., H. Mo, S. Vasavanonda, L. Han, C. T. Lin, A. Hsu, and D. J. Kempf.** 2002. In vitro antiviral interaction of lopinavir with other protease inhibitors. Antimicrob.Agents Chemother. **46**:2249-2253.
22. **Mehta, S., R. D. Moore, and N. M. H. Graham.** 1997. Potential factors affecting adherence with HIV therapy. AIDS **11**:1665-1670.
23. **Stone, V. E.** 2001. Strategies for optimizing adherence to highly active antiretroviral therapy: lessons from research and clinical practice. Clin.Infect.Dis. **33**:865-872.
24. **Eron, J. J., J. Feinberg, H. A. Kessler, H. W. Horowitz, M. D. Witt, F. F. Carpio, D. A. Wheeler, P. Ruane, D. Mildvan, B. G. Yangco, R. Bertz, B. Bernstein, M. S. King, and E. Sun.** 2004. Once-daily versus twice-daily lopinavir/ritonavir in antiretroviral-naive HIV-positive patients: a 48-week randomized clinical trial. J.Infect.Dis. **189**:265-272.
25. **Voigt, E., A. Wickesberg, J. C. Wasmuth, P. Gute, L. Locher, B. Salzberger, A. Wohrmann, A. Adam, L. Weitner, and J. K. Rockstroh.** 2002. First-line ritonavir/indinavir 100/800 mg twice daily plus nucleoside reverse transcriptase inhibitors in a German multicentre study: 48-week results. HIV Med. **3**:277-282.
26. **Burger, D., P. Hugen, P. Reiss, I. Gyssens, M. Schneider, F. Kroon, G. Schreij, K. Brinkman, C. Richter, J. Prins, R. Aarnoutse, J. Lange, and for the ATHENA Cohort Study Group.** 2003. Therapeutic drug monitoring of nelfinavir and indinavir in treatment-naive HIV-1-infected individuals. AIDS **17**:1157-1165.
27. **Burger D.M., Muller R.J., van de Leur M.R., and la Porte C.J.L.** Lopinavir plasma levels are significantly higher in female than in male HIV-1 infected patients. 3rd International Workshop on Clinical Pharmacology of HIV Therapy, April 11-13, 2002, Washington DC, USA, abstract 6.5 . 2002.
28. **Burger, D. M., M. C. Siebers, P. W. Hugen, R. E. Aarnoutse, Y. A. Hekster, and P. P. Koopmans.** 2002. Pharmacokinetic variability caused by gender: do women have higher indinavir exposure than men? J Acquir Immune Defic Syndr **29**:101.

29. **Schwartz, J. B.** 2003. The influence of sex on pharmacokinetics. Clin.Pharmacokinet. **42**:107-121.
30. **Sowunmi, A., T. J. Rashid, O. O. Akinyinka, and A. G. Renwick.** 1995. Ethnic differences in nifedipine kinetics: comparisons between Nigerians, Caucasians and South Asians. Br.J.Clin. Pharmacol. **40**:489-493.
31. **Wandel, C., J. S. Witte, J. M. Hall, C. M. Stein, A. J. Wood, and G. R. Wilkinson.** 2000. CYP3A activity in African American and European American men: population differences and functional effect of the CYP3A4*1B5'-promoter region polymorphism. Clin.Pharmacol.Ther. **68**:82-91.
32. **Kinirons, M. T., C. C. Lang, H. B. He, K. Ghebreselasie, S. Shay, D. W. Robin, and A. J. Wood.** 1996. Triazolam pharmacokinetics and pharmacodynamics in Caucasians and Southern Asians: ethnicity and CYP3A activity. Br.J.Clin.Pharmacol. **41**:69-72.
33. **Mancinelli, L. M., L. Frassetto, L. C. Floren, D. Dressler, S. Carrier, I. Bekersky, L. Z. Benet, and U. Christians.** 2001. The pharmacokinetics and metabolic disposition of tacrolimus: a comparison across ethnic groups. Clin.Pharmacol.Ther. **69**:24-31.
34. **Ingelman-Sundberg, M.** 2004. Human drug metabolising cytochrome P450 enzymes: properties and polymorphisms. Naunyn Schmiedebergs Arch.Pharmacol. **369**:89-104.
35. **Lamba, V., J. Lamba, K. Yasuda, S. Strom, J. Davila, M. L. Hancock, J. D. Fackenthal, P. K. Rogan, B. Ring, S. A. Wrighton, and E. G. Schuetz.** 2003. Hepatic CYP2B6 expression: gender and ethnic differences and relationship to CYP2B6 genotype and CAR (constitutive androstane receptor) expression. J.Pharmacol.Exp.Ther. **307**:906-922.
36. **Lang, T., K. Klein, J. Fischer, A. K. Nussler, P. Neuhaus, U. Hofmann, M. Eichelbaum, M. Schwab, and U. M. Zanger.** 2001. Extensive genetic polymorphism in the human CYP2B6 gene with impact on expression and function in human liver. Pharmacogenetics **11**:399-415.
37. **Tsuchiya, K., H. Gatanaga, N. Tachikawa, K. Teruya, Y. Kikuchi, M. Yoshino, T. Kuwahara, T. Shirasaka, S. Kimura, and S. Oka.** 2004. Homozygous CYP2B6 *6 (Q172H and K262R) correlates with high plasma efavirenz concentrations in HIV-1 patients treated with standard efavirenz-containing regimens. Biochem.Biophys.Res.Commun. **319**:1322-1326.

Part I

Lopinavir

Chapter 1

Pharmacokinetics of adjusted-dose lopinavir/ritonavir combined with rifampin in healthy volunteers

C. J. L. la Porte[1,2], E. P. H. Colbers[3], R. Bertz[4], D. S. Voncken[3], K. Wikstrom[4], M. J. Boeree[2,5], P. P. Koopmans[2,6], Y. A. Hekster[1,2], D. M. Burger[1,2]

1 Department of Clinical Pharmacy, University Medical Centre Nijmegen, The Netherlands.
2 Nijmegen University Centre for Infectious Diseases, The Netherlands.
3 Farma Research BV, Nijmegen, The Netherlands.
4 Department of Clinical Pharmacokinetics and Antiviral Global Development, Abbott Laboratories, Abbott Park, Illinois, USA.
5 University Lung Centre Dekkerswald, Nijmegen, The Netherlands.
6 Department of General Medicine, University Medical Centre Nijmegen, The Netherlands.

Antimicrobial Agents and Chemotherapy. 2004; 48: 1553-1560

Abstract

Coadministration of lopinavir/ritonavir, an antiretroviral protease inhibitor, at the standard dose (400/100 mg twice a day [BID]) with the antituberculous agent rifampin is contraindicated because of a significant pharmacokinetic interaction due to induction of cytochrome P450 3A by rifampin. In the present study, two adjusted-dose regimens of lopinavir/ritonavir were tested in combination with rifampin.

Thirty-two healthy subjects participated in a randomized, two-arm, open-label, multiple-dose, within-subject controlled study. All subjects were treated with lopinavir/ritonavir at 400/100 mg BID from days 1 to 15. From days 16 to 24, the subjects in arm 1 received lopinavir/ritonavir at 800/200 mg BID in a dose titration, and the subjects in arm 2 received lopinavir/ritonavir at 400/400 mg BID in a dose titration. Rifampin was given at 600 mg once daily to all subjects from days 11 to 24. The multiple-dose pharmacokinetics of lopinavir, ritonavir, and rifampin were assessed.

Twelve of 32 subjects withdrew from the study. For nine subjects lopinavir/ritonavir combined with rifampin resulted in liver enzyme level elevations. Pharmacokinetic data for 19 subjects were evaluable. Geometric mean ratios for the lopinavir minimum concentration in serum and the maximum concentration in serum (C_{max}) on day 24 versus that on day 10 were 0.43 (90% confidence interval [CI], 0.19 to 0.96) and 1.02 (90% CI, 0.85 to 1.23), respectively, for arm 1 (n=10) and 1.03 (90% CI, 0.68 to 1.56) and 0.93 (90% CI, 0.81 to 1.07), respectively, for arm 2 (n=9). Ritonavir exposure increased from days 10 to 24 in both arms. The geometric mean C_{max} of rifampin was 13.5 mg/liter (day 24) and was similar between the two arms.

Adjusted dose regimens of lopinavir/ritonavir in combination with therapeutic drug monitoring and monitoring of liver function may allow concomitant use of rifampin.

Introduction

The treatment of human immunodeficiency virus (HIV)-infected individuals has improved greatly over the past several years. With the development of antiretroviral agents in different classes, more options for the effective suppression of the virus have become available[1]. However, many problems remain to be solved. One of them is the treatment of patients presenting with HIV infection and coinfections. Tuberculosis is a significant opportunistic infection in HIV-infected individuals in developing countries and, to a lesser extent, in developed countries[2-5]. For public health reasons, active tuberculosis must be treated immediately[5]. The treatment of HIV infection can be postponed on the basis of CD4 cell counts and the viral load. However, depending on the clinical and biochemical parameters for coinfected patients, simultaneous treatment of both infections can become indicated in particular situations. The combination of antiretroviral therapy with therapy with antituberculous agents is complex. In particular, the use of rifampin is hampered due to significant drug drug interactions. Rifampin is a first-line antibacterial agent for the treatment of tuberculosis and acts by inhibiting the DNA-dependent RNA polymerase of the microorganism[6]. Rifampin is a strong inducer of cytochrome P450 (CYP)-mediated metabolism of other agents; in particular, the CYP3A isoenzyme is subject to induction. Rifampin metabolism itself is not dependent on CYP3A; nevertheless, autoinduction of cholinesterase- and B esterase-mediated metabolism of rifampin has been shown[6].

Because of its CYP3A-inducing effects, rifampin is known to produce significant pharmacokinetic interactions with HIV protease inhibitors (PIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs)[7]. These pharmacokinetic interactions may lead to subtherapeutic levels of these antiretroviral agents in plasma, and for this reason, rifampin in combination with most PIs and NNRTIs is contraindicated. This clearly limits the options for highly active antiretroviral therapy in *Mycobacterium tuberculosis*-coinfected HIV-infected patients. Data on the adjusted dosages that generally result in therapeutic levels of the antiretrovirals in plasma have been published for the PI saquinavir[8] and the NNRTI efavirenz[9]. Pharmacokinetic interactions between rifampin and nucleoside reverse transcriptase inhibitors (NRTIs) are less pronounced, since these agents do not undergo appreciable oxidative metabolism[10,11]. A study of the interaction of rifampin with T-20 (enfuvirtide) did not reveal clinically significant changes in the pharmacokinetics of T-20[12].

Lopinavir/ritonavir is a formulation of two PIs approved for the treatment of HIV infection at a standard dosage of 400/100 mg twice a day (BID) in combination with other antiretrovirals. The coformulation of lopinavir/ritonavir is available as capsules with a dose of 133 mg of lopinavir and 33 mg of ritonavir per capsule. Lopinavir is mainly dependent on CYP3A for its metabolism, and ritonavir is a strong inhibitor of CYP3A. For this reason, ritonavir is coformulated with lopinavir[13], resulting in sustained and elevated plasma lopinavir levels. The pharmacokinetic interaction between rifampin and lopinavir/ritonavir has been studied previously[14]. The area under the concentration-time curve (AUC) and the minimum concentration in

plasma (C_{min}) for lopinavir in healthy subjects were reduced 75 and 99%, respectively, as a result of coadministration of rifampin at 600 mg once daily (QD) with lopinavir/ritonavir at 400/100 mg BID. This is the direct result of the strong induction of CYP3A by rifampin, which overcomes the inhibition of CYP3A by low-dose ritonavir. Therefore, rifampin combined with lopinavir/ritonavir in the standard-dose regimen is contraindicated. The objective of the present study was to investigate in healthy subjects the pharmacokinetics of two adjusted-dose regimens of lopinavir/ritonavir in combination with rifampin in comparison to the standard dose of lopinavir/ritonavir without rifampin.

Materials and methods

Study design

The study was designed as a randomized, phase I, open-label, two-arm, within-subject controlled study with 32 healthy subjects, both males and females. See Table 1 for details of the study design. The study consisted of a run-in period of 10 days in which lopinavir/ritonavir at the standard dose (400/100 mg BID as three coformulated capsules) was given to all subjects. On study day 10, the steady-state pharmacokinetics of lopinavir and ritonavir were determined over the daytime 12-h dosing interval. During study days 11 to 15, subjects were dosed with lopinavir/ritonavir at the standard dosage, and rifampin at 600 mg QD was added to the regimen. After study day 15, subjects were randomized to either arm 1 or arm 2. From day 16, a dose-titration phase was started in order to diminish dose-related toxicity. In the last phase of the study (study days 18 to 24), the subjects in arm 1 were dosed with lopinavir/ritonavir at 800/200 mg BID plus rifampin at 600 mg QD, while subjects in arm 2 were dosed with lopinavir/ritonavir at 400/400 mg BID plus rifampin at 600 mg QD. The steady-state pharmacokinetics of lopinavir, ritonavir, and rifampin were studied on study day 24.

During the study both rifampin and lopinavir/ritonavir were to be taken together immediately after breakfast in the morning; 12 h later, lopinavir/ritonavir was to be taken after dinner. For the pharmacokinetic analysis, the study subjects were confined on the day prior to blood draw (days 9 and 23) until after the last blood draw on study days 10 and 24. From the day before the start of confinement, the subjects were not allowed to consume alcohol. During confinement, subjects consumed only the standardized scheduled meals and beverages provided at the research unit. The subjects fasted from midnight on study days 9 and 23 to the time of breakfast on study days 10 and 24. Water intake was not allowed from 1 h before until 2 h after drug intake. The study medication was taken orally after breakfast (550 kcal, 28% fat) with 200 ml of noncarbonated water. After the intake of medication the subjects had to remain in an upright position for at least 2 h. After dosing, the subjects continued fasting (no food or beverages) until 5 h after drug intake, at which time the subjects received a

standardized lunch. The subjects received a standardized snack at 9 h following drug ingestion, and dinner was served after the last blood draw. Beverages (i.e., water, orange juice, apple juice, coffee, tea, and milk) were allowed ad libitum from 5 h after dosing until the end of the confinement period.

Table 1. Study design

Timescale (Days)					
1–10[a]	11–15	randomisation	16	17	18–24[a]
LPV/r 400/100 mg BID[b]	LPV/r 400/100 mg BID, RIF 600 mg QD[c]	Arm 1	LPV/r 533/133 mg BID, RIF 600 mg QD	LPV/r 667/167 mg BID RIF 600 mg QD	LPV/r 800/200 mg BID RIF 600 mg QD
		Arm 2	LPV/r 400/200 mg BID[d], RIF 600 mg QD	LPV/r 400/300 mg BID[d] RIF 600 mg QD	LPV/r 400/400 mg BID[d] RIF 600 mg QD

LPV/r: lopinavir/ritonavir, RIF: rifampin
[a]on days 10 and 24, blood sampling was performed up to 12 hours post ingestion.
[b] LPV/r was coformulated in capsules containing 133 mg lopinavir and 33 mg ritonavir each. A dose of 400/100 mg lopinavir/ritonavir BID therefore consisted of 3 capsules.
[c] RIF was dosed as 2 capsules of containing 300 mg rifampin each.
[d] In the combinations LPV/r 400/200 mg BID, LPV/r 400/300 mg BID and LPV/r 400/400 mg BID the extra ritonavir was dosed as 100 mg capsules ritonavir, in addition to the normal LPV/r 400/100 mg combination.

Selection of subjects

This study was performed with healthy subjects. The inclusion criteria were the ability to sign voluntary informed consent; age 18 years or older; good health (i.e., the subject was not suffering from an acute or chronic illness and was not using medications); and a body mass index (BMI) lower than 30.0 for men and lower than 28.6 for women (body mass index is equal to weight [in kilograms]/height^2 [in square meters]). Female subjects could not be of childbearing potential, defined as being postmenopausal for at least 1 year or surgically sterile (by bilateral tubal ligation, bilateral oophorectomy, or hysterectomy), or could be of childbearing potential but practicing one of the following methods of birth control: condoms, sponge, foams, jellies, diaphragm or intrauterine device, vasectomy for the sexual partner, or total abstinence from sexual intercourse. Exclusion criteria were as follows: known hypersensitivity to lopinavir, ritonavir, or rifampin; positive test result for HIV; positive test result for hepatitis B or C virus; a tuberculin skin test reaction of more than 15 mm or a tuberculin skin test reaction of 1 to 15 mm with a chest X-ray with abnormalities consistent with tuberculosis; pregnancy or breastfeeding; body weight <50 kg; use of contact lenses; a history of pancreatitis; a history of alcohol abuse; and one or more of the following laboratory

test results: hemoglobin concentration, <7.5 mM; leukocyte count, <3 x 10^9/liter; aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT) levels more than two times the upper limit of normal (ULN); γ-glutamyltransferase (GGT) levels more than two times the ULN; alkaline phosphatase levels more than two times the ULN; serum creatinine levels more than 1.5 times the ULN; (pancreatic) amylase levels more than two times the ULN; or total bilirubin levels more than two times the ULN.

Blood sampling procedure

For determination of lopinavir and ritonavir concentrations, blood samples (5 ml) were collected predosing and at 2, 4, 6, 8, 10, and 12 h postingestion on days 10 and 24. Rifampin concentrations were determined with the same samples used for determination of lopinavir and ritonavir concentrations on day 24. However, additional blood samples (5 ml) for the more precise determination of the maximum concentration in plasma (C_{max}) for rifampin were drawn at 1 and 3 h postingestion on day 24. The subjects returned to the study location on study days 1, 3, 7, 13, 16, 17, 18, 20, and 22 for predose blood sampling (5 ml) for determination of lopinavir and ritonavir concentrations. Blood samples were centrifuged at approximately 1,000 x *g* for 10 min at 4°C. Plasma for determination of lopinavir and ritonavir concentrations was transferred to a labeled polypropylene tube and stored at ≤−18°C within 2 h after collection. Plasma for determination of rifampin concentrations was transferred to a labeled polypropylene tube containing ascorbic acid and was stored at ≤−80°C within 2 h after collection.

Bioanalysis

Plasma lopinavir and ritonavir levels were determined by a validated high-performance liquid chromatography (HPLC) method, which was a modified version of a previously published method[15]. The modification consisted of a switch of the UV detection wavelength from 245 to 215 nm at 15.5 min, with retention times of 14.4 min for ritonavir and 15.8 min for lopinavir. The concentration of each agent could be measured without interference from the other drug. The lopinavir and ritonavir calibration curves were linear over a range of 0.045 to 30.0 mg/liter. The lower limit of quantification was 0.04 mg/liter for both lopinavir and ritonavir. Rates of recovery after extraction from plasma were 95% for lopinavir and 94% for ritonavir. The accuracies ranged from 99 to 101% for lopinavir and from 92 to 100% for ritonavir, and the intraday precisions ranged from 0.92 to 5.16% for lopinavir and from 1.51 to 5.14% for ritonavir. The interday precisions ranged from 0 to 1.57% for lopinavir and from 0 to 5.00% for ritonavir.

Plasma rifampin levels were measured by a validated HPLC method that was developed in the University Medical Centre Nijmegen but that has not yet been published. The method consisted of protein precipitation followed by reversedphase HPLC with UV detection. Two hundred microliters of acetonitrile was added to 200 μl of plasma to precipitate protein. This mixture was vortexed for 20 s, and afterwards the mixture

was centrifuged for 5 min. Fifty microliters of the clear supernatant was used for injection. Chromatographic analysis was performed on an Inertsil 5 ODS 2 analytical column (250 by 4.6 mm [inner diameter]; Varian, Bergen op Zoom, The Netherlands) protected with a Chromguard HPLC column (10 by 3 mm[inner diameter]; Varian). The mobile phase was a mixture of 0.01 M potassium dihydrogen phosphate (62%) and acetonitrile (38%). The flow rate was 1 ml/min, and the wavelength for UV detection was 334 nm. The rifampin retention time was 7.3 min. The rifampin calibration curve was linear over a range of 0.50 to 30.0 mg/liter. The lower limit of quantification for rifampin was 0.50 mg/liter. Recovery after extraction from plasma was 108.5%. Accuracy ranged from 101.3 to 102.2%, and intraday and interday precisions ranged from 2.84 to 3.65% and from 1.59 to 3.68%, respectively.

Safety monitoring and laboratory safety

The medical history, vital signs, a physical examination, and an electrocardiogram for each subject were obtained at screening. Laboratory tests were done at screening and all study visits (days 1, 3, 7, 10, 13, 16, 17, 18, 20, 22, and 24). Laboratory tests included tests for sodium, potassium, creatinine, total bilirubin, cholesterol, triglycerides, glucose, alkaline phosphatase, ASAT, ALAT, GGT, and amylase (pancreatic) levels; a wholeblood cell count; and urinalysis.

Additionally, subjects were asked about the occurrence of adverse events at each visit. Adverse events were assessed for intensity, according to the AIDS Clinical Trials Group classifications, mild (symptoms do not interfere with daily activities), moderate (symptoms interfere with daily activities), and severe (symptoms markedly interrupt daily activities), and seriousness. Serious adverse events were defined as any untoward medical occurrence that at any dose resulted in death, that was life threatening, that required in-patient hospitalization or prolongation of existing hospitalization, that resulted in a persistent or significant disability or incapacity, or that was a congenital anomaly or birth defect. During the study, the occurrence of grade 2 toxicity, according to World Health Organization scales, would result in discontinuation of a subject from the study medication. For cholesterol and triglycerides, grade 3 toxicity was a reason to discontinue study medication.

Pharmacokinetic analysis

Values for the pharmacokinetic parameters of lopinavir, ritonavir, and rifampin were estimated by noncompartmental methods. The C_{max} and the time to C_{max} (T_{max}) were determined directly from the plasma concentration-time data. C_{min} and the morning predosing observed trough concentration in plasma (C_0) were also determined directly from the plasma concentration-time data. The area under the plasma concentration-time curve from time zero to 12 h postdosing (AUC_{12}) was calculated by use of the linear trapezoidal rule. The value of the peak-to-trough rate constant (β) was obtained from the slope of the least-squares regression of the logarithms of the plasma concentration-versus-time data for the 12-h interval, which was then used to calculate the half-life ($T_{1/2}$). The dosing interval or peak-to-trough $T_{1/2}$ was calculated as ln

2/β. The apparent oral clearance value (CL/*F*), where *F* is the bioavailability, was calculated by dividing the administered dose in a dosing interval by AUC_{12}. CL/*F* was normalized for body weight (CL/*F*.kg) by dividing by the weight (in kilograms). The apparent volume of distribution (V/*F*) was calculated by dividing CL/*F* by β. V/*F* was normalized for body weight (V/*F*.kg) by dividing by the weight (in kilograms).

Statistical analysis

The pharmacokinetic data for lopinavir, ritonavir, and rifampin are presented as arithmetic means ± standard deviations and geometric means. The data were logarithmically transformed for the calculation of geometric means. The median and interquartile ranges are presented for T_{max}. The change in a pharmacokinetic variable for lopinavir or ritonavir from the administration of lopinavir/ritonavir alone to the administration of the combination regimen with rifampin was analyzed by a paired *t* test for each of the study arms. A Wilcoxon signed-ranks test was used for T_{max}. *P* values less than 0.05 were considered statistically significant. Variables included logarithmically transformed AUC_{12}, C_{min}, C_0, and C_{max} and nontransformed T_{max} and $T_{1/2}$. The bioavailability ratio for the combination regimen relative to that for lopinavir alone was assessed by the two one-sided-tests procedure with 90% confidence intervals for AUC_{12}, C_{min}, C_0, and C_{max}. For this purpose geometric mean ratios were calculated by dividing the geometric mean values for study day 24 by the geometric mean values for study day 10. The 90% confidence intervals of the geometric mean ratios were obtained by exponentiating the confidence limits for the differences in logarithmic means. The geometric mean ratios together with the 90% confidence intervals were compared to the range of 0.80 to 1.25 to determine whether the lopinavir/ritonavir dose regimens combined with rifampin met the criteria for bioequivalence to the standard clinical dose of lopinavir/ritonavir.

Additionally, lopinavir C_0s on study day 7 versus those on study day 10 and lopinavir C_0s on study day 22 versus those on study day 24 were tested by the paired-samples *t* test to evaluate whether steady state was achieved. A power calculation was performed in the development phase of the study. The calculation, based on the lopinavir C_0, indicated that data for nine subjects were needed in each study arm. As a dropout rate of 40 to 50% was assumed, 16 subjects were included in each study arm.

Results

Subjects

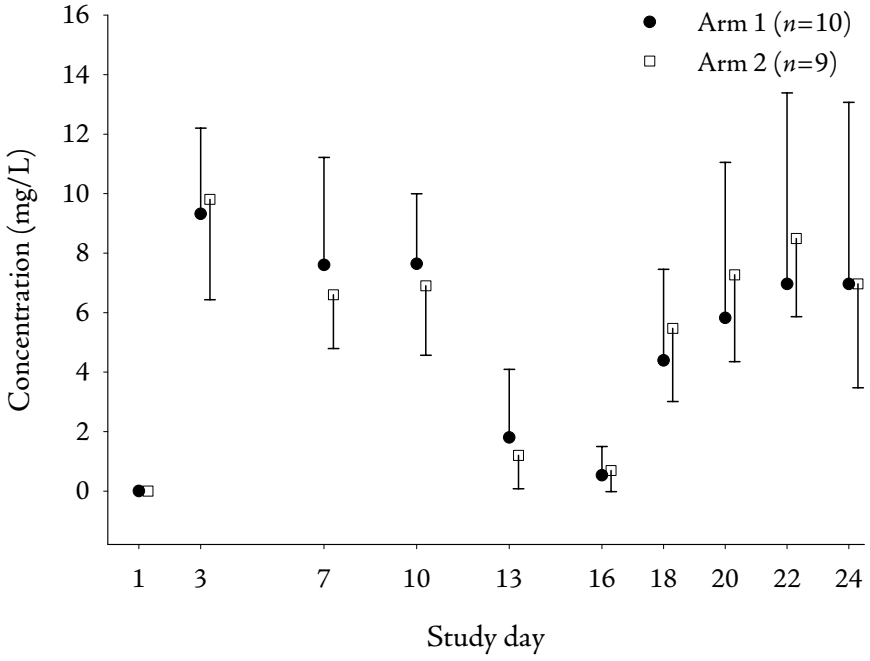
Thirty-two subjects (18 males, 14 females) were included in the study, of which 20 completed the study. Twelve subjects dropped out for reasons of adverse events or laboratory abnormalities. Data for all 32 subjects participating in the study were included in the safety analyses. Pharmacokinetic data for 1 of the 20 subjects who completed the study were not evaluable due to vomiting shortly after drug administration on

day 24. For this reason, statistical analyses for pharmacokinetics were performed with data for 19 subjects, 10 in arm 1 and 9 in arm 2. The 10 subjects in arm 1 (4 males, 6 females) had a mean age of 37 years (range, 22 to 70 years), a mean height of 1.70 m (range, 1.61 to 1.85 m), and a mean weight of 70.6 kg (range, 61.5 to 77.0 kg). Of these 10 subjects, 1 was black; all others were Caucasian. The nine subjects in arm 2 (7 males, 2 females) had a mean age of 36 years (range, 25 to 47 years), a mean height of 1.80 m (range, 1.58 to 1.90 m), and a mean weight of 75.4 kg (range, 60.5 to 85.4 kg). All nine subjects in arm 2 were Caucasian.

Lopinavir pharmacokinetics

Figure 1 shows the arithmetic mean ± standard deviation trough lopinavir levels in plasma in the morning obtained during the study. Trough lopinavir levels in plasma were not statistically different between days 7 and 10, suggesting that in both arms steady state was reached after 10 days of treatment with lopinavir/ritonavir at 400/100 mg BID (P = 0.5 and P = 0.5 for comparison of C_0s on day 7 versus that on day 10 for arms 1 and 2, respectively).

Figure 1. Lopinavir trough levels throughout the study



Day 1-10; lopinavir/ritonavir (LPV/r) 400/100 mg BID; Day 11-15; LPV/r 400/100 mg BID + rifampin (RIF); Day 16-17; LPV/r dose escalation + RIF; Day 18-24; Arm 1: LPV/r 800/200 mg BID + RIF, Arm 2: LPV/r 400/400 mg BID + RIF. Data are presented as aritmetic mean values with standard deviations in error bars.

Table 2. Summary of steady state pharmacokinetics of lopinavir

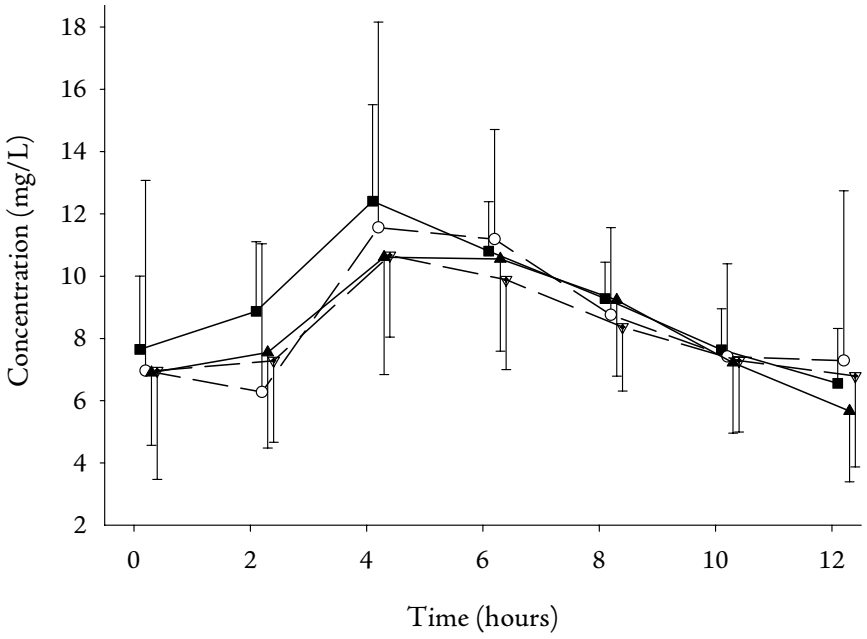
Parameter[a]	Aritmetic mean ± standard deviation (Geometric mean)		*P*-value[b]	Geometric mean ratio (day 24 / day 10) and 90% CI[c]
	Study day 10	Study day 24		
Arm 1[d] (*n*=10)				
AUC_{12} (h.mg/L)	111.8 ± 19.03 (110.1)	104.5 ± 46.86 (92.3)	0.27	0.84 [0.64-1.10]
C_{min} (mg/L)	6.5 ± 1.83 (6.2)	5.1 ± 4.17 (2.7)	0.08	0.43 [0.19-0.96]
C_0 (mg/L)	7.6 ± 2.36 (7.3)	7.0 ± 6.11 (3.4)	0.14	0.46 [0.19-1.10]
C_{max} (mg/L)	12.9 ± 2.50 (12.6)	13.8 ± 4.89 (12.9)	0.84	1.02 [0.85-1.23]
T_{max} (h)	4.0 (4.0-5.4)[e]	4.1 (4.0-6.0)[e]	0.40[f]	-
$T_{1/2}$ (h)	6.8 ± 1.81 (6.6)	7.2 ± 4.28 (6.1)	0.72	-
Cl/*F*.kg (L/h.kg)	0.06 ± 0.01 (0.06)	0.18 ± 0.18 (0.13)	-	-
Vd/*F*.kg (L/kg)	0.56 ± 0.15 (0.55)	2.16 ± 3.77 (1.13)	-	-
Arm 2[d] (*n*=9)				
AUC_{12} (h.mg/L)	102.9 ± 26.09 (99.9)	100.7 ± 26.81 (97.4)	0.81	0.98 [0.81-1.17]
C_{min} (mg/L)	5.2 ± 1.88 (4.9)	5.9 ± 2.73 (5.1)	0.91	1.03 [0.68-1.56]
C_0 (mg/L)	6.9 ± 2.33 (6.5)	7.0 ± 3.50 (5.8)	0.64	0.89 [0.56-1.40]
C_{max} (mg/L)	12.3 ± 3.22 (11.9)	11.5 ± 3.07 (11.1)	0.35	0.93 [0.81-1.07]
T_{max} (h)	4.0 (4.0-6.0)[e]	6.0 (4.0-6.0)[e]	0.12[f]	-
$T_{1/2}$ (h)	6.2 ± 2.41 (5.8)	8.4 ± 3.34 (7.9)	0.05	-
Cl/*F*.kg (L/h.kg)	0.06 ± 0.01 (0.06)	0.06 ± 0.02 (0.06)	-	-
Vd/*F*.kg (L/kg)	0.51 ± 0.18 (0.48)	0.72 ± 0.32 (0.66)	-	-

[a] AUC_{12}: the area under the plasma concentration-time curve from time 0 to 12 hours post-dose, C_{min}: the minimum observed plasma concentration, C_0: the pre-dose observed plasma concentration, C_{max}: the maximum observed plasma concentration, T_{max}: the time to C_{max}, $T_{1/2}$: the dosing interval or peak to trough half-life, CL/*F*.kg: the apparent oral clearance value normalized for body weight, Vd/*F*.kg: volume of distribution corrected for body weight.
[b] *P*-value for the difference between the 2 study periods, two-sided t-test for paired data.
[c] CI: confidence interval
[d] Arm 1 (*n*=10): lopinavir/ritonavir 800/200 mg BID, Arm 2 (*n*=9): lopinavir/ritonavir 400/400 mg BID
[e] median and interquartile range
[f] Wilcoxon signed-ranks test

On study day 16 (when lopinavir/ritonavir at 400/100 mg BID was combined with rifampin at 600 mg QD), trough lopinavir levels decreased 93% in arm 1 and 90% in arm 2 in comparison to those on study day 10. Trough lopinavir levels increased in both study arms after lopinavir/ritonavir dosages were titrated to 800/200 mg BID in arm 1 and 400/400 mg BID in arm 2 and administered in combination with rifampin at 600 mg QD (days 18, 20, 22, and 24). Trough lopinavir levels in plasma were not statistically different between days 22 and 24, suggesting that steady state was reached (P = 0.7 and P = 0.15 for comparison of C_0s on day 22 versus those on

day 24 for arms 1 and 2, respectively). The values for the lopinavir pharmacokinetic parameters are summarized in Table 2. Arithmetic means ± standard deviations and geometric means are included for study days 10 and 24 for both arm 1 and arm 2. Note that these geometric means result in values different from the arithmetic means in Figure 1 and 2. The *P* values for the within-subject differences between days 10 and 24 are presented in Table 2 as well. Table 2 also presents the geometric mean ratio (day 24/day 10) and the 90% confidence interval for AUC_{12}, C_{min}, C_0, and C_{max}. For arm 1 (n = 10; lopinavir/ritonavir at 800/200 mg BID in combination with rifampin at 600 mg QD), no statistically significant differences were observed between the values of the pharmacokinetic parameters for days 10 and 24. Due to intersubject variability, the geometric mean ratio and matching confidence interval met the criteria for bioequivalence only for C_{max}. Geometric mean ratios for C_{min} and C_0 showed decreases of 57 and 54%, respectively, from days 10 to 24, and the lopinavir AUC_{12} for arm 1 decreased by 16%. The total variability (coefficient of variation) in the lopinavir C_{min} in arm 1 was 28% on day 10, whereas it was 81% on day 24.

Figure 2. Lopinavir steady-state concentration-time profiles



Arm 1(n=10); lopinavir/ritonavir 400/100 mg BID (Day 10)
Arm 1(n=10); lopinavir/ritonavir 800/200 mg BID + rifampin (Day 24)
Arm 2 (n=9); lopinavir/ritonavir 400/100 mg BID (Day 10)
Arm 2 (n=9); lopinavir/ritonavir 400/400 mg BID + rifampin (Day 24)
Data are presented as aritmetic mean values with standard deviations in error bars

Figure 2 displays the arithmetic mean plasma-concentration time profiles for lopinavir in arm 1 for both study day 10 and study day 24. The error bars in Figure 2 show this larger variability on day 24. In arm 2 (n = 9; lopinavir/ritonavir at 400/400 mg BID in combination with rifampin at 600 mg QD), the geometric mean ratios and their matching confidence intervals meet the criteria for bioequivalence for AUC_{12} and C_{max} (Table 2). Although the geometric mean ratios are within 11% of unity for C_{min} (+3%) and C_0 (–11%), the 90% confidence intervals for the geometric mean ratios of C_{min} and C_0 exceed both the upper and the lower limits of the predefined bioequivalence range of 0.80 to 1.25. No statistically significant differences between the values of the pharmacokinetic parameters obtained on days 10 and 24 were observed. The total variabilities (coefficients of variation) in lopinavir C_{min}s in arm 2 on days 10 and 24 were 36 and 46%, respectively. Figure 2 displays the arithmetic mean plasma-concentration time profiles for lopinavir in arm 2 for both study day 10 and study day 24. The error bars in Figure 2 represent the standard deviation of the mean and show variabilities of the same magnitude on both study days.

Ritonavir pharmacokinetics

The values of the steady-state pharmacokinetic parameters for ritonavir are summarized in Table 3. Arithmetic means ± standard deviations and geometric means are included for study days 10 and 24 for both arm 1 and arm 2. The P values for the within-subject differences between days 10 and 24 are displayed as well. The geometric mean ratio (day 24/day 10) and 90% confidence interval are given for AUC_{12}, C_{min}, C_0, and C_{max}. Geometric mean ratios in arm 1 showed increases in AUC_{12}, C_{min}, C_0, and C_{max} of 42, 11, 17, and 75%, respectively, with an increase in the ritonavir dosage from 100 to 200 mg BID from days 10 to 24. A statistically significant difference in the ritonavir C_{max} was found between study days 10 and 24 (P = 0.01). No statistically significant differences in C_{min}, C_0, and AUC_{12} were observed. In arm 2, geometric mean ratios showed increases in the ritonavir AUC_{12}, C_{min}, C_0, and C_{max} of 7.1-, 4.9-, 4.7-, and 8.4-fold, respectively, with the increase in the ritonavir dosage from 100 to 400 mg BID from days 10 to 24. These differences were statistically significant for AUC_{12}, C_{min}, C_0, and C_{max} from days 10 to 24 ($P < 0.001$ for all comparisons) (Table 3). No significant differences in T_{max} or $T_{1/2}$ were observed in either arm.

Rifampin pharmacokinetics

The values of the steady-state pharmacokinetic parameters for rifampin on day 24 are presented in Table 4. Arithmetic means ± standard deviations and geometric means are displayed. Data are grouped by study arm; arm 1 denotes lopinavir/ritonavir at 800/200 mg BID in combination with rifampin at 600 mg QD, and arm 2 denotes lopinavir/ritonavir at 400/400 mg BID in combination with rifampin at 600 mg QD. No statistically significant differences in rifampin pharmacokinetics between arms 1 and 2 were found.

Table 3. Summary of steady state pharmacokinetics of ritonavir

Parameter[a]	Aritmetic mean ± standard deviation (Geometric mean)		*P*-value[b]	Geometric mean ratio (day 24 / day 10) and 90% CI[c]
	Study day 10	Study day 24		
Arm 1[d] (*n*=10)				
AUC_{12} (h.mg/L)	6.6 ± 2.26 (6.3)	10.7 ± 5.83 (8.9)	0.05	1.42 [1.07-1.90]
C_{min} (mg/L)	0.19 ± 0.10 (0.17)	0.24 ± 0.32 (0.18)	0.67	1.11 [0.72-1.73]
C_0 (mg/L)	0.28 ± 0.15 (0.24)	0.47 ± 0.58 (0.28)	0.64	1.17 [0.65-2.13]
C_{max} (mg/L)	1.37 ± 0.73 (1.20)	2.50 ± 1.37 (2.10)	0.01	1.75 [1.31-2.34]
T_{max} (h)	4.0 (4.0-5.4)[e]	4.0 (4.0-5.5)[e]	0.67[f]	-
$T_{1/2}$ (h)	2.7 ± 1.09 (2.6)	2.7 ± 1.58 (2.3)	0.56	-
Cl/*F*.kg (L/h.kg)	0.25 ± 0.09 (0.24)	0.40 ± 0.30 (0.31)	-	-
Vd/*F*.kg (L/kg)	0.98 ± 0.37 (0.89)	1.26 ± 0.80 (1.03)	-	-
Arm 2[d] (*n*=9)				
AUC_{12} (h.mg/L)	5.6 ± 1.92 (5.3)	41.5 ± 16.83 (37.9)	<0.001	7.12 [5.85-8.66]
C_{min} (mg/L)	0.17 ± 0.05 (0.16)	0.96 ± 0.57 (0.79)	<0.001	4.89 [3.22-7.43]
C_0 (mg/L)	0.24 ± 0.09 (0.22)	1.39 ± 0.97 (1.06)	<0.001	4.73 [2.89-7.73]
C_{max} (mg/L)	1.16 ± 0.60 (1.01)	9.63 ± 4.86 (8.47)	<0.001	8.38 [6.59-10.64]
T_{max} (h)	4.0 (4.0-6.0)[e]	4.0 (4.0-4.0)[e]	0.52[f]	-
$T_{1/2}$ (h)	3.1 ± 0.71 (3.1)	2.3 ± 0.99 (2.2)	0.07	-
Cl/*F*.kg (L/h.kg)	0.29 ± 0.14 (0.27)	0.16 ± 0.10 (0.14)	-	-
Vd/*F*.kg (L/kg)	1.39 ± 0.90 (1.18)	0.56 ± 0.43 (0.46)	-	-

[a] AUC_{12}: the area under the plasma concentration-time curve from time 0 to 12 hours post-dose, C_{min}: the minimum observed plasma concentration, C_0: the pre-dose observed plasma concentration, C_{max}: the maximum observed plasma concentration, T_{max}: the time to C_{max}, $T_{1/2}$: the dosing interval or peak to trough half-life, CL/*F*.kg: the apparent oral clearance value normalized for body weight, Vd/*F*.kg: volume of distribution corrected for body weight.

[b] *P*-value for the difference between the 2 study periods, two-sided t-test for paired data.

[c] CI: confidence interval

[d] Arm 1 (*n*=10): lopinavir/ritonavir 800/200 mg BID, Arm 2 (*n*=9): lopinavir/ritonavir 400/400 mg BID

[e] median and interquartile range

[f] Wilcoxon signed-ranks test

Table 4. Steady state pharmacokinetics of rifampin on study day 24

Parameter[a]	Aritmetic mean ± standard deviation (Geometric mean)	
	Arm 1[b] (*n*=10)	**Arm 2[b] (*n*=9)**
AUC_{12} (h.mg/L)	79.2 ± 33.84 (72.2)	76.6 ± 31.87 (70.3)
C_{max} (mg/L)	14.2 ± 5.61 (13.0)	15.0 ± 3.80 (14.5)
T_{max} (h)	4.0 (3.0-4.0)[c]	4.0 (3.0-4.0)[c]
$T_{1/2}$ (h)	3.12 ± 1.75 (2.8)	2.5 ± 1.33 (2.2)
Cl/*F*.kg (L/h.kg)	0.12 ± 0.06 (0.11)	0.11 ± 0.06 (0.10)
Vd/*F*.kg (L/kg)	0.45 ± 0.16 (0.43)	0.34 ± 0.10 (0.33)

[a] AUC_{12}: the area under the plasma concentration-time curve from time 0 to 12 hours post-dose, C_{max}: the maximum observed plasma concentration, T_{max}: the time to C_{max}, $T_{1/2}$: the dosing interval or peak to trough half-life, CL/*F*.kg: the apparent oral clearance value normalized for body weight, Vd/*F*.kg: volume of distribution corrected for body weight.
[b] Arm 1 (*n*=10): lopinavir/ritonavir 800/200 mg BID, Arm 2 (*n*=9): lopinavir/ritonavir 400/400 mg BID
[c] median and interquartile range

Adverse events

Most (87%) of the adverse events were mild. Three adverse events (not related to a study medication) were reported to be severe; these were cases of gastroenteritis, influenza, and headache. Serious adverse events did not occur. Twelve of 32 subjects (38%) were prematurely discontinued from the study; 3 of these subjects discontinued the study prior to randomization (while receiving lopinavir/ritonavir at 400/100 mg BID alone). One subject was prematurely discontinued from the study for grade 2 total bilirubin level elevations (>31 μmol/liter), which predominantly consisted of indirect bilirubin. However, this subject did not have concurrent grade 2+ ALAT, ASAT, or alkaline phosphatase level elevations. One additional subject was prematurely discontinued from the study for grade 3 elevations in cholesterol levels (>7.77 mmol/liter) and triglyceride levels (>8.48 mmol/liter), both of which subsequently declined to below grade 3 elevations following discontinuation of the study medication. A third subject was prematurely discontinued from the study for a complex of vomiting and abdominal pain. These complaints disappeared after the study medication was discontinued. There were half as many subject discontinuations in arm 1 (lopinavir/ritonavir at 800/200 mg BID and rifampin at 600 mg QD; three subjects discontinued the medication; one subject developed grade 2 elevations in ASAT, ALAT, and GGT levels; one subject developed a grade 2 elevation in ASAT levels and a grade 3 elevation in ALAT levels; and one subject suffered from vomiting and diarrhoea) as in arm 2 (lopinavir/ritonavir at 400/400 mg BID with rifampin at 600 mg QD; six subjects discontinued the medication; three subjects developed grade 2 elevations in ASAT and ALAT levels; two subjects developed grade 2 elevations in ALAT levels; and one subject suffered from nausea, abdominal pain, fatigue, shivers, and increased sweating).

During the study, six subjects (two in arm 1, four in arm 2) had grade 2 elevations in ALAT levels (>2.6 times the ULN) and three subjects (one in arm 1, two in arm 2) had grade 3 elevations in ALAT levels (>5.1 times the ULN). Five of these subjects experienced concurrent grade 2 elevations in ASAT levels (>2.6 times the ULN). Seven of the nine subjects with grade 2 to 3 elevations in ALAT levels (two in arm 1, five in arm 2) were prematurely discontinued from the study. The other two subjects developed elevations in liver enzyme levels on or after study day 24. The onset of all grade 2 or 3 elevations in ALAT and ASAT levels was after the initiation of rifampin treatment, but none of these were associated with grade 2+ elevations in total bilirubin or alkaline phosphatase levels. After discontinuation of the study medication, all such elevations declined below those for grade 2 toxicity, with only two remaining above the ULN at the final study evaluation.

Laboratory measurements

The mean change from the baseline values to the maximum values as well as the mean change from the baseline values to the final values was determined for a number of laboratory parameters. The baseline was day 1 for the period from day 1 to 10, and the baseline was day 10 for the period from days 11 to 24. For ASAT, the mean changes from the baseline value (day 10) to the maximum value (final value) were 39.9 (2.4) and 39.9 (1.1) IU/liter in arms 1 and 2, respectively, for study days 11 to 24. For ALAT, the mean changes from the baseline value (day 10) to the maximum value (final value) were 72.6 (13.5) and 89.3 (6.9) IU/liter in arms 1 and 2, respectively, for study days 11 to 24. For alkaline phosphatase, the mean changes from the baseline value (day 10) to the maximum value (final value) were 12.9 (3.0) and 16.3 (5.0) IU/liter in arms 1 and 2, respectively, for study days 11 to 24. No clinically relevant changes in ASAT, ALAT, and alkaline phosphatase levels were seen on study days 1 to 10 (lopinavir/ritonavir at 400/100 mg BID alone). For total bilirubin, the mean change from the baseline value (day 1) to the maximum value (final value) was 10 (4.7) μmol/liter for study days 1 to 10. For study days 11 to 24, the mean changes in the total bilirubin level from the baseline value (day 10) to the maximum value (final value) were 0.9 (–9.1) and 1.3 (–3.9) μmol/ liter in study arms 1 and 2, respectively.

Discussion

In the present study, two adjusted-dose regimens of lopinavir/ritonavir in combination with rifampin were compared to the standard dose of lopinavir/ritonavir without rifampin. The steady-state pharmacokinetics of lopinavir and ritonavir were determined after 10 days of treatment with the standard dose of lopinavir/ritonavir (400/100 mg BID). In the second part of the study, the steady-state pharmacokinetics of lopinavir/ritonavir at 800/200 mg BID with rifampin at 600 mg QD (arm 1) and lopinavir/ritonavir at 400/400 mg BID with rifampin at 600 mg QD (arm 2) were assessed.

Lopinavir

Lopinavir exposure was substantially higher in both study arms compared to the historical data obtained for lopinavir/ritonavir at 400/100 mg BID in combination with rifampin at 600 mg QD. This historical interaction study with a standard dose of lopinavir/ritonavir with rifampin was conducted with 22 healthy subjects to assess the effects of multiple doses of rifampin at 600 mg QD on the pharmacokinetics of lopinavir after treatment with lopinavir/ritonavir at 400/100 mg BID. The values of the pharmacokinetic parameters for lopinavir were substantially reduced by the coadministration of lopinavir/ritonavir with rifampin, as follows: C_{max} by 45%, AUC by 75%, and C_{min} by 99%[14].

While the concentrations of lopinavir are dramatically increased when lopinavir/ritonavir is used in combination with rifampin with both of the dosing regimens, it could not be demonstrated that the adjusted-dose regimens with rifampin evaluated in the present study were equivalent, particularly with respect to C_{min}, to the standard dose of lopinavir/ritonavir without rifampin. This indicates that the adjusted-dose regimens may in some cases not be capable of completely compensating for the accelerated metabolism of lopinavir by rifampin. Data are also limited by the relatively small number of subjects who completed the study and for whom pharmacokinetic data were evaluable. However, the level of lopinavir exposure in arm 2 was more comparable to that obtained with a standard dose lopinavir/ritonavir without rifampin than was the level of lopinavir exposure in arm 1. The pharmacokinetics of lopinavir in arm 1 were more variable than those in arm 2. It could be that the higher dose of ritonavir given in arm 2 (400 mg BID) resulted in a more consistent inhibition of lopinavir metabolism compared to that achieved with the ritonavir dose given in arm 1 (200 mg BID). Note that on study day 24, a total of 4 of 10 subjects (40%) in arm 1 had a C_{min} lower than the lowest value observed on study day 10 (C_{min}, <3.7 mg/liter). In contrast, only one of the nine subjects (11%) in arm 2 had a C_{min} lower than the lowest value observed on study day 10 (C_{min}, <3.1 mg/liter). The study was not designed to show a difference in lopinavir exposures between study arms. However, by taking into account the greater variability in lopinavir pharmacokinetics in arm 1, therapeutic drug monitoring might prove to be useful in clinical practice to monitor for possible subtherapeutic C_{min}s of lopinavir in plasma and individually optimize the lopinavir/ritonavir dosing regimen in a given patient. In arm 2, the C_{min}s of lopinavir in plasma tended to be higher, possibly making therapeutic drug monitoring of less importance.

Ritonavir

For ritonavir, it is apparent that in arm 1 a twofold increase in the ritonavir dose from 200 mg/day in the absence of rifampin to 400 mg/day in the presence of rifampin resulted in a less than proportional increase in plasma ritonavir concentrations. In arm 2, considerably higher plasma ritonavir concentrations were achieved during treatment with lopinavir/ritonavir at 400/400 mg BID in combination with rifampin compared to those achieved with lopinavir/ritonavir at 400/100 mg BID alone. In fact, when ritonavir is administered in combination with lopinavir, the ritonavir C_{max} and AUC_{12}

increase more than proportionally due to nonlinear pharmacokinetics[16] when the total daily dose is increased fourfold from 200 mg/day in the absence of rifampin to 800 mg/day in the presence of rifampin. The ritonavir AUC_{12} was approximately fourfold higher when lopinavir/ritonavir at 400/400 mg BID was coadministered with rifampin than when lopinavir/ritonavir at 800/200 mg BID was coadministered with rifampin. These observed effects of ritonavir exposure indicate that the inhibition of CYP3A by ritonavir is more complete and less subject to induction by rifampin when ritonavir is dosed at 400 mg BID than when it is dosed at 200 mg BID.

Rifampin

Data for rifampin in the literature[6] report a mean C_{max} and a mean AUC of 8 to 20 mg/liter and 60 to 80 mg x h/liter, respectively. The mean values for C_{max} and AUC observed in this study are within these ranges (Table 4). This indicates that lopinavir/ritonavir does not affect the pharmacokinetics of rifampin. The fact that no statistically significant differences in the pharmacokinetic parameters for rifampin were observed between arm 1 and arm 2, as shown in Table 4, provides further evidence that these different doses of lopinavir and ritonavir had no influence on rifampin exposure. The literature also reports[6,17] that rifampin intake with food can decrease the rifampin C_{max}. It is noteworthy that in the present study rifampin was administered with lopinavir/ritonavir at breakfast (550 kcal, 28% fat). Nevertheless, in this study the rifampin C_{max} did not show the decrease that has been reported before. Data in the literature indicate that the T_{max} is 1.5 to 2.0 h under fasting conditions. In a trial studying the single-dose pharmacokinetics of rifampin under fasting conditions[17], with food, and with antacids, the observed T_{max} was 4.43 h after a high-fat breakfast (792 kcal, 57% fat). In the present study, the median T_{max} was about 4 h in both study arms; this delay of T_{max} was probably the result of the intake with food. However, in clinical practice, the rifampin C_{max} is the main pharmacokinetic parameter of interest[6]. Therefore, the clinical relevance of the delay in T_{max} is limited, as in the present study the mean C_{max}s were well within the previously reported ranges[6].

Safety

The most common adverse events, reported by 50% of subjects, included urine discoloration, which is a known effect of rifampin therapy[18]; nausea; headache; diarrhoea; abdominal pain and cramps; and fatigue. The majority of all adverse events were mild (87%), with approximately 13% judged to be of moderate severity and only three events (gastroenteritis, influenza, and headache) reported to be severe. None of the adverse events met the regulatory definition of serious. A number of subjects had to discontinue the study prematurely due to elevations in liver function test results, with the onset of the elevations occurring after the initiation of combination lopinavir/ritonavir and rifampin dosing. A greater number of discontinuations occurred among the subjects in the arm receiving lopinavir/ritonavir at 400/400 mg BID plus rifampin at 600 mg QD. However, the study design did not allow an assessment of whether the frequency or magnitude of the elevations in the liver function test results seen

with lopinavir/ritonavir in combination with rifampin was different between the study arms. No clinically significant hematology or urinalysis values were observed in the study.

Overall, tolerability limitations were observed with the coadministration of lopinavir/ritonavir and rifampin in healthy subjects. The high rate of discontinuations observed was primarily a result of the elevations in the liver function test results that occurred after the initiation of lopinavir/ritonavir and rifampin coadministration. However, there was no dosing segment with rifampin alone to allow determination of whether the liver function test abnormalities observed during combination lopinavir/ritonavir and rifampin treatment were of a greater magnitude or incidence than would have been observed with rifampin administration alone to healthy subjects. Nevertheless, the increased rate of elevations in hepatic transaminase levels seen in both combination-treatment arms warrants the use of caution when these two drugs are administered concurrently to patients infected with both HIV and *M. tuberculosis*.

Conclusions

The present recommendations from the Centers for Disease Control and Prevention indicate that rifampin can be used in conjunction with efavirenz at 800 mg QD[10]. The combination of efavirenz with rifampin has been studied in a group of 24 HIV-infected patients coinfected with *M. tuberculosis*[9]. Other combination regimens that have been considered for use for the simultaneous treatment of HIV and *M. tuberculosis* infections are limited.

The combination of saquinavir/ritonavir at 400/400 mg BID with rifampin, as mentioned in the Introduction, has its parallels with the combination evaluated in the present study. However, the data for the saquinavir/ritonavir combination were only presented as a case report.

The product Trizivir combines three NRTIs, namely, zidovudine, lamivudine, and abacavir, and could be an option for use in combination with rifampin. Nevertheless, the pharmacokinetics of this combination of NRTIs in combination with rifampin were not studied, and recently, this combination of NRTIs was shown to be less effective than an efavirenz-based regimen[19] and therefore will not be an option of first choice.

Centers for Disease Control and Prevention guidelines also suggest the use of rifabutin[5,10]. However, complex bidirectional interactions are to be expected when rifabutin is combined with PIs[6]. To compensate for these interactions, rifabutin doses must be decreased in some cases, or the PIs should be given at higher doses to compensate for accelerated metabolism. The combination of rifabutin with the NNRTIs efavirenz and delavirdine results in pharmacokinetic interactions as well[10]; however, nevirapine can be used in combination with rifabutin, although no data from clinical studies have been published[10]. It was reported from a study with HIV-infected inmates during a tuberculosis outbreak in a prison that, regardless of the rifabutin dosage, rifabutin concentrations are highly unpredictable, probably due to drug-drug interactions[20].

From these data it becomes clear that the combination of rifabutin with PIs or NNRTIs remains a therapeutic challenge.
The presently studied combination of lopinavir/ritonavir, dosed as either 800/200 mg BID or 400/400 mg BID with rifampin, may be considered for the treatment of HIV-infected persons who are coinfected with *M. tuberculosis*. The toxicity observed when higher-dose lopinavir/ritonavir and rifampin were administered together led to discontinuation in 31% (9 of 29) of the healthy subjects during this study. When these drugs are used to treat patients who use other hepatically metabolized drugs, who are receiving long-term chronic treatment, and who have concomitant disease, the adverse event profile observed in the present study might even worsen. Therefore, the treatment of HIV-infected patients with tuberculosis with these agents should be approached with caution, and close monitoring of liver function will be needed. Therapeutic monitoring of the pharmacokinetics of lopinavir may be useful for the detection of minimal levels in plasma that are markedly below the expected mean, particularly in those patients treated with 800/200 mg BID, as well as to optimize the dosing regimen of lopinavir/ritonavir in combination with rifampin. If therapeutic drug monitoring is not possible, the combination of lopinavir and ritonavir at 400/400 mg BID may be preferred, although the rates of elevations in liver function test results achieved with that regimen tended to be higher than those achieved with the lopinavir/ritonavir regimen of 800/200 mg BID.

Acknowledgements

We thank the subjects who participated in this study and Alina Bergshoeff, Jackie Droste, Marga de Graaff, Rosan Rosmalen, and the laboratory technicians of the Department of Clinical Pharmacy for help during performance and analysis of the results of this study. Kevin Niemi of the Antiviral Global Team at Abbott Laboratories, Abbott Park, Ill., is thanked for logistical and operational support to the study report.
This study was funded by a grant from Abbott Laboratories.

References

1. **Egger, M., M. May, G. Chene, A. N. Phillips, B. Ledergerber, F. Dabis, D. Costagliola, M. A. D'Arminio, F. de Wolf, P. Reiss, J. D. Lundgren, A. C. Justice, S. Staszewski, C. Leport, R. S. Hogg, C. A. Sabin, M. J. Gill, B. Salzberger, and J. A. Sterne**. 2002. Prognosis of HIV-1-infected patients starting highly active antiretroviral therapy: a collaborative analysis of prospective studies. Lancet **360**:119-129.
2. **Bowen, E. F., P. S. Rice, N. T. Cooke, R. J. Whitfield, and C. F. Rayner**. 2000. HIV seroprevalence by anonymous testing in patients with Mycobacterium tuberculosis and in tuberculosis contacts. Lancet **356**:1488-1489.

3. **Msamanga, G. I. and W. W. Fawzi**. 1997. The double burden of HIV infection and tuberculosis in sub-Saharan Africa. N.Engl.J.Med. **337**:849-851.
4. **Schluger, N. W.** 1999. Issues in the treatment of active tuberculosis in human immunodeficiency virus-infected patients. Clinical Infectious Diseases **1999**:130-135.
5. **Centers for Disease Control and Prevention**. 1998. Prevention and treatment of tuberculosis among patients infected with human immunodeficiency virus: principles of therapy and revised recommendations. Morb.Mortal.Wkly.Rep. **47**(RR-20), 1-58.
6. **Burman, W. J., K. Gallicano, and C. Peloquin**. 2001. Comparative pharmacokinetics and pharmacodynamics of the rifamycin antibacterials. Clin.Pharmacokinet. **40**:327-341.
7. **Piscitelli, S. C. and K. D. Gallicano**. 2001. Interactions among drugs for HIV and opportunistic infections. N.Engl.J.Med. **344**:984-996.
8. **Veldkamp, A., R. M. W. Hoetelmans, J. H. Beijnen, J. W. Mulder, and P. L. Meenhorst**. 1999. Ritonavir enables combined therapy with rifampin and saquinavir. Clinical Infectious Diseases **29**:1586.
9. **Lopez-Cortes, L. F., R. Ruiz-Valderas, P. Viciana, A. Alarcon-Gonzalez, J. Gomez-Mateos, E. Leon-Jimenez, M. Sarasanacenta, Y. Lopez-Pua, and J. Pachon**. 2002. Pharmacokinetic interactions between efavirenz and rifampicin in HIV-infected patients with tuberculosis. Clin. Pharmacokinet. **41**:681-690.
10. **Centers for Disease Control and Prevention**. 2000. Updated guidelines for the use of rifabutin or rifampin for the treatment and prevention of tuberculosis among HIV-infected patients taking protease inhibitors or nonnucleoside reverse transcriptase inhibitors. Morb.Mortal.Wkly.Rep. **49**:185-189.
11. **Pozniak, A. L., R. Miller, and L. P. Ormenod**. 1999. The treatment of tuberculosis in HIV-infected persons. AIDS **1999**:435-445.
12. Fuzeon® Summary of Product Characteristics. 2003. Roche Registration Limited, Hertfordshire, United Kingdom.
13. Kaletra® Summary of Product Characteristics. 2000. Abbott, Chicago, IL, USA.
14. **Bertz, R., A. Hsu, W. Lam, L. Williams, C. Renz, M. D. Karol, S. Dutta, A. Carr, Y. Zhang, Q. Wang, S. Schweitzer, C. Foit, A. Andre, B. Bernstein, G. Granneman, and E. Sun**. 2000. Pharmacokinetic interactions between lopinavir/ritonavir (ABT-378/r) and other non-HIV drugs. 5th International Congress on Drug Therapy in HIV Infection.Glasgow, UK, October 2000. Abstract P291.
15. **Hugen, P. W. H., C. P. W. G. M. Verwey-van Wissen, D. M. Burger, E. W. Wuis, P. P. Koopmans, and Y. A. Hekster**. 1999. Simultaneous determination of the HIV-protease inhibitors indinavir, nelfinavir, saquinavir and ritonavir in human plasma by reversed-phase high-performance liquid chromatography. Journal of Chromatography B Biomed Sci Appl **727**:139-149.
16. **Hsu, A., G. R. Granneman, G. Witt, C. Locke, J. Denissen, A. Molla, J. Valdes, J. Smith, K. Erdman, N. Lyons, P. Niu, J. P. Decourt, J. B. Fourtillan, J. Girault, and J. M. Leonard**. 1997. Multiple-dose pharmacokinetics of ritonavir in human immunodeficiency virus-infected subjects. Antimicrob.Agents Chemother. **41**:898-905.
17. **Peloquin, C. A., R. Namdar, M. D. Singleton, and D. E. Nix**. 1999. Pharmacokinetics of rifampin under fasting conditions, with food, and with antacids. Chest **115**:12-18.
18. **Douglas, J. G. and M. J. McLeod**. 1999. Pharmacokinetic factors in the modern drug treatment of tuberculosis. Clin.Pharmacokinet. **37**:127-146.
19. **Gulick, R. M., Ribaudo, H. J., Shikuma, C. M., Lustgarten, S., Meyer, W. A., Klingman, K., Squires, K. E., Snyder, S., and Kuritzkes, D. R**. 2003. ACTG 5095: A comparative study of 3 protease inhibitor-sparing antiretroviral regimens for the initial treatment of HIV infection. Antiviral Therapy **8**(**Suppl. 1**), S194.
20. **Spradling, P., D. Drociuk, S. McLaughlin, L. M. Lee, C. A. Peloquin, K. Gallicano, C. Pozsik, I. Onorato, K. G. Castro, and R. Ridzon**. 2002. Drug-drug interactions in inmates treated for human immunodeficiency virus and Mycobacterium tuberculosis infection or disease: an institutional tuberculosis outbreak. Clin.Infect.Dis. **35**:1106-1112.

Chapter 2

Lopinavir/ritonavir plus saquinavir in salvage therapy; pharmacokinetics, tolerability and efficacy

C.J.L. la Porte[1], J-C. Wasmuth[2], K. Schneider[2], J.K. Rockstroh[2], D.M. Burger[1]

1 Department of Clinical Pharmacy, University Medical Centre, Nijmegen, The Netherlands.
2 Department of Internal Medicine I, University of Bonn, Germany.

AIDS. 2003; 17: 1700-1702

Abstract

Patients receiving a lopinavir/ritonavir and saquinavir dual protease inhibitor-based antiretroviral salvage regimen were studied to evaluate the pharmacokinetics, tolerability and efficacy of the regimen. Pharmacokinetic curves were obtained for lopinavir and saquinavir. Patient records were studied for adverse events and efficacy data. The pharmacokinetics of lopinavir and saquinavir were comparable with literature data, except for the saquinavir 0–12 h area under the curve and maximum concentration. The tolerability of the regimen was good and efficacy was encouraging.

Introduction

HIV-infected patients are treated with highly active antiretroviral therapy (HAART) for longer periods. Second-line and further regimens are seen more often, as patients fail their initial HAART regimen. This development underlines the need for more options in salvage therapies. Dual protease inhibitor (PI)-based HAART might lead to a powerful suppression of HIV infection and improve outcomes in salvage therapy. Accordingly, a combination of lopinavir/ritonavir and saquinavir in standard dosages could be used in the treatment of heavily pre-treated HIV-1-infected patients. Combining saquinavir with lopinavir/ritonavir has the advantage that ritonavir has a 'double boosting' function for both lopinavir and saquinavir. Another advantage of this combination might be the described in-vitro synergy of lopinavir and saquinavir[1]. Negative pharmacokinetic interactions, as seen for lopinavir/ritonavir plus amprenavir[2], are not expected for lopinavir/ritonavir plus saquinavir. Increasing interest in the combination of lopinavir/ritonavir and saquinavir for the treatment of HIV infection has led to several studies on this combination of drugs. However, only sparse data have so far been published. The objectives of the study were to evaluate the pharmacokinetics, tolerability and efficacy of salvage regimens containing lopinavir/ritonavir plus saquinavir.

Methods

Patients older than 18 years who had failed at least three previous antiretroviral regimens including non-nucleoside reverse transcriptase inhibitors and PI were eligible. Selected patients were treated with lopinavir/ritonavir 400/100 mg twice a day and saquinavir soft gel capsules (Fortovase) 1000 mg twice a day; backbone therapy was at the physician's discretion. All patients had been on the current treatment for at least 4 weeks before the pharmacokinetics were studied. To evaluate the pharmacokinetics of lopinavir and saquinavir, patients received a morning dose together with standard breakfast at the clinic. Blood samples were drawn immediately before and up to 12 h after dosing. All available plasma samples were measured using a modified version of a validated high-performance liquid chromatography method[3]. Non-compartmental methods were used to evaluate drug concentration versus time data. The pharmacokinetic parameters evaluated were: area under the curve (AUC_{0-12h}), maximum concentration (C_{max}), minimum concentration (C_{min}), apparent clearance (Cl/F) and half-life ($T_{1/2}$). Pharmacokinetic data were compared with literature data of lopinavir/ritonavir dosed as 400/100 mg twice a day and saquinavir plus ritonavir dosed as 1000/100 mg twice a day. Patient characteristics including concomitant medication and data on viral loads and CD4 cell counts were obtained from patient records in the hospital. All viral loads were quantified using the Versant HIV-1 RNA 3.0 test kit (Bayer Diagnostics, Leverkusen, Germany).

Results

Seven male patients with a mean age of 45 years (range 32–55) were included, all were in Centers for Disease Control and Prevention (CDC) class C3. Lamivudine was part of the backbone therapy for all patients; six patients also used tenofovir and five patients used T20. Three patients used stavudine. Abacavir, didanosine and zidovudine were used by one patient each.

Six out of seven patients used concomitant medication (fluconazol, pyrimethamin, sulfadiazin, folinate, loperamide, ganciclovir, alprazolam, bezafibrate and L-thyroxine). None of these medications are known to interact with either lopinavir or saquinavir.

For lopinavir the median and interquartile ranges (IQR) of pharmacokinetic parameters were: AUC_{0-12h} 88.0 h.mg/L (68.6–118.9 h.mg/L), C_{max} 9.9 mg/L (7.9–12.5 mg/L), C_{min} 4.8 mg/L (3.9–5.6 mg/L), Cl/*F* 5.8 l/h (4.3–6.1 l/h) and $T_{1/2}$ 5.0 h (4.7–9.8 h). See Figure 1 for individual plasma concentration-time profiles of lopinavir. Lopinavir product information[4] reported similar mean AUC_{0-12h} 82.8 h.mg/L, C_{max} 9.6 mg/L, C_{min} 5.5 mg/L, Cl/*F* 6–7 l/h and $T_{1/2}$ 5–6 h. Other studies[5,6] also reported that lopinavir levels were not negatively affected by saquinavir when co-administered.

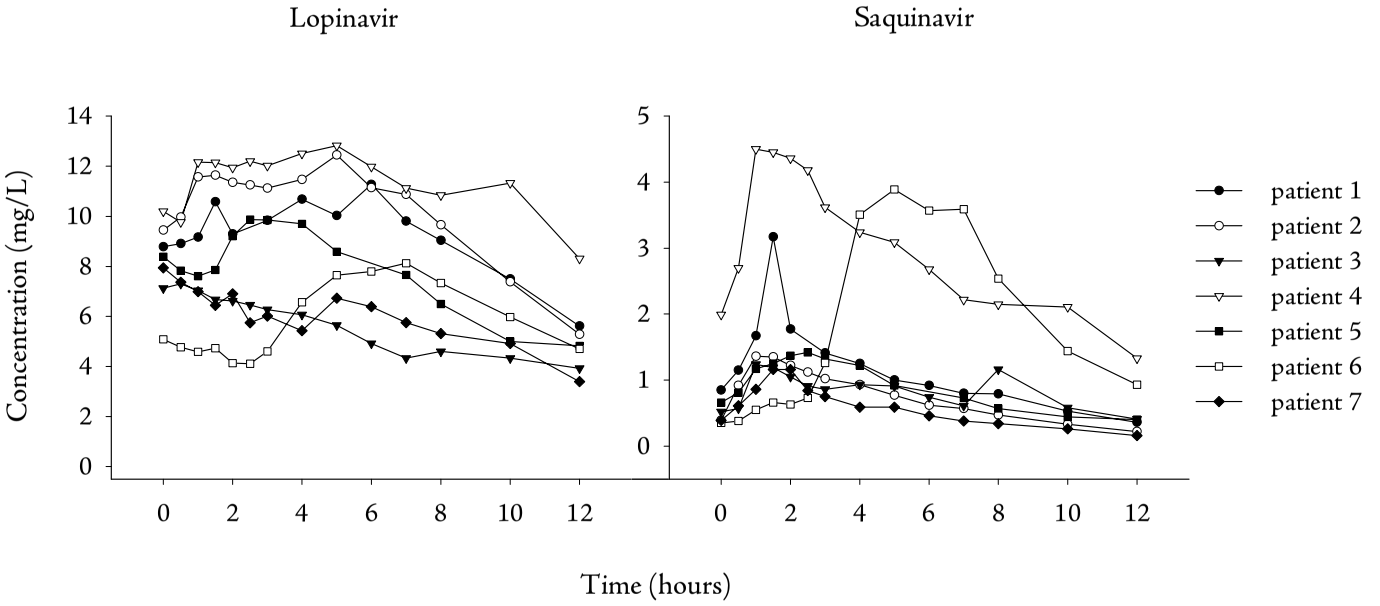
For saquinavir the median and IQR of pharmacokinetic parameters were: AUC_{0-12h} 9.8 h.mg/L (8.3–24.5 h.mg/L), C_{max} 1.6 mg/L (1.4–3.9 mg/L), C_{min} 0.40 mg/L (0.22–0.93 mg/L), Cl/*F* 105.8 l/h (67.7– 121.8 L/h) and $T_{1/2}$ 3.6 h (2.9–4.1 h). Figure 1 gives an overview of individual saquinavir plasma concentration- time profiles. Veldkamp *et al.*[7] reported a higher median AUC_{0-12h} and C_{max} of: 18.8 h.mg/L and 3.7 mg/L, a similar median C_{min} and $T_{1/2}$: 0.40 mg/L and 3.0 h. The median Cl/*F* was lower at 54.4 l/h. Ribera *et al.*[6] reported higher values, Kurowski *et al.*[8] reported higher AUC_{0-12h} but similar C_{min}. Stephan *et al.*[5] reported that lopinavir/ritonavir was able to boost saquinavir levels as effectively as ritonavir alone.

Adverse events reported by five out of seven patients were mainly gastrointestinal and mild in nature (vomiting, two; nausea, one; diarrhoea, one; heartburn, one; herpes labialis, one; exanthema, one; cutaneous herpes zoster, one). It has to be taken into account that the patients studied were experienced with regard to antiretroviral therapy. Good tolerability of this combination was also reported in other studies[9,10].

The median and IQR of the viral load in log10 copies/ml at baseline, week 12 and week 24 were 5.2 (4.9–5.5), 4.5 (2.9–5.0) and 3.9 (2.7–4.9). Two patients reached an undetectable viral load (< 400 copies/ml), one at week 12 the other at week 24. Five out of seven patients showed a decreased viral load (> 0.5 log) during 24 weeks of treatment.

The median and IQR of the CD4 cell counts x 10^6/l at baseline, week 12 and week 24 were 72 (31–109), 194 (59–242) and 134 (44–238). All patients showed an increased CD4 cell count during the first 12 weeks of treatment.

Figure 1. Lopinavir and saquinavir individual plasma concentration-time profiles



Conclusion

The chosen dosages for lopinavir/ritonavir and saquinavir may be used for the combination of these agents in a dual PI-based antiretroviral regimen. The pharmacokinetic data of seven patients obtained from this study corresponded with literature data for lopinavir[4,5] and saquinavir[5,7,8] when used separately, with the exception of saquinavir AUC_{0-12h} and C_{max}, which appeared to be somewhat lower in this study. Therapeutic drug monitoring remains an important tool in regimens like this, with or without concomitant medications. The tolerability of the studied treatment was good. The efficacy of this regimen, measured as a decline in viral load and an increase in CD4 cell count was encouraging, as also reported by others[9-11].

References

1. **Molla, A., H. Mo, S. Vasavanonda, L. Han, C. T. Lin, A. Hsu, and D. J. Kempf**. 2002. In vitro antiviral interaction of lopinavir with other protease inhibitors. Antimicrob.Agents Chemother. **46**:2249-2253.
2. **Bertz, R. J., Foit, C., Ashbrenner, E., Burt, D., Williams, L. A., Chira, T., Bernstein, B., Wieboldt, R., Tillmann, E., Heuser, R., Rode, R., Hsu, A., Granneman, R., and Sun, E**. Effect of amprenavir on the steady-state pharmacokinetics of lopinavir/ritonavir in HIV+ and healthy subjects. 42nd Interscience Conference on Antimicrobial Agents and Chemotherapy, September 27-30, 2002, San Diego, CA, USA, Abstract A-1823. 2002.
3. **Hugen, P. W. H., C. P. W. G. M. Verwey-van Wissen, D. M. Burger, E. W. Wuis, P. P. Koopmans, and Y. A. Hekster**. 1999. Simultaneous determination of the HIV-protease inhibitors indinavir, nelfinavir, saquinavir and ritonavir in human plasma by reversed-phase high-performance liquid chromatography. Journal of Chromatography B Biomed Sci Appl **727**:139-149.
4. Kaletra® Summary of Product Characteristics. 2000. Abbott, Chicago, IL, USA.
5. **Stephan, C. J., Lutz, T., Kurowski, M., Gute, P., and Staszewski, S**. [TuPeB4561] Lopinavir/Ritonavir versus Rinonavir boosted Saquinavir plasma levels. XIV International AIDS Conference. 2002.
6. **Ribera, E., Diaz, M., Pou, L., Ruiz, L., Ruiz, I., Ocana, I., and Falco, V**. [TuPeB4545] Steady-state Pharmaccokinetics of double boosting regimen of Lopinavir, plus Minidose Ritonavir, plus Saquinavir Soft-Gel in HIV-infected adults. XIV International AIDS Conference . 2002.
7. **Veldkamp, A. I., R. P. van Heeswijk, J. W. Mulder, P. L. Meenhorst, G. Schreij, G. S. van der, J. M. Lange, J. H. Beijnen, and R. M. Hoetelmans**. 2001. Steady-state pharmacokinetics of twice-daily dosing of saquinavir plus ritonavir in HIV-1-infected individuals. J.Acquir.Immune.Defic. Syndr. **27**:344-349.
8. **Kurowski, M., A. Arslan, K. Arasteh, C. Moecklinghoff, and A. Hill**. 2001. Comparative pharmacokinetics of twice daily Fortovase/ritonavir and Invirase/ritonavir. 2nd International Workshop on Clinical Pharmacology of HIV Therapy, April 2-4, 2001.Noordwijk, the Netherlands [abstract 3.2].
9. **Smith, G. H. R., Klien, M. B., Murphy, T., Macleod, J. D., Routy, J. P., LeBlanc, R. P., Rene, P., Gilmore, N., and Lalonde, R. G**. [TuPeB4547] Double, boosted salvage therapy with lopinavir(LOP)/ritonavir(RIT) and saquinavir-sgc(SQR) in HIV-1 infected patients having failed 3 antiretroviral classes. XIV International AIDS Conference . 2002.

10. **Zala, C., Patterson, P., Coll, P., Bouzas, M. B., Kaufman, S., Gun, A., Perez, H., and Cahn, P**. [TuPeB4492] Virological response and safety at 48 weeks of double boosted protease inhibitors with Lopinavir/R plus either Saquinavir or Amprenavir in heavily pretreated HIV infected patients. XIV International AIDS Conference . 2002.
11. **Staszewski, S., Dauer, B., Stephan, C., Gute, P., Klauke, S., and Sturmer, M**. [TuPeB4474] Switch to a simple boosted double protease inhibitor regimen of lopinavir/r and saquinavir without reverse transcriptase inhibitors after multiple therapy failures. XIV International AIDS Conference. 2002.

Chapter 3

Pharmacokinetics of once daily lopinavir/ritonavir as part of a regimen also containing two nucleosides administered once daily: the influence of dose modifications

C.J.L. la Porte[1,2], E.F. Schippers[3], M.E. van der Ende[4], P.P. Koopmans[5,2], W.L. Blok[6], R.H. Kauffmann[7], F.P. Kroon[3], D.M. Burger [1,2]

1 Department of Clinical Pharmacy, University Medical Centre Nijmegen, The Netherlands.
2 Nijmegen University Centre for Infectious Diseases, The Netherlands.
3 Department of Infectious Diseases, Leiden University Medical Center, The Netherlands.
4 Erasmus Medical Centre Rotterdam, The Netherlands.
5 Department of General Medicine, University Medical Centre Nijmegen, The Netherlands.
6 Walcheren Hospital, Vlissingen, The Netherlands.
7 Leyenburg Hospital, The Hague, The Netherlands.

Submitted

Abstract

Background: There is an increasing interest in once daily highly active antiretroviral therapy (HAART) regimens. For lopinavir/ritonavir, only sparse data are available on the pharmacokinetic profile and interpatient variability of once daily administration. No data have been reported so far that evaluate the effect of dose modifications in case of subtherapeutic exposure to lopinavir.

Methods: 20 HIV-1-infected patients (1 female), 10 of whom were treatment-naive, were started on a once daily HAART regimen consisting of lopinavir/ritonavir 800/200 mg once daily and two nucleosides once daily (didanosine/lamivudine: 13; didanosine/tenofovir: 3; lamivudine/tenofovir: 3; abacavir/lamivudine: 1 patient). A 24h pharmacokinetic curve was recorded after at least 2 weeks of treatment. Plasma samples for lopinavir were analyzed by a validated HPLC method.

Results: The mean ± standard deviation AUC_{24}, C_{max}, and C_{trough} for lopinavir were 197.9 ± 64.5 mg/L.h, 12.94 ± 4.47 mg/L, and 2.94 ± 2.48 mg/L, respectively. These values are 120%, 135%, and 53% of the respective pharmacokinetic parameters of lopinavir in the licensed dose of 400/100 mg twice daily. The data were similar with data presented on once daily lopinavir/ritonavir with nucleosides administered twice daily. We found no significant correlation between body weight and lopinavir exposure in our patients. In 6/20 (30%) patients a lopinavir C_{trough} below the target threshold of 1.0 mg/L was observed; these patients were eligible for a dose modification. From two patients no follow-up sample was available. Despite the increased dosage of 7 lopinavir/ritonavir capsules once daily, none of the other 4 patients met the target lopinavir threshold of 1.0 mg/L. In three patients the dose was increased further to 8 lopinavir/ritonavir capsules, only 1 patient reached a lopinavir plasma trough level above 1.0 mg/L (1.7 mg/L). Data on viral load, CD4 cell counts and adverse events were available for the 10 naive patients.

Conclusions: once daily administration of lopinavir/ritonavir results in exposure to lopinavir that is on average similar to twice daily administration with the exception of a somewhat lower C_{trough}. Therapeutic drug monitoring may be helpful in identifying patients with lower-than-expected lopinavir exposure. However, dose modifications will not lead to C_{trough} levels above 1.0 mg/L in the majority of the patients.

Introduction

The treatment of HIV-infected patients has gained success with the introduction of HAART[1,2]. Combinations of two nucleoside reverse transcriptase inhibitors (NRTIs) with either a protease inhibitor (PI) or a non-nucleoside reverse transcriptase inhibitor (NNRTI) have become standard of care nowadays[3]. Adverse events, both short- and long-term, and complexity of the regimen are important predictors of non-compliance[4,5], and subsequent virological failure[6]. Once daily (QD) dosed HAART regimens contribute to the development of less complex regimens.

Lopinavir/ritonavir is a formulation of two PIs that is approved for the treatment of HIV infection at a standard dose of 400/100 mg twice daily (BID)[7]. Ritonavir is an inhibitor of cytochrome-P450-isoenzyme CYP3A4[8]. Lopinavir is metabolised by CYP3A4 and inhibition of CYP3A4 by ritonavir results in higher plasma concentrations of lopinavir.

Recently, a study comparing lopinavir/ritonavir 800/200 mg QD with 400/100 mg BID in 38 antiretroviral-naive HIV-infected patients has been published[9]. Pharmacokinetics were studied in both treatment groups as well as antiviral activity and adverse events. A number of patients receiving lopinavir/ritonavir 800/200 mg QD had lower lopinavir trough concentrations (C_{trough}) as compared to patients receiving lopinavir/ritonavir 400/100 mg BID. Nevertheless, no dose adjustments were made to achieve higher lopinavir plasma concentrations in these particular patients. No statistically significant differences were observed in antiviral activity between the BID and QD treatment groups, up to 72 weeks of treatment[10].

The inhibitory quotient (IQ) has been shown to be a predictor of virological response to HIV therapy[11]. The inhibitory quotient is calculated by dividing the lopinavir trough concentration (C_{trough}) by the concentration needed to suppress the virus for 50% (IC50) in vitro, corrected for protein binding. In experienced patients receiving HAART consisting of lopinavir/ritonavir 400/100 mg BID with efavirenz and NRTIs, IQ values for lopinavir were determined. It was shown that patients with IQ >15 showed 100% response (<400 HIV-RNA copies at week 24). Taking an IQ of 15 as starting point, in patients infected with wild-type virus (IC50 estimated at 0.07 mg/L), the target C_{trough} of lopinavir can be calculated by the same formula; Target C_{trough} = IQ x IC50 = 15 x 0.07 = 1.0 mg/L. Based on this calculation, for antiretroviral-naive patients a target lopinavir C_{trough} of 1.0 mg/L has been proposed[12]. One could hypothesize that a QD dose of lopinavir/ritonavir 800/200 mg should have a target C_{trough} level of at least 1.0 mg/L to ensure antiviral activity.

In the present study the primary objective was to study the pharmacokinetics of lopinavir/ritonavir dosed as 800/200 mg QD. Furthermore, the effect of a dose increase in patients with lopinavir C_{trough} plasma concentrations below 1.0 mg/L was studied. Antiviral activity and safety of the studied regimen were considered secondary objectives.

Methods

Study design

This study was a 48-week, open-label, uncontrolled, multi-centre study in 10 HIV-1 infected patients with an indication to start HAART. Patients were treated with lopinavir/ritonavir 800/200 mg QD in combination with two nucleosides, which were dosed QD as well. After at least 14 days of treatment the patients were admitted for 24 hour blood sampling. Upon admission, study medication was taken with a standard breakfast. Blood samples were drawn at 0, 1, 2, 3, 4, 5, 6, 7, 8, and 24 hours after observed intake of the drugs. In some patients additional blood samples were drawn at 10, 12, 16 and 20 hours after intake of medication, for a better estimation of terminal half-life.

The target for C_{trough} was 1.0 mg/L. Dosage increase by 1 lopinavir/ritonavir 133/33 mg capsule, in addition to the daily dosage, was made when the target C_{trough} level of 1.0 mg/L was not reached. After another 2 weeks a C_{trough} was again measured. If the C_{trough} was again below 1.0 mg/L the procedure of dosage adjustment could be repeated (up to 3 times).

Patients were followed for 48 weeks after inclusion in the study. Visits were planned at weeks 4, 8, 12, 24, 36 and 48 after start of treatment. Upon these visits, blood samples were collected for routine measurements and lopinavir plasma concentrations. Additionally the occurrence of adverse events was evaluated during each visit.

Subsequently 10 non-naive patients were included to extend the pharmacokinetic information of this study.

Selection of patients

Naive patients were selected on the basis of the following inclusion criteria: able and willing to sign informed consent, age 18 years or older, documented HIV-1 infection, CD4 cell count ≤ 500 cells/mm^3. The following exclusion criteria were applicable: pregnancy or breast-feeding, known hypersensitivity to lopinavir or ritonavir, concomitant use of flecainide, propafenone, astemizole, terfenadine, rifampin, ergotamine, dihydoergotamine, ergonovine, methylergonovine, cisapride, lovastatin, simvastatin, pimozide, midazolam, triazolam, carbamazepine, phenobarbital, phenytoin, one or more of the following laboratory results: haemoglobin < 6 mM (≅ < 90 g/L), leukocytes < 2.10^9/L, ASAT or ALAT > 10 times upper limit of normal, serum creatinine > 2 times upper limit of normal, active opportunistic infection.

Non-naive patients were selected on their preference for a once daily regimen at the discretion of the treating physician.

Bioanalysis

Plasma levels of lopinavir were determined using a validated high performance liquid chromatography method, that was previously published[13]. The lopinavir calibration curve was linear over a range of 0.10 to 30.0 mg/L. The lower limit of quantification was 0.10 mg/L for lopinavir. Recovery after extraction from plasma was 95%. Accuracy

ranged from 99% to 101% and intra-day precision ranged from 0.92% to 5.16%. The inter-day precision of lopinavir ranged from 0% to 1.57%.

Pharmacokinetic analysis

Values for the pharmacokinetic parameters of lopinavir were estimated using noncompartmental methods. The maximum observed plasma concentration (C_{max}) and the time to C_{max} (T_{max}) were determined directly from the plasma concentration-time data. The plasma concentration observed 24 hours after intake of lopinavir (C_{trough}) was also determined directly from the plasma concentration-time data. The area under the plasma concentration-time curve from time 0 to 24 hours post-dose (AUC_{24}) was calculated by the linear trapezoidal rule. The value of the peak to trough rate constant (β) was obtained from the slope of the least squares regression of the logarithms of the plasma concentration versus time data for the 24-hour interval, which was then used to calculate half-life ($T_{1/2}$). The dosing interval or peak to trough $T_{1/2}$ was calculated as ln(2)/β. The apparent oral clearance value (CL/*F*), where *F* is the bioavailability, was calculated by dividing the administered dose in a dosing interval by AUC_{24}. CL/*F* was normalized for body weight by dividing by the weight in kg (CL/*F*.kg). The apparent volume of distribution (Vd/*F*) was calculated by dividing the CL/*F* by β. Vd/*F* was normalized for body weight by dividing by the weight in kg (Vd/*F*.kg).

Safety

During the scheduled study visits, blood samples were drawn for the determination of laboratory safety parameters. Additionally, patients were asked for the occurrence of adverse events during the past period on each study visit. For this purpose a questionnaire was used. Adverse events were assessed for intensity according to AIDS Clinical Trial Group (ACTG) classifications: mild (symptoms do not interfere with daily activities), moderate (symptoms interfere with daily activities) or severe (symptoms markedly interrupt daily activities).

Results

Patient characteristics

A total of 20 patients, at five different study centres, were included in the study. One of them was female, 10 patients were treatment naive and 10 were experienced. The mean age was 38 ± 7 years, the mean weight and length was 74 ± 15 kg and 1.79 ± 0.09 m, respectively. The route of transmission was homosexual in 15 patients, heterosexual in 2 patients, iv-drug use in 3 patients. Backbone therapy consisted of 2 nucleosides QD (didanosine/lamivudine in 13 patients; didanosine/tenofovir in 3 patients; lamivudine/tenofovir in 3 patients; and abacavir/lamivudine in 1 patient). Out of the 10 naive patients, 3 discontinued study medication during the 48-week follow-up. One patient discontinued between week 12 and 24 for reasons of severe

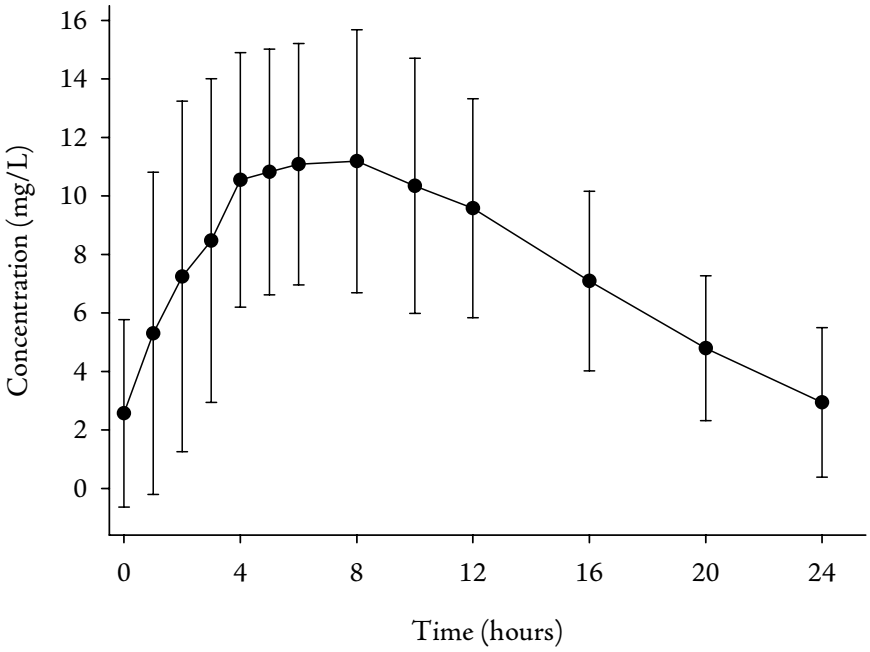
diarrhoea. A second patient discontinued between week 12 and 24 due to the large pill load as a result of 2 dose adjustments. A third patient discontinued because of a persisting detectable viral load up to week 36.

Lopinavir pharmacokinetics

In Table 1, lopinavir pharmacokinetic parameters are summarized. Furthermore, the pharmacokinetic parameters of lopinavir once daily from a previous study[9] are presented as comparison. In Figure 1 the mean lopinavir concentration-time plot for all 20 patients is presented.

The mean ± standard deviation AUC_{24}, C_{max}, and C_{trough} for lopinavir were 197.9 ± 64.5 mg/L.h, 12.94 ± 4.47 mg/L, and 2.94 ± 2.48 mg/L, respectively. These values are 120%, 135%, and 53% of the respective pharmacokinetic parameters of lopinavir in the licensed dose of 400/100 mg BID[7]. The data were similar to data presented on QD lopinavir/ritonavir with nucleosides administered BID[9].

Figure 1. Mean lopinavir concentration-time plot



We found no significant correlation between body weight and lopinavir exposure in our patients. In six out of twenty (30%) patients, a lopinavir C_{trough} below the target threshold of 1.0 mg/L was observed. Four of these patients were naive patients, two of them were experienced. These six patients were eligible for a dose modification. From the two non-naive patients no follow-up sample was available. After an increase in the lopinavir/ritonavir dosage to 7 capsules QD (933/233 mg), none of the other 4 patients met the target lopinavir C_{trough} level of 1.0 mg/L. Three patients received a further dose increase to 1066/266 mg of lopinavir/ritonavir (8 capsules), with only 1 patient reaching a lopinavir plasma level above 1.0 mg/L (1.7 mg/L).
Follow up lopinavir plasma concentrations were measured in nine of the 14 patients reaching a C_{trough} level of 1.0 mg/L on the standard dose. The lopinavir plasma concentrations in these patients remained at the same level as compared to the 24-hour curve at week 2. Lopinavir plasma concentrations were temporally lower in only one patient.

Table 1. Lopinavir pharmacokinetic parameters

Parameter[a]	Present study[b] (n=20)	Literature data[b](n=17)[9]
AUC_{24} (h.mg/L)	197.9 ± 64.5	164.9 ± 67.5
C_{max} (mg/L)	12.94 ± 4.47	10.94 ± 2.81
C_{trough} (mg/L)	2.94 ± 2.48	2.46 ± 2.63
T_{max} (h)	5.5 ± 2.7	6.6 ± 2.8
$T_{1/2}$ (h)	9.1 ± 8.3	NA
CL/*F*•kg (L/h•kg)	0.07 ± 0.03	NA
Vd/*F*•kg (L/kg)	0.73 ± 0.59	NA

Data are mean ± standard deviation. NA = not available.
[a] AUC_{24}: the area under the plasma concentration-time curve from time 0 to 24 hours post-dose, C_{max}: the maximum observed plasma concentration, T_{max}: the time to C_{max}, $T_{1/2}$: the dosing interval or peak to trough half-life, CL/*F*.kg: the apparent oral clearance value normalized for body weight, Vd/*F*.kg: volume of distribution corrected for body weight.
[b] lopinavir/ritonavir 800/200 mg once daily.

Antiviral activity and immunological results

From 10 naive patients follow up viral load and CD4 cell counts were available. At baseline 6 out 10 patients had a viral load (VL) >100,000 copies/ml, the other 4 patients had viral loads between 19,100 and 95,900 copies/ml. After 48 weeks six of ten treatment naive patients had reached undetectable plasma HIV-RNA levels (<50 copies/ml). One patient had a viral load of 106 copies/ml and three patients discontinued the study. Mean CD4 cell count in the 10 naive patients at baseline was 183 cells/μl. An increase in CD4 cell counts towards 295 cells/μl was observed by 4

weeks of treatment and stabilized at 388 cells/μl by week 12. At week 48 mean CD4 cell counts were 366 cells/μl.

Adverse events and safety

Adverse events and safety data were available for the 10 naive patients. The lipid spectra (total cholesterol, low density lipoproteins (LDL), high density lipoproteins (HDL) and triglycerides) showed increases in time (Table 2). The blood samples were drawn without regard to food intake. However these increases were small and no concomitant medication or treatment interruption was necessary.

Adverse event data are summarized in Table 3. In one patient, with lopinavir/ritonavir dosage of 1066/266 mg QD, severe persisting diarrhoea was a reason to stop the study medication. Severe fatigue and somnolence occurred in one patient, however this was not a reason to stop study medication.

Table 2. Summary of lipid profiles in the 10 naive patients

Week	Total cholesterol	Low density lipoproteins	High density lipoproteins	Triglycerides
0 (n=10)	4.58	2.61	0.93	1.81
4 (n=10)	5.37	2.73	0.96	3.19
8 (n=10)	5.98	3.20	0.95	3.95
12 (n=10)	6.19	2.94	1.22	3.30
24 (n=8)	6.63	3.31	1.18	4.78
36 (n=7)	6.86	4.61	1.24	2.77
48 (n=7)	6.40	3.79	1.17	3.10

Data given as mean values in mmol/L

Discussion

The main finding of this study was that the pharmacokinetic profile of QD lopinavir/ritonavir 800/200 mg in 20 HIV-infected patients was similar to data reported previously[9]. More importantly, the present study gives insight in the effect of dose adjustment in naive patients that did not reach a predefined lopinavir trough plasma concentration upon treatment with lopinavir/ritonavir 800/200 mg QD. This minimum concentration for antiviral activity has been proposed as 1.0 mg/L[12]. We found lopinavir C_{trough} levels <1.0 mg/L in 6 out of 20 patients, as compared to 3 out of 18 patients previously reported[9]. Following dose adjustments only 1 out of these 4 patients finally reached a lopinavir C_{trough} >1.0 mg/L. This is a rather poor success

Table 3. Adverse events in the 10 naive patients

Description	Number[a]
Diarrhoea[b]	8
Fatigue and somnolence[b]	8
Skin reactions	6
Headache	5
Peripheral paresthesias	5
Asthenia	5
Flatulence	3
Insomnia	3
Per oral paresthesia	3
Abdominal pain	3
Nightmares	3
Taste perversions	2

[a] Number represents number of patients that experienced a specific adverse event at least once during 48 weeks follow up. Numbers reflect mild to moderate adverse events, unless stated otherwise.
[b] These data include 1 case of severe diarrhoea and 1 case of severe fatigue and somnolence.

rate and the reason why remains puzzling. It is recommended that lopinavir/ritonavir is taken with food to ensure absorption[7]. Differences in food composition between studies might explain the differences of the trough levels in both studies. In our study the medication was taken with a standard breakfast, whereas in the previous study possibly a meal with a higher amount of fat and a higher caloric value was used. In the present study patients with low plasma levels were reinforced to ensure their fed status before intake of lopinavir/ritonavir, to exclude the negative effect of intake on an empty stomach. For the PI nelfinavir dosed at 1250 mg BID it has also been reported that dose adjustments in case of low nelfinavir plasma concentrations were only effective in a minority of patients[14]. For nelfinavir the same applies with regard to food intake as for lopinavir, and the advice given to patients with low nelfinavir plasma concentrations. This observation indicates that dose adjustments may not have the desired effect for other PIs as well. Another explanation could be that absorption from the gastrointestinal tract is limited. In some patients this limitation might result in a decreasing bioavailability of lopinavir at higher dosages. In a multiple dose escalating study, in healthy subjects, increasing lopinavir and/or ritonavir doses provided less than dose proportional increases in lopinavir concentrations[15]. Although the mechanism of this remains unclear, the occurrence of diarrhoea at higher dosages could be an important factor.

In the present study 3 out of the 4 patients that did not reach a lopinavir C_{trough} >1.0 mg/L, stopped the study medication, one of them because of virological failure. The

fourth patient had an undetectable VL from week 12 on. In an ITT analysis of the once daily versus twice daily study 74% of 19 patients had an undetectable viral load at 48 and 72 weeks[9,10]. Five patients did not reach an undetectable viral load by that moment. At week 72, two patients had dropped out of the study for reasons of adverse events and site closure. It would have been interesting to know whether the three remaining patients were the patients with the lowest lopinavir C_{trough} as well.

Lopinavir/ritonavir dosed as 800/200 mg QD was well tolerated by the patients in our study. One patient stopped therapy for reasons of severe diarrhoea. In comparison to lopinavir/ritonavir 400/100 mg BID the occurrence of diarrhoea might be more frequent after QD intake of lopinavir/ritonavir 800/200 mg. In the previous study, diarrhoea was reported more frequent in the QD group than in the BID group (5/19 versus 3/19) as well[9].

Our study had its limitations. Firstly, since a control group with another non-lopinavir based QD regimen or a lopinavir based BID regimen was not included, no direct comparisons with regard to antiviral activity and adverse events could be made. Secondly, efficacy and adverse events were only descriptive in the follow up from the 10 naive patients. A third limitation was the entrance of both naive and non-naive patients to the study, making the study population heterogeneous. Furthermore, the study was too small to investigate the correlations between pharmacokinetic parameters and sustained virological suppression. Certainly, based on our data such correlations cannot be ruled out. Large studies are needed to address this question.

In conclusion, lopinavir dosed as 800/200 mg QD together with nucleosides given once daily is a safe combination with respect to side effects and viral suppression. However, attention should be paid to the occurrence of lopinavir C_{trough} levels that are below 1.0 mg/L. Therapeutic drug monitoring is necessary to detect these likely subtherapeutic plasma levels. Dose modification, as first line intervention, in patients with C_{trough} levels <1.0 mg/L, is not likely to result in higher plasma levels. Also, dose escalation might induce more side effects, higher pill burden and subsequent non-compliance. For these patients, switching to BID lopinavir/ritonavir or replacing lopinavir/ritonavir for another drug might be better alternatives. Further studies are needed that address the question to what extend lopinavir pharmacokinetic parameters, i.e. C_{trough}, are predictors of sustained virological suppression.

Acknowledgements

The authors would like to thank the patients for their participation in this study. The doctors and HIV nurses at the outpatient clinics of the participating centres are thanked for their cooperation. The laboratory technicians of the Department of Clinical Pharmacy are thanked for analysing all plasma samples.

References

1. **Hogg, R. S., K. V. Heath, B. Yip, K. J. Craib, M. V. O'Shaughnessy, M. T. Schechter, and J. S. Montaner**. 1998. Improved survival among HIV-infected individuals following initiation of antiretroviral therapy. JAMA **279**:450-454.
2. **Palella, F. J. J., K. M. Delaney, A. C. Moorman, M. O. Loveless, J. Fuhrer, J. E. Satten, D. E. Aschman, and S. D. Holmberg**. 1998. Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. New England Journal of Medicine **338**:853-860.
3. **Anonymous**. Guidelines for the Use of Antiretroviral Agents in HIV-1-Infected Adults and Adolescents. www.aidsinfo.nih.gov/guidelines/adult. 2003.
4. **Mehta, S., R. D. Moore, and N. M. H. Graham**. 1997. Potential factors affecting adherence with HIV therapy. AIDS **11**:1665-1670.
5. **Stone, V. E.** 2001. Strategies for optimizing adherence to highly active antiretroviral therapy: lessons from research and clinical practice. Clin.Infect.Dis. **33**:865-872.
6. **Paterson, D. L., S. Swindells, J. Mohr, M. Brester, E. N. Vergis, C. Squier, M. M. Wagener, and N. Singh**. 2000. Adherence to protease inhibitor therapy and outcomes in patients with HIV infection. Annals of Internal Medicine **133**:21-30.
7. Kaletra® Summary of Porduct Characteristics. 2000. Abbott, Chicago, IL, USA.
8. **Hsu, A., G. R. Granneman, and R. J. Bertz**. 1998. Ritonavir. Clinical pharmacokinetics and interactions with other anti-HIV agents. Clinical Pharmacokinetics **35**:275-291.
9. **Eron, J. J., J. Feinberg, H. A. Kessler, H. W. Horowitz, M. D. Witt, F. F. Carpio, D. A. Wheeler, P. Ruane, D. Mildvan, B. G. Yangco, R. Bertz, B. Bernstein, M. S. King, and E. Sun**. 2004. Once-daily versus twice-daily lopinavir/ritonavir in antiretroviral-naive HIV-positive patients: a 48-week randomized clinical trial. J.Infect.Dis. **189**:265-272.
10. **Feinberg, J., Eron, J., Bernstein, B., King, M., Manning, L., Bertz, R., Carpio-Cedraro, F., Horowitz, H., Wheeler, D., Kessler, H., Mildvan, D., Ruane, P., Yangco, B., and Sun, E**. Once-daily vs. twice-daily Kaletra (lopinavir/ritonavir) in antiretroviral-naive HIV+ patients: 72 week follow-up. XIVth International Aids Conference, July 7-12 2002, Barcelona, Spain, abstract TuPeB4445. 2002.
11. **Hsu, A., J. Isaacson, S. Brun, B. Bernstein, W. Lam, R. Bertz, C. Foit, K. Rynkiewicz, B. Richards, M. King, R. Rode, D. J. Kempf, G. R. Granneman, and E. Sun**. 2003. Pharmacokinetic-pharmacodynamic analysis of lopinavir-ritonavir in combination with efavirenz and two nucleoside reverse transcriptase inhibitors in extensively pretreated human immunodeficiency virus-infected patients. Antimicrob.Agents Chemother. **47**:350-359.
12. **Anonymous**. 2003. Optimising TDM in HIV clinical care. A practical guide to performing therapeutic drug monitoring (TDM) for antiretroviral agents. Available: http://www.HIVpharmacology.com. Last visited at May 24, 2004.
13. **Droste, J. A. H., C. P. W. G. M. Verweij-van Wissen, and Burger D.M.** 2003. Simultaneous Determination of the HIV Drugs Indinavir, Amprenavir, Saquinavir, Ritonavir, Lopinavir, Nelfinavir, the Nelfinavir hydroxymetabolite M8 and Nevirapine in Human Plasma by Reversed Phase High Performance Liquid Chromatography. Therapeutic Drug Monitoring **25**:393-399.
14. **la Porte, C. J. L. and Burger D.M**. Is nelfinavir (NFV) 1500 mg BID an effective intervention for patients on NFV 1250 mg BID who have low NFV exposure? 2nd International Workshop on Clinical Pharmacology of HIV Therapy, April 2-4, 2001. Noordwijk, the Netherlands [abstract 6.8]. 2001.
15. **Pharmaceutical Products Division, Research and Development of Abbott Laboratories**. ABT-378 Investigator Brochure. Abbott Park, Illinois 60064 U.S.A.Edition 5 (04 May 2000). 4-5-2000.

Part II

Indinavir

Chapter 4

Pharmacokinetic interaction study of indinavir/ritonavir and the enteric-coated capsule formulation of didanosine in healthy volunteers

C.J.L. la Porte[1,2], C.P.W.G.M. Verweij-van Wissen[1], N. van Ewijk[1], R.E. Aarnoutse[1,2], P.P. Koopmans[3,2], P. Reiss[4], M. Stek Jr[5], Y.A. Hekster[1,2], D.M. Burger[1,2]

1 Department of Clinical Pharmacy, University Medical Centre, Nijmegen, The Netherlands.
2 Nijmegen University Centre for Infectious Diseases, Nijmegen, The Netherlands.
3 Department of General Medicine, University Medical Centre, Nijmegen, The Netherlands.
4 Department of Infectious Diseases and National AIDS Therapy Evaluation Centre (NATEC), Academic Medical Centre, Amsterdam, The Netherlands.
5 Merck & Co., Whitehouse Station, NJ, USA.

Journal of Clinical Pharmacology. 2005; 45: 211-218

Abstract

Didanosine enteric-coated (EC) should be taken on an empty stomach, but the once daily (QD) combination of indinavir/ritonavir can be taken with food. Because these drugs are frequently included in 1 regimen, the food effects on the pharmacokinetics were evaluated. This was a randomized, 4 way crossover study of single doses of didanosine EC 400 mg and indinavir/ritonavir 1200/400 mg in 8 healthy subjects. The following regimens were given: didanosine EC 2 hours after breakfast (reference regimen A), indinavir/ritonavir with breakfast (reference regimen B), didanosine EC + indinavir/ritonavir 2 hours after breakfast (test regimen C), and didanosine EC + indinavir/ritonavir with breakfast (test regimen D). Breakfast was 550 kcal, 28% fat. Blood samples were drawn before and up to 24 hours after ingestion. Statistical comparisons of test regimens C and D with reference regimens A and B were made using the equivalence approach for indinavir and didanosine AUC and C_{max} (0.80-1.25). Eight subjects (5 men, 3 women) were enrolled and completed the study. Indinavir AUCs were bioequivalent in test regimens C and D compared to reference regimen B. A 14% increased C_{max} was observed in test regimen C. Didanosine AUC in test regimen D was 4% lower and suggestive of bioequivalence compared to reference regimen A. However, regimen C didanosine AUC was 23% lower and bioinequivalent compared to reference regimen A. Didanosine C_{max} decreased 42% and 46% in test regimens C and D, respectively, in comparison to reference regimen A. In this study, dosing didanosine EC 400 mg QD + indinavir/ritonavir 1200/400 mg QD with breakfast indicated no decrease in the amount of absorption for either didanosine and indinavir and that this regimen could be administered with food.

Introduction

The treatment of HIV-infected persons with highly active antiretroviral therapy (HAART) has greatly improved the prognosis of patients. A high rate (>95%) of adherence to therapy is important to maintain long-term viral suppression[1]. Unfortunately, due to the complexity of the HAART regimens, compliance is low because both dose frequency and restrictions on concomitant food intake must be followed[2,3]. To make HAART regimens less complex, efforts are being focused on designing combination regimens suitable for once daily use, regardless of food restrictions. The protease inhibitor indinavir is approved for 800 mg thrice daily[4]. However, the combination with ritonavir, another protease inhibitor, allows decreased dosing frequency to twice-a-day (BID) dosage due to inhibition of cytochrome P450 3A enzymes by ritonavir, which are responsible for the metabolism of indinavir[5-7]. In addition, once daily indinavir with ritonavir was studied in healthy subjects[8], showing that 1200 mg indinavir with 400 mg ritonavir resulted in pharmacokinetic parameters that were promising for the once daily treatment of patients. The combination was best taken with food to avoid high peak plasma levels that are associated with nephrotoxicity. The preliminary results of a study investigating this combination in patients showed good virological and immunological response[9]. Didanosine, a nucleoside reverse transcriptase inhibitor (NRTI), has a recommended dose of 400 mg once daily. Didanosine decomposes in an acid environment and therefore is unstable in the acid environment of the stomach[10]. To protect didanosine from decomposition, it was previously formulated in tablets with a buffer to increase gastric pH after intake. However, this formulation of didanosine was not well tolerated due to the buffer included in the tablets. A new formulation of didanosine has become available with encapsulated enteric-coated (EC) beads. The EC formulation dissolves once the low gastric pH is neutralized in the gut lumen. The EC capsules are much better tolerated and are approved for intake on an empty stomach, defined as at least 2 hours before or after a meal[10]. Combining indinavir/ritonavir with didanosine EC in a once daily regimen yields problems with regard to food intake. To take full advantage of a once daily regimen, it is important that all drugs involved can be taken at the same time. This study was undertaken to characterize the pharmacokinetics of both indinavir and didanosine when given together with or without food.

Methods

Study design

This was a randomized, 4-way, crossover, single-dose pharmacokinetic study in 8 healthy subjects. Four different drug regimens were randomly assigned to the subjects, using a Latin square design. The different drug regimens were as follows: didanosine EC 400 mg 2 hours after breakfast (reference regimen A), indinavir 1200

mg + ritonavir 400 mg with breakfast (reference regimen B), didanosine EC 400 mg + indinavir 1200 mg + ritonavir 400 mg 2 hours after breakfast (test regimen C), and didanosine EC 400 mg + indinavir 1200 mg + ritonavir 400 mg with breakfast (test regimen D). A washout period of 3 or 4 days was implemented between the 4 regimens. In preparation for study days, participants had to remain fasted for 8 hours. Beverages containing alcohol were prohibited from 15 hours before the start of each study day. Medication was administered with 420 mL of tap water. After intake of medication, blood and urine samples were collected for 24 hours. On study days, participants received a standardized breakfast (550 kcal; 28% fat) at the clinical research unit. Lunch (4 hours after breakfast) and dinner (9 hours after breakfast) were also standardized and provided at the clinical research unit. For up to 12 hours after administration, participants had to drink 2.5 L of fluid, according to a prescribed schedule. The intake of grapefruit (juice) was prohibited throughout the whole study period.

Selection of subjects

Subjects were eligible for inclusion if they met the following inclusion criteria: aged 18 years or older and healthy (ie, not suffering from an acute or chronic illness and not using medications). Subjects meeting any of the following criteria were excluded: documented hypersensitivity to indinavir, ritonavir, or didanosine; positive HIV test; pregnancy; history of pancreatitis; history of alcohol abuse; or 1 or more prespecified laboratory abnormalities. Written informed consent was obtained from all subjects, and the Regional Ethical Review Board approved the study. The study was conducted at the Department of Clinical Pharmacy of the University Medical Centre, St. Radboud Nijmegen, in collaboration with the Department of General Medicine.

Blood and urine sampling procedures

On study days, 10 mL blood samples were collected in heparinized tubes by an indwelling catheter or venipuncture. The first sample was collected immediately before dosing; the other samples were taken at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, 6.0, 8.0, 10.0, 12.0, and 24.0 hours after ingestion. Plasma for the determination of indinavir, ritonavir, and didanosine was transferred to labeled polypropylene tubes and stored at –20°C.

Urine samples were collected predose and during intervals from 2.0, 4.0, 6.0, 8.0, 10.0, 12.0, and 24.0 hours after administration of medication. Total urine volume was measured for each interval. Urine samples (5.0 mL) for the determination of indinavir and ritonavir were transferred to labeled polypropylene tubes and stored at –20°C. Urine samples (1.00 mL) for the determination of didanosine were transferred to labeled polypropylene tubes containing 2.00 mL phosphate buffer (0.2 M, pH 8.0) and stored at –20°C.

Bioanalysis

Indinavir and ritonavir were analyzed in plasma using a previously described high-performance liquid chromatography (HPLC) method[11]. For indinavir, accuracy ranged from 104% to 108%, depending on the concentration level, and intraday and interday precision ranged from 2.1% to 7.5% and 0.4% to 3.5%, respectively.

For ritonavir, accuracy ranged from 102% to 108%, and intraday and interday precision ranged from 2.0% to 8.1% and 0.4% to 3.5%, respectively. For the analysis of indinavir urine levels, a modification of the method used for plasma was used, as described elsewhere[12]. For this method, the accuracy ranged from 92% to 101%, and intraday and interday precision ranged from 0.3% to 1.1% and 0.5% to 4.4%, respectively. Didanosine plasma levels were measured using solid-phase extraction followed by reversed-phase HPLC with ultraviolet detection. Solid-phase extraction was performed with Waters Oasis MAX columns (Waters, Etten-Leur, The Netherlands). The columns were washed with 500 µL of methanol followed by 250 µL of water. Then, 500 µL of the plasma sample was loaded on the column together with 500 µL of HPLC-analyzed water (Baker, Deventer, The Netherlands). After loading the sample, the column was flushed twice with 150 µL HPLC-analyzed water (Baker, Deventer, The Netherlands) and vacuumed to dryness. Elution was performed by adding 0.5 mL of a mixture of methanol and water (80/20 vol/vol). The eluate was vaporized under a gentle stream of nitrogen at 37°C and reconstituted in 0.2 mL 95/5 vol/vol water/acetonitrile. Then, 50 µL of this solution was injected into the HPLC system. Chromatographic analysis was performed at ambient temperature on a Symmetry Shield RP18 3.5-µm analytical column (150 × 4.6 mm i.d., Waters, Etten-Leur, The Netherlands), protected by a Symmetry Shield RP18 3.5-µm column (3.9 × 20 mm i.d., Waters, Etten-Leur, The Netherlands). Mobile phase was a mixture of 0.020 M acetate buffer (pH 4.6) (94%) and acetonitrile (6%) vol/vol. From 10 to 24 minutes, the composition of the mobile phase gradually changed to 74% acetate buffer with 26% acetonitrile. The gradient was back to the original values by 26 minutes. The flow rate was set at 1 mL/min, and the wavelength for ultraviolet detection was 260 nm. Didanosine retention time was 6 minutes. The didanosine calibration curve was linear over a range of 0.017 to 5.58 mg/L. Recovery after extraction from plasma was 97%. Accuracy ranged from 100% to 102%, and intraday and interday precision ranged from 1.8% to 2.1% and 1.5% to 2.4%, respectively.

Didanosine urine levels were measured using solid-phase extraction followed by reversed-phase HPLC with ultraviolet detection. Solid-phase extraction was performed with Waters Oasis MAX columns (Waters, Etten-Leur, The Netherlands), which were pretreated with 100 µL of methanol followed by 100 µL of water. Then, 1 mL 0.1 M ammonium hydroxide was loaded on the column together with 200 µL of the urine sample. After loading the sample to the column, it was flushed with 1 mL 0.02 M ammonium hydroxide and 1 mL methanol and vacuumed to dryness. Elution was performed with 0.5 mL 2% acetic acid in methanol in 15-mL glass tubes. The eluate was vaporized under nitrogen at 37°C and resolved in 0.5 mL 0.2 M disodium monohydrogen phosphate (pH 8.0), and 20 µL of this solution was used for injection to

the HPLC system. Chromatographic analysis was performed on a Platinum EPS C18 300 A 5-µ analytical column (150 × 4.6 mm i.d., Alltech, Breda, The Netherlands), protected by a Platinum EPS C18 300 A5-µ All-Guard column (7.5 × 4.6 mm i.d., Alltech, Breda, the Netherlands). Mobile phase was a mixture of 0.025M potassium dihydrogenphosphate (97%) and acetonitrile (3%) vol/vol. The flow rate was 1 mL/min, and the wavelength for ultraviolet detection was 250 nm. Didanosine retention time was 10.5 minutes. The didanosine calibration curve was linear over a range of 1.56 to 467 mg/L. Recovery after extraction from urine was 100.3%. Accuracy ranged from 101% to 105%, and intraday and interday precision ranged from 3.1% to 4.0% and 0% to 0.6%, respectively.

Safety monitoring and laboratory measurements

Twenty-four hours after administration, the clinical laboratory tests performed during screening were repeated on each study day. Adverse events were recorded and graded as mild, moderate, or severe according to World Health Organization (WHO) grading scales.

Pharmacokinetic analysis

The pharmacokinetic parameters of indinavir, ritonavir and didanosine were calculated by noncompartmental methods using Excel version 2000 (Microsoft Corporation 1985-1999). The highest observed plasma concentration was defined as C_{max}, with the corresponding sampling time as T_{max}. C_{min} was the concentration at 24 hours after ingestion of the drugs. The terminal, log-linear period (log C versus t) was defined by visual inspection of the last data points ($n \geq 3$). The absolute value of the slope (β/2.303) was calculated by least squares linear regression analysis (β is the first-order elimination rate constant). The elimination half-life ($T_{1/2}$) was calculated by the equation 0.693/β. The area under the concentration versus time curve (AUC) was calculated using the trapezoidal rule from 0 to 24 hours. The $AUC_{0-\infty}$ value was calculated by extrapolating to infinity by the addition of the last measured plasma concentration divided by β. The apparent clearance (CL/*F*, where *F* is bioavailability) was calculated by dividing dose (D) by AUC, and apparent volume of distribution (Vd/*F*) was obtained by dividing CL/*F* by β. Clearance and volume of distribution were corrected for weight of the subject by dividing these parameters by the subject's body weight in kilograms. For this purpose, body weight was measured on each study day.

The cumulative renal excretion of indinavir and didanosine (Ae) was approximated by the total amount of indinavir and didanosine that was excreted unchanged in the urine during the dosing interval; Ae = S (volume urine x concentration indinavir or didanosine in urine). Renal clearance (CL_R) was calculated using the formula Ae/AUC. The fraction of the total amount excreted unchanged (fe) was calculated using the following formula: fe x *F* =Ae/D = CL_R/CL.

Statistical analysis

All statistical evaluations were performed with SPSS for Windows, version 10 (SPSS, Inc, Chicago). Prior to statistical analysis, the pharmacokinetic parameters of indinavir, ritonavir, and didanosine were logarithmically transformed. Geometric means were calculated for all transformed pharmacokinetic parameters. T_{max} values were summarized as medians and ranges and compared by regimen using the Wilcoxon signed rank test. A P value ≤ 0.05 was considered to be significant in all analyses. AUC and C_{max} were tested for bioequivalence over the different regimens. For indinavir, AUC and C_{max} of test regimens C and D were compared to the AUC and C_{max} of reference regimen B. For didanosine, AUC and C_{max} of test regimens C and D were compared to the AUC and C_{max} of reference regimen A. A general linear method was used to calculate the geometric mean ratios of the AUC and C_{max} of test regimens over the reference regimen. Conclusions with regard to bioequivalence were drawn as described by Williams et al[13]. The 90% confidence intervals of the geometric mean ratios were compared to the predefined range of 0.80 to 1.25. Bioequivalence was concluded when the geometric mean ratio and the 90% confidence interval fell within the limits of 0.80 to 1.25. Bioequivalence was suggested if the geometric mean ratio fell within the limits of 0.80 to 1.25, but either the lower or the upper limit of the 90% confidence interval failed the limits of 0.80 to 1.25. Bioinequivalence was concluded if the geometric mean ratio and the 90% confidence interval fell outside the limits of 0.80 to 1.25. Bioinequivalence was suggested if the geometric mean ratio fell outside the range of 0.80 to 1.25, but one of the limits of the 90% confidence interval fell within the limits of 0.80 to 1.25.

A power calculation was performed in the development phase of the study. The calculation, based on indinavir C_{min}, indicated that data of 6 subjects were needed to detect a 50% difference. As a low dropout rate was assumed, 8 subjects were included in the study.

Results

Subjects

Eight subjects(5 men, 3 women) were enrolled and could be evaluated. There were no dropouts in this study. Their median age was 33 years (range = 19–57 years), and their median weight was 73.6 kg (range = 66.7–106 kg). All subjects were Caucasians.

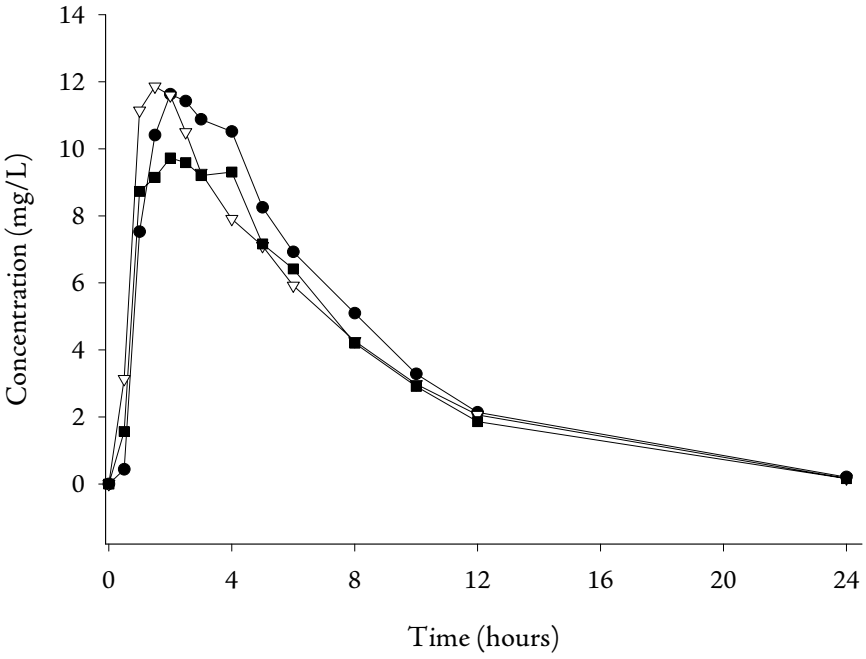
Adverse events and laboratory measurements

Two out of 8 subjects did not experience any adverse events at all. The single doses given in this study were well tolerated by the subjects. No serious adverse events occurred. During the study, no clinically significant changes were observed in biochemical and hematological parameters.

Pharmacokinetics

The indinavir pharmacokinetic parameters are listed in Table 1. Indinavir plasma concentration versus time profiles based on median values for the reference regimen B and the test regimens C and D are shown in Figure 1. Note that data are presented as geometric means in the table, which may read differently from the medians used in Figure 1. Test regimens C and D relative to reference regimen B showed bioequivalence, with the exception of indinavir C_{max} in test regimen C, in which bioequivalence was only suggested. The 14% higher observed indinavir C_{max}, when combined with didanosine 2 hours after breakfast, was accompanied by a statistically nonsignificant (P= 0.12) decreased median indinavir T_{max} of 1.3 hours when compared to reference regimen B. Reference regimen B (indinavir/ritonavir with breakfast) resulted in a T_{max} of 2.3 hours. Test regimen D, in which indinavir/ritonavir and didanosine were given simultaneously with breakfast, showed a T_{max} of 2.0 hours. No statistically significant differences were observed in indinavir renal excretion.

Figure 1. Indinavir plasma concentration-time profiles



—●— Reference regimen B, indinavir/ritonavir with breakfast
—▽— Test regimen C, didanosine EC + indinavir/ritonavir 2 hours after breakfast
—■— Test regimen D, didanosine EC + indinavir/ritonavir with breakfast
Curves are median values of 8 subjects

Table 1. Summary of indinavir pharmacokinetic parameters (n=8)

Parameter	Geometric mean and range			Geometric mean ratio and 90% CI			
	Regimen B[†]	Regimen C[‡]	Regimen D[#]	Regimen C/B[$]	*P*-value	Regimen D/B[&]	*P*-value
AUC (h.mg/L)	86.6 (60.2-118.5)	82.6 (61.3-97.3)	82.6 (62.0-98.9)	0.95 [0.81-1.13]	0.61	0.95 [0.85-1.07]	0.48
C_{min} (mg/L)	0.20 (0.10-0.35)	0.16 (0.07-0.35)	0.17 (0.07-0.43)				
C_{max} (mg/L)	11.8 (7.9-15.0)	13.4 (10.9-17.4)	11.5 (9.2-13.6)	1.14 [1.01-1.28]	0.07	0.97 [0.86-1.10]	0.68
T_{max} (h)[*]	2.3 (1.0-4.0)	1.3 (1.0-2.6)	2.0 (1.0-4.0)	-	0.12	-	0.62
$T_{1/2}$ (h)	3.6 (2.8-4.1)	3.6 (3.0-4.4)	3.5 (3.0-4.2)				
CL/*F*.kg (L/h.kg)	0.18 (0.14-0.28)	0.19 (0.14-0.27)	0.19 (0.14-0.27)				
Vd/*F*.kg (L/kg)	0.91 (0.77-1.35)	0.98 (0.64-1.32)	0.95 (0.69-1.21)				
Ae (mg)	564 (501-613)	545 (462-644)	538 (435-646)				
CL_R/*F*.kg (L/h.kg)	0.08 (0.06-0.13)	0.08 (0.06-0.12)	0.08 (0.06-0.12)				
Fe.*F*	0.47 (0.42-0.51)	0.45 (0.38-0.54)	0.45 (0.36-0.54)				

CI, Confidence interval; AUC, area under the concentration-time curve; C_{min}, trough concentration at 24 hours; C_{max}, highest observed plasma concentration; T_{max}, sampling time for C_{max}; CL/*F*.kg, total clearance corrected for weight; Vd/*F*.kg, volume of distribution corrected for weight; $T_{1/2}$, elimination half-life; *F*, bioavailability; Ae, total amount excreted with urine; CL_R/*F*.kg, renal clearance corrected for weight; Fe.*F*, fraction excreted by urine

* Median and range; Wilcoxon-signed rank test

† Combination of 1200 mg indinavir and 400 mg ritonavir with breakfast

‡ Combination of 1200 mg indinavir and 400 mg ritonavir and 400 mg didanosine 2 hours after breakfast

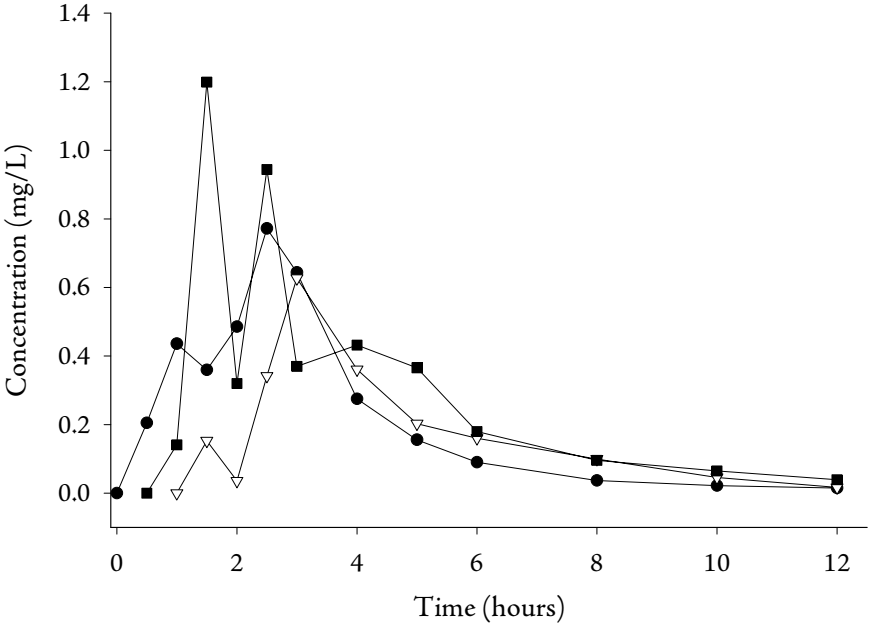
Combination of 1200 mg indinavir and 400 mg ritonavir and 400 mg didanosine with breakfast

$ Geometric mean ratio plus 90% CI for regimen C versus B

& Geometric mean ratio plus 90% CI for regimen D versus B

Didanosine plasma concentration-time profiles can be found in Figure 2, with pharmacokinetic parameters listed in Table 2. Note that data are presented as geometric means in the table, whereas medians were used in Figure 2. For didanosine, it was not possible to determine a reliable C_{min} value because no subjects had detectable didanosine levels in plasma more than 12 hours after intake. The didanosine AUC in test regimen D was 4% lower and suggestive of bioequivalence compared to reference regimen A. However, didanosine AUC in test regimen C was suggestive of bioinequivalence compared to reference regimen A (geometric mean ratio and 90% confidence interval = 0.77 [0.60–0.98]). Didanosine C_{max} was not bioequivalent in both test regimens C and D relative to reference regimen A. For C_{max}, geometric mean ratios and 90% confidence intervals were 0.58 [0.40–0.83] for test regimen C and 0.54 [0.36–0.81] for test regimen D. As such, there was a significant average decrease in C_{max} of 42% and 46% for the respective test regimen relative to the reference regimen. A statistically significant increase for T_{max} was observed for both test regimens as well. Other didanosine pharmacokinetic parameters did not show any statistically significant changes. The total renal excretion was significantly (P = 0.04) decreased in test regimen C (48.9 versus 64.7 mg in reference regimen A).

Figure 2. Didanosine plasma concentration-time profiles



—●— Reference regimen A, didanosine EC 2 hours after breakfast
—▽— Test regimen C, didanosine EC + indinavir/ritonavir 2 hours after breakfast
—■— Test regimen D, didanosine EC + indinavir/ritonavir with breakfast
Curves are median values of 8 subjects

Table 2. Summary of didanosine pharmacokinetic parameters (n=8)

Parameter	Geometric mean and range			Geometric mean ratio and 90% CI			
	Regimen A[†]	Regimen C[‡]	Regimen D[#]	Regimen C/A	*P*-value	Regimen D/A	*P*-value
AUC (h.mg/L)	2.01 (1.11-3.15)	1.55 (0.52-3.11)	1.93 (1.16-4.93)	0.77 [0.60-0.98]	0.08	0.96 [0.79-1.17]	0.69
C_{min} (mg/L)	nd	nd	nd				
C_{max} (mg/L)	0.90 (0.39-1.49)	0.52 (0.10-1.13)	0.48 (0.14-2.26)	0.58 [0.40-0.83]	0.02	0.54 [0.36-0.81]	0.02
T_{max} (h)[*]	2.5 (1.0-3.0)	3.0 (2.5-8.0)	4.0 (2.5-5.0)	-	0.02	-	0.03
$T_{1/2}$ (h)	1.3 (0.6-2.0)	1.8 (0.9-3.7)	1.8 (0.9-4.5)				
CL/*F*.kg (L/h.kg)	2.6 (1.6-3.4)	3.3 (1.6-7.2)	27 (1.0-3.8)				
Vd/*F*.kg (L/kg)	4.7 (2.7-9.3)	8.5 (3.7-38.6)	6.8 (1.8-18.9)				
Ae (mg)	64.7 (44.8-98.5)	48.9 (27.2-82.5)	53.4 (30.9-95.1)				
CL_R/*F*.kg (L/h.kg)	0.41 (0.18-0.67)	0.41 (0.24-0.52)	0.36 (0.23-0.52)				
Fe.*F*	0.16 (0.11-0.25)	0.13 (0.07-0.21)	0.15 (0.08-0.24)				

CI, Confidence interval; AUC, area under the concentration-time curve; C_{min}, trough concentration at 24 hours; C_{max}, highest observed plasma concentration; T_{max}, sampling time for C_{max}; CL/*F*.kg, total clearance corrected for weight; Vd/*F*.kg, volume of distribution corrected for weight; $T_{1/2}$, elimination half-life; *F*, bioavailability; Ae, total amount excreted with urine; CL_R/*F*.kg, renal clearance corrected for weight; Fe.*F*, fraction excreted by urine

* Median and range; Wilcoxon-signed rank test

[†] 400 mg didanosine 2 hours after breakfast

[‡] Combination of 1200 mg indinavir and 400 mg ritonavir and 400 mg didanosine 2 hours after breakfast

[#] Combination of 1200 mg indinavir and 400 mg ritonavir and 400 mg didanosine with breakfast

[$] Geometric mean ratio plus 90% CI for regimen C versus A

[&] Geometric mean ratio plus 90% CI for regimen D versus A

Ritonavir pharmacokinetic parameters are displayed in Table 3. Apart from a decreased T_{max} in regimen C ($P = 0.04$), all regimens (C and D) were bioequivalent to reference A for ritonavir pharmacokinetic parameters.

Table 3. Summary of ritonavir pharmacokinetic parameters (n=8)

Parameter	Geometric mean and range		
	Regimen B[†]	Regimen C[‡]	Regimen D[#]
AUC (h.mg/L)	66.1 (27.5-119.3)	66.2 (36.4-116.3)	58.7 (31.0-119.4)
C_{min} (mg/L)	0.42 (0.09-1.50)	0.32 (0.12-1.40)	0.27 (0.03-1.43)
C_{max} (mg/L)	6.2 (3.8-11.2)	6.9 (4.6-10.5)	5.9 (2.8-11.5)
T_{max} (h)[*]	4.0 (4.0-10.0)	2.5 (1.5-8.0)	4.0 (2.0-8.1)
$T_{1/2}$ (h)	4.4 (3.3-7.2)	4.3 (3.6-6.4)	4.4 (2.4-6.8)
CL/*F*.kg (L/h.kg)	0.08 (0.05-0.20)	0.08 (0.05-0.15)	0.09 (0.05-0.17)
Vd/*F*.kg (L/kg)	0.50 (0.35-1.16)	0.48 (0.35-0.88)	0.56 (0.40-1.28)

CI, Confidence interval; AUC, area under the concentration-time curve; C_{min}, trough concentration at 24 hours; C_{max}, highest observed plasma concentration; T_{max}, sampling time for C_{max}; CL/*F*.kg, total clearance corrected for weight; Vd/*F*.kg, volume of distribution corrected for weight; $T_{1/2}$, elimination half-life; *F*, bioavailability

* Median and range; Wilcoxon-signed rank test

† Combination of 1200 mg indinavir and 400 mg ritonavir with breakfast

‡ Combination of 1200 mg indinavir and 400 mg ritonavir and 400 mg didanosine 2 hours after breakfast

Combination of 1200 mg indinavir and 400 mg ritonavir and 400 mg didanosine with breakfast

Discussion

In the current study, we investigated the utility of the combination of indinavir/ritonavir with didanosine EC for once daily use in healthy subjects. The combination of these 3 drugs was given together with or 2 hours after breakfast to investigate possible food effects on the pharmacokinetics of indinavir and didanosine. These test regimens were compared to reference regimens of indinavir/ritonavir administered with breakfast or didanosine administered 2 hours after breakfast, respectively. Indinavir exposure (both AUC and C_{max}), when administered with didanosine and breakfast, was bioequivalent to reference regimen B. However, when indinavir was given with didanosine 2 hours after breakfast, C_{max} was 14% higher but still suggestive of bioequivalence in comparison to reference regimen B. From this study, it becomes apparent that the intake of indinavir/ritonavir with breakfast lowers the indinavir C_{max}. This is a desirable effect as indinavir toxicity is at least partly related to the magnitude of C_{max}[14]. Lowered indinavir C_{max} in the fed state has been reported by others as well[5,7,15]. Food is also known to delay

the absorption of indinavir, resulting in a delayed T_{max}[5,7,15]. The observed differences in indinavir pharmacokinetics following the different study regimens in the current study show that there were differences in the fed state of the subjects following the regimens of being dosed 2 hours after breakfast or together with breakfast. Combining indinavir/ritonavir with didanosine with breakfast did not change indinavir AUC or C_{max} relative to the same combination without didanosine, suggesting that there is no pharmacokinetic effect of didanosine on indinavir exposure. A lack of such effect has previously been reported[16]. When didanosine is given with breakfast, as in test regimen D, a decreased C_{max} and an increased T_{max} can be expected[17]. Given 2 hours after breakfast (regimen C), didanosine exposure decreased, indicating an interaction between absence of food and concomitant indinavir/ritonavir administration on didanosine absorption. However, such an effect has not been reported elsewhere. No statistical comparisons were made between regimens C and D. Nevertheless, didanosine AUC in regimen D is favorable over that in regimen C, derived from the statistical comparison with regimen A. Didanosine C_{max}, however, seems to be of the same magnitude in regimens C and D. Decreased absorption with regimen C in comparison to regimen A was further supported by the decrease in total renal excretion in regimen C (48.9 versus 64.7 mg in reference regimen A). In a previous study[18] comparing didanosine buffered tablets with enteric-coated didanosine in both healthy subjects and patients, a decreased C_{max} was observed for enteric-coated didanosine. However, AUC showed bioequivalence for both formulations, indicating that the absorption rate was slower, but total absorption remained constant. In this study, data from regimen D show the most favorable didanosine pharmacokinetics in that AUC values were similar to the reference regimen. Although this study was not conducted to investigate possible differences in ritonavir pharmacokinetics, the only statistical significant change observed was a decreased T_{max} in regimen C (P= 0.04), indicating faster absorption. These data indicate that the use of didanosine has no effect on ritonavir pharmacokinetics.

This study had its limitations; first, the sample size was small, with only 8 subjects. In addition, considerable variability in pharmacokinetic parameters was observed. Second, we cannot draw conclusions for a steady-state situation. However, the studied effects here mainly concern the absorption phase of the drugs, and these are not likely to be different when steady-state conditions apply. Third, at the time we conducted this study, no analytical assay to analyze intracellular triphosphate levels of didanosine was available. Didanosine needs to be converted intracellularly to the active triphosphate. To date, no data are available relating plasma didanosine levels to their intracellular triphosphate levels.

In conclusion, didanosine EC + indinavir/ritonavir with breakfast (test regimen D) shows the most favorable pharmacokinetics and could serve as the basis of a HAART regime. Didanosine exposure was slightly lower in test regimens C and D. However, the confidence intervals for the didanosine AUC ratios (regimens D and A) were within 1% of the bioequivalence range, suggesting that a large sample size may have demonstrated bioequivalence. Indinavir exposure was bioequivalent for the 2 fed

conditions compared to reference, with the exception of C_{max} in test regimen C. These results indicate that this HAART regimen could be administered with food without decreased bioavailability.

Acknowledgements

The subjects are kindly thanked for their participation in this study. All laboratory technicians of the Department of Clinical Pharmacy are gratefully acknowledged for their assistance in the analysis of the plasma and urine samples. This study was funded by a grant from Merck & Co. (Whitehouse Station, NJ).

References

1. **Paterson, D. L., S. Swindells, J. Mohr, M. Brester, E. N. Vergis, C. Squier, M. M. Wagener, and N. Singh.** 2000. Adherence to protease inhibitor therapy and outcomes in patients with HIV infection. Annals of Internal Medicine **133**:21-30.
2. **Mehta, S., R. D. Moore, and N. M. H. Graham.** 1997. Potential factors affecting adherence with HIV therapy. AIDS **11**:1665-1670.
3. **Stone, V. E.** 2001. Strategies for optimizing adherence to highly active antiretroviral therapy: lessons from research and clinical practice. Clin.Infect.Dis. **33**:865-872.
4. Crixivan® Summary of Product Characteristics. 2002. Merck and Co. Inc., Whitehouse Station, NJ, USA.
5. **Hsu, A., G. R. Granneman, G. Cao, L. Carothers, A. Japour, T. El Shourbagy, S. Dennis, J. Berg, K. Erdman, J. M. Leonard, and E. Sun.** 1998. Pharmacokinetic interaction between ritonavir and indinavir in healthy volunteers. Antimicrob.Agents Chemother. **42**:2784-2791.
6. **Heeswijk van, R. P. G., A. I. Veldkamp, R. M. W. Hoetelmans, J. W. Mulder, G. Schreij, A. Hsu, J. M. A. Lange, J. H. Beijnen, and P. L. Meenhorst.** 1999. The steady-state pharmacokinetics of indinavir alone and in combination with a low dose of ritonavir in twice daily dosing regimens in HIV-1-infected individuals. AIDS **13**:F95-F99.
7. **Burger, D. M., P. W. H. Hugen, R. E. Aarnoutse, J. P. Dieleman, J. M. Prins, T. v. d. Poll, J. H. 't Veen, J. W. Mulder, P. L. Meenhorst, W. J. Blok, J. T. M. Meer van der, P. Reiss, and J. M. A. Lange.** 2001. A retrospective, cohort-based survey of patients using twice daily indinavir + ritonavir combinations: pharmacokinetics, safety and efficacy. JAIDS **26**:218-224.
8. **Hugen, P. W., D. M. Burger, H. J. ter Hofstede, P. P. Koopmans, M. Stek, Y. A. Hekster, P. Reiss, and J. M. Lange.** 2000. Dose-finding study of a once-daily indinavir/ritonavir regimen. J.Acquir.Immune Defic.Syndr. **25**:236-245.
9. **Burger, D. M., R. E. Aarnoutse, J. P. Dieleman, I. C. Gyssens, J. Nouwen, S. de Marie, P. P. Koopmans, M. Stek, Jr., and M. E. Van Der Ende.** 2003. A once-daily HAART regimen containing indinavir + ritonavir plus one or two nucleoside reverse transcriptase inhibitors (PIPO study). Antivir.Ther. **8**:455-461.
10. Videx EC® Summary of Product Characteristics. Bristol-Myers Squibb B.V., Woerden, The Netherlands. 29-8-2003.

11. **Hugen, P. W. H., C. P. W. G. M. Verwey-van Wissen, D. M. Burger, E. W. Wuis, P. P. Koopmans, and Y. A. Hekster.** 1999. Simultaneous determination of the HIV-protease inhibitors indinavir, nelfinavir, saquinavir and ritonavir in human plasma by reversed-phase high-performance liquid chromatography. Journal of Chromatography B Biomed Sci Appl **727**:139-149.
12. **Aarnoutse, R. E., K. J. Grintjes, D. S. Telgt, M. Stek, Jr., P. W. Hugen, P. Reiss, P. P. Koopmans, Y. A. Hekster, and D. M. Burger.** 2002. The influence of efavirenz on the pharmacokinetics of a twice-daily combination of indinavir and low-dose ritonavir in healthy volunteers. Clin Pharmacol Ther. **71**:57-67.
13. **Williams, R. L., M. L. Chen, and W. W. Hauck.** 2002. Equivalence approaches. Clin.Pharmacol. Ther. **72**:229-237.
14. **Dieleman, J. P., I. C. Gyssens, v. d. M. E. Ende, d. S. Marie, and D. M. Burger.** 1999. Urological complaints in relation to indinavir plasma concentrations in HIV-infected patients. AIDS **13**:473-478.
15. **Saah, A., Winchell, G., Seniuk, M., Mehrotra, D., and Deutsch, P.** 1999. Multiple-dose pharmacokinetics and tolerability of indinavir-ritonavir combinations in healthy volunteers. 6th Conference on Retroviruses and Opportunistic Infections, 31 January-4 February 1999, Chicago, IL, USA, abstract 362.
16. **Damle, B. D., V. Mummaneni, S. Kaul, and C. Knupp.** 2002. Lack of effect of simultaneously administered didanosine encapsulated enteric bead formulation (Videx EC) on oral absorption of indinavir, ketoconazole, or ciprofloxacin. Antimicrob.Agents Chemother. **46**:385-391.
17. **Damle, B. D., J. H. Yan, D. Behr, E. O'Mara, P. Nichola, S. Kaul, and C. Knupp.** 2002. Effect of food on the oral bioavailability of didanosine from encapsulated enteric-coated beads. J.Clin. Pharmacol. **42**:419-427.
18. **Damle, B. D., S. Kaul, D. Behr, and C. Knupp.** 2002. Bioequivalence of two formulations of didanosine, encapsulated enteric-coated beads and buffered tablet, in healthy volunteers and HIV-infected subjects. J.Clin.Pharmacol. **42**:791-797.

Chapter 5

Comparison of two reduced-dose regimens of indinavir (600 mg vs 400 mg twice daily) and ritonavir (100 mg twice daily) in healthy volunteers (COREDIR)

J-C Wasmuth[1], C.J.L. la Porte[2], K. Schneider[1], D.M. Burger[2], J.K. Rockstroh[1]

1 Department of Internal Medicine I, University of Bonn, Germany.
2 Department of Clinical Pharmacy and Nijmegen University Centre for Infectious diseases, University of Nijmegen, The Netherlands.

Antiviral Therapy. 2004; 9: 213-220

Abstract

Objective: To assess the pharmacokinetics and tolerability of reduced dosages of twice daily indinavir boosted by low-dose ritonavir in healthy volunteers.
Methods: Pharmacokinetics and tolerability of indinavir/ritonavir twice daily (600/100 mg and 400/100 mg) were assessed in a randomized crossover design in 16 healthy volunteers. Each dosage was taken twice daily for 2 weeks before 12 h pharmacokinetics were obtained.
Results: Sixteen subjects were included, with a mean age ±SD of 30 ±4 years; seven female, nine male. Fifteen subjects completed the study. After dose reduction of indinavir AUC, C_{max} and C_{min} decreased significantly. In the 400 mg group three out of 15 subjects had indinavir levels below 0.10 mg/L versus none in the 600 mg group.
All subjects reported mild to moderate side effects throughout the study period, which were more severe in the 600 mg group (mostly renal, dry skin/lips, paresthesias/oral discomfort). In the 600 mg group four subjects reported dysuria and one subject discontinued because of flank pain, whereas two subjects reported dysuria and no subject discontinued in the 400 mg group, respectively. Eight subjects developed crystalluria without a significant difference between both groups. No significant change in serum creatinine was observed.
Conclusions: indinavir/ritonavir 400/100 mg twice daily resulted in significant lower indinavir exposure, with three out of 15 subjects revealing C_{min} values below the recommended threshold for wild-type virus of 0.10 mg/L. Tolerability, however, was lower in the 600 mg indinavir group. Therapeutic drug monitoring in the individual patient appears to be necessary to guarantee appropriate drug levels and simultaneously minimize toxicity.

Introduction

The introduction of highly active antiretroviral therapy has markedly improved the treatment of HIV infection. Although drug therapy may have reached near maximal efficacy, toxicity and adherence issues are the most important obstacles to long-term treatment, which is a lifelong treatment at the moment. In addition, most HIV-positive patients in the world do not have access to current treatments due to the high costs of antiretroviral drugs. Therefore, it is important to explore drug regimens that are easier to adhere to, have less toxicity than current regimens and are made cheaper by reducing the amount of pills necessary, while the antiviral activity is preserved.

This pharmacokinetic study focuses on protease inhibitor combinations of indinavir and ritonavir. Combinations of these two protease inhibitors are widely used in clinical practice[1,2]. The two most common regimens are indinavir/ritonavir 400/400 mg and 800/100 mg twice daily, respectively[3,4]. The latter combination has the advantage of fewer pills and less ritonavir toxicity, which might be beneficial in the long term. However, a shortcoming of this combination is a higher rate of nephrotoxicity due to the development of kidney stones as compared to the indinavir monotherapy or the 400/400 mg twice daily combination[5]. Therefore, a dose reduction of indinavir might mitigate the rate and severity of side effects, on top of that reducing the costs of antiretroviral treatment. Preliminary clinical data with combinations of indinavir/ritonavir of 600/100 mg and 400/100 mg twice daily suggest that such a dose reduction will not affect the antiviral potency of the combination[6-8]. However, pharmacokinetic data on these particular indinavir/ritonavir combinations do not exist in detail so far and indinavir cannot simply be reduced in the indinavir/ritonavir combination, since it cannot be excluded that dose reduction may result in subtherapeutic plasma levels of indinavir. Therefore, we studied the steady state pharmacokinetics of the 600/100 mg and 400/100 mg twice daily indinavir/ritonavir combinations in healthy volunteers.

Methods

Subjects and treatment

Sixteen healthy volunteers were recruited for this study. All volunteers were HIV-negative, had not received any antiretroviral treatment before and had no known underlying disease. All volunteers gave written informed consent. All study procedures were done in accordance with the current revision of the Helsinki declaration of 1975, and the Ethics committee of the University of Bonn, Faculty of Medicine, Bonn, Germany approved the study.

Following a crossover design, volunteers were randomly assigned to start either with a dose of 400 mg indinavir twice daily boosted by baby-dose ritonavir (100 mg twice daily; treatment 1) or with a dose of 600 mg indinavir twice daily boosted by baby-dose ritonavir (treatment 2). They were instructed to ingest the drugs in the morning and

in the evening at 12-h intervals together with a light meal[9]. Furthermore, participants had to drink 1.5 l of water in addition to their normal daily fluid intake to prevent possible nephrotoxicity. After 2 weeks a complete 12-h pharmacokinetic assessment was done, and volunteers switched to the other dose regimen for another 2 weeks.
The study was conducted on an outpatient basis at the Medizinische Klinik, University of Bonn, Germany. On days 4, 8, 14, 18, 22 and 28, drug administration was performed under staff supervision. Compliance with study medication at home was evaluated at every study visit by inspection of drug-taking diaries, counting of capsules and measurement of plasma drug concentrations.
Intensive blood and urine sampling was performed during days 14 and 28 after an overnight fast. Subjects had taken the last dose of indinavir and ritonavir the preceding evening. A pre-dose blood sample was taken, and subjects then ingested indinavir and ritonavir with a standardized medium-fat medium-calorie breakfast (610 kcal in total, 16% of which was attributable to protein and 33 and 51% to fat and carbohydrates, respectively). Serial plasma sampling was performed at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, 6.0, 8.0, 10.0 and 12.0 h after drug administration. In four randomly selected participants urine samples were collected after initial voiding of the bladder at 2-h intervals up to 12 h after ingestion of the drugs. Standard meals were served at lunchtime (4 h after ingestion of the drugs) and dinnertime (10 h after ingestion of the drugs). Blood samples were processed within 6 h after drawing. Plasma and urine samples were stored at –70°C until analysis.

Safety and tolerability
Safety and tolerability were assessed by a self-developed questionnaire that presented 17 possible adverse events that may occur during treatment with indinavir or ritonavir. The volunteers were questioned six times (on days 4, 8, 14, 18, 22 and 28). They could also mention adverse events that were not on the list. Participants were asked to grade every complaint as mild (symptoms do not interfere with daily activities), moderate (symptoms may interfere with daily activities) or severe (symptoms interrupt daily activities). Subjects were physically examined on every study visit and questioned about the reported adverse events in order to validate the self-reporting. On the same 6 study days an extensive blood chemistry and haematology screen, and urine analysis were performed.

Bioanalysis of indinavir and ritonavir
Plasma samples were analysed simultaneously for indinavir and ritonavir, and urine samples for indinavir concentrations. A validated reversed-phase HPLC method was used for determination of indinavir and ritonavir plasma levels[10]. Urine samples were analysed for indinavir with another HPLC method that has been described previously[11], but with a modified sample pre-treatment procedure that has been described elsewhere[12].

Pharmacokinetic analysis

The pharmacokinetic parameters of indinavir and ritonavir were calculated with standard non-compartmental methods[13]. The terminal, log-linear period (log C versus t) was defined by visual inspection of the last data points ($n \geq 3$). The absolute value of the slope (β/2.303) was calculated by least squares linear regression analysis (β is the first-order elimination rate constant). The elimination half-life ($T_{1/2}$) was calculated by the equation 0.693/β. The area under the concentration-versus-time curve (AUC) – calculated using the trapezoidal rule – was extrapolated to infinity by adding C_{min}/β and was corrected for the contribution of the predose AUC by subtraction of C_0/β, in which C_0 is initial plasma drug concentration. The apparent clearance (CL/*F*, where *F* is bioavailability) was calculated by dividing dose (D) by AUC, and apparent volume of distribution (V/*F*) was obtained by dividing CL/*F* by β.

The cumulative renal excretion of indinavir (Ae) was approximated by the total amount of indinavir that was excreted unchanged in the urine during the dosing interval; Ae=Σ (volume urine x concentration indinavir in urine). Renal clearance (CL_R) of indinavir was calculated using the formula Ae/AUC. The fraction of the total amount of indinavir excreted unchanged (fe) was calculated using the formula: fe x *F*=Ae/D=CL_R/CL.

Data analysis

All statistical evaluations were performed with SPSS for Windows, version 10 (SPSS, Inc., Chicago, Ill., USA) and the SAS software package. Prior to statistical analysis, the pharmacokinetic parameters of indinavir and ritonavir (apart from T_{max}) were logarithmically transformed. The effect of dose-reduction of indinavir on the steady-state pharmacokinetics of indinavir and ritonavir was evaluated by comparison of the pharmacokinetic parameters of days 14 and 28 with the use of the two-sided Student t test for paired samples. Furthermore, geometric mean ratios with 95% confidence intervals were calculated for every comparison, and the model used included adjustment for the treatment sequence. The values for C_{max} sampling time (T_{max}) were not transformed and were compared with the Wilcoxon signed-rank test. A *P*-value ≤ 0.05 was considered to be significant in all analyses.

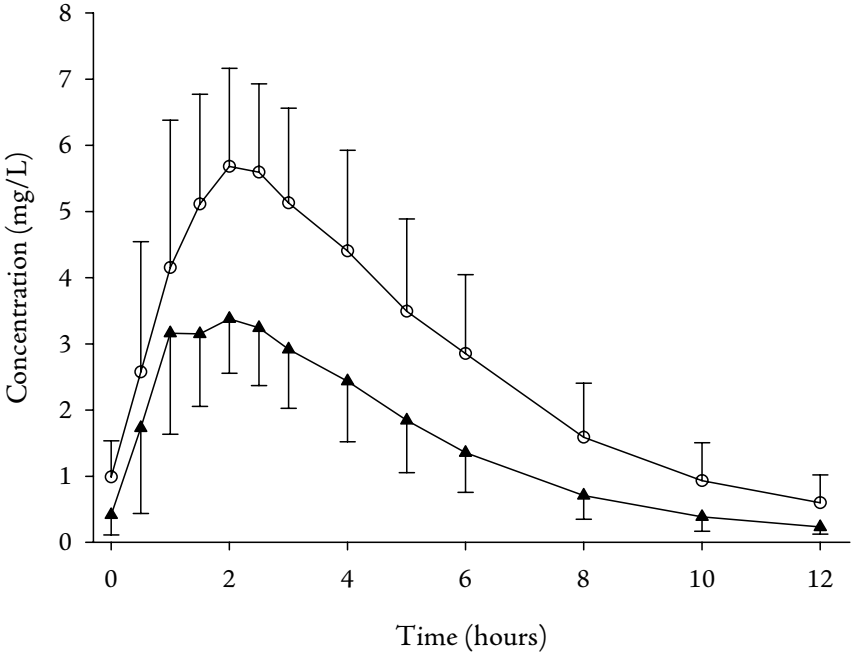
The incidence of adverse events was calculated separately for the first and second study period according to both dosages. It was expressed as the percentage of participants that reported a particular adverse event at least one time during the six consecutive reporting times in every study period. Statistical significance between groups was tested with the McNemar-Test for dichotomized paired samples. Consequently, every reported mild, moderate or severe adverse event was ascribed a severity score of 1, 2 or 3 points, respectively. All scores were added up for every participant and were divided by the number of reporting times. In this way the mean toxicity scores for both dose groups were obtained for all individual participants. Differences in toxicity scores between groups were calculated with the use of the Wilcoxon test for paired samples. Correlation between parameters was calculated with the Pearson correlation coefficient (r) or Spearman's correlation coefficient (rs).

Results

Study subjects

Sixteen subjects were included in the study (seven female, nine male); 15 of those completed both study periods. One participant had to be withdrawn after switching to the higher indinavir dose due to an adverse event (flank pain and crystalluria). Accordingly, this subject was included into the analysis of safety and tolerability, but was excluded from the calculation of pharmacokinetic parameters. The mean age of subjects was 30 years (age range, 24–41 years) and mean weight was 71 kg (weight range, 49–90 kg). The combination of methods for measurement of compliance allowed for a reliable estimation of adherence to study medication. Compliance was excellent in all volunteers. There were no reported doses missed. Three subjects reported a deviation in time of drug intake (one deviation each); two subjects reported two deviations each. None of these time deviations occurred prior to 3 days before study days 14 and 28.

Figure 1. Indinavir steady-state plasma concentration-time profiles



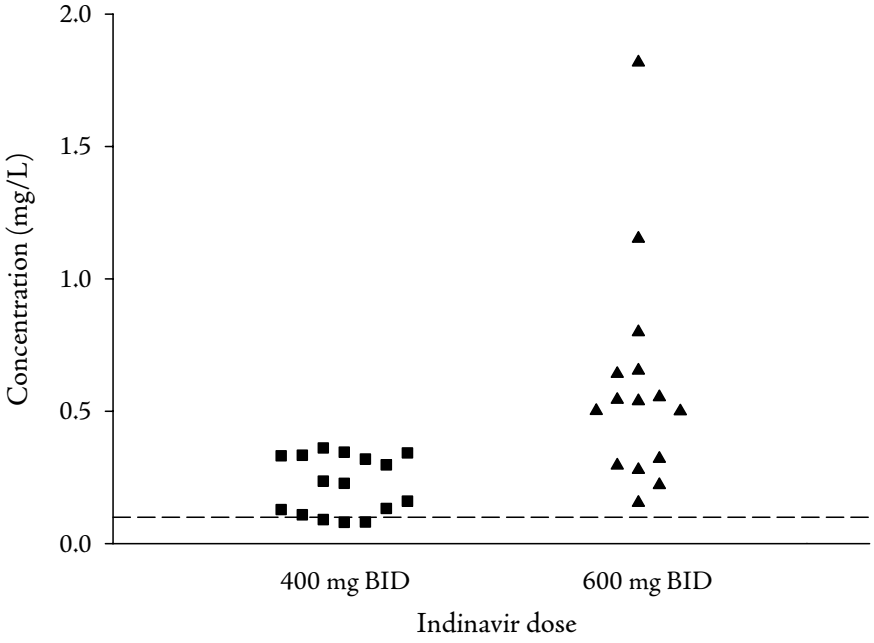
Indinavir/ritonavir 600/100 mg BID
Indinavir/ritonavir 400/100 mg BID
Data from 15 subjects are presented as means with standard deviation in error bars

Pharmacokinetics of indinavir

Dose reduction of indinavir resulted in a decrease in indinavir $AUC_{0-\infty}$, C_{max} and C_{min} (Table 1, Figures 1 and 2).

Indinavir $AUC_{0-\infty}$ decreased in all subjects; C_{max} decreased in all but one subject, in whom it increased from 4.82 to 5.28 mg/L despite dose reduction of indinavir; similarly, C_{min} decreased in all but one subject, in whom it increased from 0.28 to 0.35 mg/L. After dose reduction three out of 15 subjects in the 400 mg group had C_{min} values below the recommended threshold of 0.10 mg/L (Figure 2).

Figure 2. Indinavir minimum plasma concentration



– – – Recommended treshold of 0.1 mg/L indinavir
Data presented are Cmin values of 15 subjects

There was a significant linear correlation between C_{min} values and the weight of the participants after dose reduction of indinavir to 400 mg (r_s=–0.719, P<0.001), but not for the higher indinavir dose (r_s=–0.491, P=0.063). Accordingly, the three subjects with too low C_{min} values all were heavy men (89, 83 and 90 kg, respectively). Marked variability in C_{min} values narrowed after dose reduction of indinavir.

There exist several upper limit thresholds that are linked with an increase in indinavir toxicity[1,7,14,15]. With treatment 2 (600 mg indinavir twice daily) there were eight subjects with indinavir C_{min} values above 0.5 mg/L[14] and three subjects with indinavir

C_{min} above 0.675 mg/L[7], whereas all subjects with treatment 1 (400 mg indinavir twice daily) had C_{min} values below those thresholds. In the 600 mg indinavir group there were two subjects with indinavir C_{max} values above 8 mg/L[1]. No subject in this group had indinavir C_{max} values above 10 mg/L[15], as all subjects in the 400 mg indinavir group had C_{max} values below those thresholds.
Total clearance increased significantly after dose reduction of indinavir. However, no significant changes were seen in either renal clearance of indinavir or the fraction of indinavir excreted unchanged with urine. The cumulative renal excretion of indinavir was higher in the 600 mg twice daily group than in the 400 mg twice daily group.

Table 1. Summary of steady-state pharmacokinetic parameters of indinavir (n=15)

Parameter	Geometric mean and range			
	Treatment 1[†]	Treatment 2[‡]	geometric mean ratio (treatment 1 /treatment 2) [95% CI]	*P* value[#]
$AUC_{0\text{-infinity}}$ (h.mg/L)	16.18 (9.98-29.63)	30.15 (17.47-56.52)	0.54 [0.48-0.61]	<0.001
C_{min} (mg/L)	0.19 (0.08-0.36)	0.49 (0.16-1.82)	0.38 [0.28-0.54]	0.002
C_{max} (mg/L)	3.84 (2.68-5.28)	6.03 (4.82-8.42)	0.63 [0.56-0.71]	<0.001
T_{max} (h)[*]	1.75 (0.50-4.0)	2.0 (1.0-4.0)		0.094
CL/*F* (L/h)	24.42 (13.75-39.97)	19.93 (11.13-34.94)		0.001
CL/*F*.kg (L/h.kg)	0.34 (0.20-0.46)	0.28 (0.19-0.43)		
Vd (L)	74.88 (43.58-124.79)	68.12 (45.95-110.65)		
Vd/*F*.kg (L/kg)	1.07 (0.63-1.85)	0.96 (0.72-1.27)		
$T_{1/2}$ (h)	2.20 (1.68-2.79)	2.41 (1.94-3.64)		
Ae (mg)[$]	211 (152-229)	313 (249-345)		
CL_R/*F*.kg (L/h.kg)[$]	0.18 (0.16-0.26)	0.16 (0.15-0.23)		
Fe.*F*[$]	0.53 (0.38-0.57)	0.52 (0.42-0.57)		

CI, Confidence interval; AUC, area under the concentration-time curve; C_{min}, trough concentration at 12 hours; C_{max}, highest observed plasma concentration; T_{max}, sampling time for C_{max}; CL, total clearance; CL/*F*.kg, total clearance corrected for weight; Vd (L), volume of distribution; Vd/*F*.kg (L/kg), volume of distribution corrected for weight; $T_{1/2}$, elimination half-life; *F*, bioavailability
* Median and range; Wilcoxon-signed rank test
# *P* value for the difference between PK parameters in the two study periods; 2-sided t-test for paired data
† Combination of 400 mg indinavir and 100 mg ritonavir twice a day
‡ Combination of 600 mg indinavir and 100 mg ritonavir twice a day
$ Median and range; based on data of 4 subjects

Pharmacokinetics of ritonavir

Dose reduction of indinavir resulted in a significant decrease of ritonavir C_{min} and T_{max} (Table 2). There was a trend to a lower $AUC_{0-\infty}$ of ritonavir, which, however, did not reach statistical significance. Ritonavir C_{max} remained unchanged after dose modification of indinavir. There was a significant correlation of C_{min} concentrations of ritonavir and indinavir in both dosage groups (600 mg group: Pharmacokinetics of indinavir/ritonavir in reduced dose rs=0.864, $P<0.001$; 400 mg group: rs=0.788, $P<0.001$) as well as of the AUC of both substances (600 mg group: rs=0.827, $P<0.001$; 400 mg group: rs=0.836, $P<0.001$).

Table 2. Summary of steady-state pharmacokinetic parameters of ritonavir (n=15)

Parameter	Geometric mean and range		
	Treatment 1†	Treatment 2‡	*P* value#
$AUC_{0\text{-infinity}}$ (h.mg/L)	9.25 (4.37-21.47)	11.47 (5.75-27.02)	0.06
C_{min} (mg/L)	0.23 (0.09-0.67)	0.42 (0.16-1.72)	<0.001
C_{max} (mg/L)	1.55 (0.67-3.64)	1.66 (0.78-5.04)	0.62
T_{max} (h) *	1.50 (0.50-5.00)	4.08 (1.00-6.00)	0.009
CL/*F* (L/h)	11.68 (4.82-26.66)	9.49 (3.60-24.54)	
CL/*F*.kg (L/h.kg)	0.16 (0.07-0.32)	0.13 (0.06-0.40)	
Vd (L)	54.99 (17.57-140.80)	45.84 (9.33-219.23)	
Vd/*F*.kg (L/kg)	0.77 (0.25-2.23)	0.64 (0.16-3.59)	
$T_{1/2}$ (h)	3.26 (2.51-5.50)	3.35 (1.80-6.52)	

CI, Confidence interval; AUC, area under the concentration-time curve; C_{min}, trough concentration at 12 hours; C_{max}, highest observed plasma concentration; T_{max}, sampling time for C_{max}; CL, total clearance; CL/*F*.kg, total clearance corrected for weight; Vd (L), volume of distribution; Vd/*F*.kg (L/kg), volume of distribution corrected for weight; $T_{1/2}$, elimination half-life; *F*, bioavailability

* Median and range; Wilcoxon-signed rank test

\# *P* value for the difference between PK parameters in the two study periods; 2-sided *t*-test for paired data

† Combination of 400 mg indinavir and 100 mg ritonavir twice a day

‡ Combination of 600 mg indinavir and 100 mg ritonavir twice a day

Safety and tolerability

One subject discontinued medication after switching to the higher indinavir dose of 600 mg twice daily because of flank pain and crystalluria. When taking the 400 mg indinavir dosage C_{max} and AUC values for indinavir were not significantly higher in this subject than in the other subjects (C_{max} 4.3 mg/L, geometric mean for the other 15 subjects was 3.9 mg/L; AUC 22.8 mg/L, geometric mean 17.5 mg/L). The C_{min} value of this subject was 0.34 mg/L. There were two other adverse events grade 3 (diarrhoea, taste disturbance) that occurred in two subjects after beginning medication with the

lower indinavir dose of 400 mg twice daily. Both did not lead to discontinuation and resolved within a few days without intervention.

The incidence of adverse events as assessed by repeated questioning of the participants is summarized in Table 3. The mean toxicity score for treatment 1 (400 mg indinavir twice daily) was 2.5 (range, 0–10). In 14 of 16 subjects the severity score increased with the higher dose of indinavir (600 mg twice daily) during treatment 2. Mean toxicity score for this treatment period was 4.2 (range, 0.3–10.3; P=0.008). In the 600 mg group four subjects reported dysuria and one subject discontinued due to flank pain as mentioned above. On the other hand, in the 400 mg group two subjects reported dysuria and no subject discontinued. Eight subjects developed crystalluria without a significant difference between both dosage groups. Close examination of Table 3 and the corresponding toxicity scores revealed that not only did the incidence of kidney-related adverse events increase, but also the median toxicity score for kidney-related adverse events. The median toxicity score for all kidney-related adverse events (dysuria, flank pain and/or crystalluria) increased from 0.35 to 0.44 (P=0.72). Primarily, an increase in the toxicity score for the clinical symptoms dysuria and flank pain contributed to this deterioration (0.06–0.23; P=0.18). On the contrary, the median toxicity score for all adverse events related to the gastrointestinal system (nausea, abdominal pain, meteorism, vomiting, diarrhoea) was 1.50 for treatment 1 and 1.56 for treatment 2, and did not differ significantly (P=0.82). There was no clear correlation between indinavir AUC, C_{max} or C_{min}, and mean toxicity score or any organ-related toxicity score, respectively.

The incidence and severity of dry skin and dry lips, which are usually regarded as adverse events related to indinavir, as well as of paresthesia, oral discomfort and taste disturbance, which are assumed to be related to ritonavir, were higher during the treatment with indinavir 600 mg twice daily.

Analysis of laboratory parameters in all 16 subjects showed no significant differences in laboratory parameters between groups at baseline apart from creatinine, which was not clinically significant (group 1: 1.1 ±0.1 mg/dl; group 2: 0.9 ±0.1 mg/dl; P=0.001; normal range for creatinine: <1.4 mg/dl). Clinically relevant changes in laboratory parameters were observed only for total bilirubin, fasting triglyceride and fasting cholesterol levels. Total bilirubin increased in all subjects from 0.57 ±0.32 mg/dl (mean ±SD) at baseline to 1.34 ±0.60 mg/dl at the maximum (P=0.004, normal range for total bilirubin: 0.1–1.2 mg/dl). Fasting triglyceride levels increased from 88 ±46 mg/dl at baseline to 134 ±65 mg/dl at the maximum (P=0.001, normal range for fasting triglyceride: <200 mg/dl). Fasting cholesterol levels increased from 187 ±38 mg/dl at baseline to 229 ±49 mg/dl at the maximum (P<0.001, normal range for fasting cholesterol: <220 mg/dl). There was a very small increase in creatinine values, which, however, was not clinically relevant (0.98 ±0.14 versus 1.06 ±0.15 mg/dl; P=0.004; normal range: 0.5–1.4 mg/dl). There were no changes in serum GGT, ALT, AST, plasma glucose, total blood leukocytes, haemoglobin or thrombocytes. There was no correlation between increases in bilirubin, cholesterol or triglyceride levels and any pharmacokinetic parameter of indinavir or ritonavir.

Table 3. Incidence of adverse events in % (n=16)

Adverse event	Treatment 1[†]	Treatment 2[‡]	*P*-value*
Abdominal Pain	44	25	0.25
Diarrhoea	56	56	1.0
Meteorism	69	56	0.625
Nausea	31	50	0.25
Vomiting	0	13	0.5
Total Gastro intestinal	40	40	1.0
Crystalluria	38	31	1.0
Dysuria	13	25	0.625
Flank Pain	0	13	0.5
Total Kidney-related	17	23	1.0
Fever	0	0	1.0
Headache	19	31	0.5
Joint Pain	0	6	1.0
Muscle Pain	13	13	1.0
Oral discomfort	38	44	1.0
Paresthesia	6	31	0.125
Skin Abnormalities	38	50	0.625
Taste disturbance	50	69	0.25
Tiredness	38	44	1.0
Weakness	31	38	1.0
Not in the questionnaire:			
Dry lips	25	44	0.25
Epigastric Pain	6	0	1.0
Pimples	0	6	1.0
Sweats	13	6	1.0
Uric urge	6	6	1.0
Vertigo	6	6	1.0

[†] Combination of 400 mg indinavir and 100 mg ritonavir twice a day
[‡] Combination of 600 mg indinavir and 100 mg ritonavir twice a day
*McNemar-Test for dichotomized paired samples

Conclusions

The results of this study describe steady-state pharmacokinetic data for two reduced dosages of twice daily combinations of indinavir and low-dose ritonavir in healthy volunteers. As expected, they show that exposure to indinavir is reduced after reduction of indinavir dose. Compared to reported pharmacokinetic parameters of indinavir when administered as indinavir/ritonavir 800/100 mg twice daily, indinavir exposure was even lower (reported values for C_{max}: 6.8–8.7 mg/L; C_{min}: 0.44–0.99 mg/L; AUC: 37.7–44)[9,10,16].

It has been shown that antiviral efficacy of indinavir is dependent on C_{min} values, which should be kept above a certain threshold in order to obtain and maintain adequate suppression of viral replication[17]. There are different thresholds for indinavir trough concentrations, but lately a threshold of 0.10 mg/L has been proposed to be adequate for indinavir in naive patients[17,18], which we have taken for the analysis in this study. There were no C_{min} values in any of the subjects below this threshold in the 600 mg indinavir group. Even the lowest indinavir C_{min} observed in this group (0.16 mg/L) was equivalent to the mean C_{min} in the regimen of indinavir three times a day[19], and it is safely above the presumed therapeutic threshold of 0.10 mg/L. However, after dose reduction of indinavir to 400 mg there were three subjects (20%) with C_{min} values of indinavir below this threshold, thereby provoking the risk of possible viral replication and resistance development in case of HIV infection. Thus, indinavir dose cannot safely be lowered without controlling the resulting C_{min} value of indinavir. All subjects with C_{min} values below the 0.10 mg/L threshold were relatively heavy men. Accordingly, there was a linear correlation between C_{min} values of indinavir and the body weight of the subjects. However, there were heavy men with adequate C_{min} values also, and, therefore, it does not seem possible to predict the safety of dose reduction in an individual patient based on body weight. Therapeutic drug monitoring (TDM) in the individual patient could overcome this uncertainty. A recent clinical study of indinavir/ritonavir 400/100 mg twice daily in HIV-infected patients showed that this dosage may be effective in the treatment of HIV infection as compared to the traditional indinavir 800 mg three times a day dosage[8]. In contrast to our study, C_{min} values were above the threshold of 0.10 mg/L in all compliant patients studied (lowest observed C_{min} value 0.14 mg/L). There are several explanations to this discrepancy. First, it is a study in HIV-infected patients and not healthy volunteers. There might be differences in tolerability that may be better in HIV-infected patients, or pharmacokinetics that contribute to the divergent results of our study and the study by Ghosn *et al.* Pharmacokinetics in HIV-infected patients may be significantly altered due to concomitant medication possibly interfering with indinavir or ritonavir metabolism, or underlying diseases such as chronic hepatitis. On the other hand, there might be a selection bias, as only patients virologically suppressed were included by Ghosn *et al.* Accordingly, patients in that study had relatively high C_{min} values with the three times a day regimen. From our results we conclude that the indinavir/ritonavir 400/100 mg twice daily regimen should not be administered without the use of TDM.

Nevertheless, the study by Ghosn *et al*. together with our data indicates that a reduced dose of ritonavir-boosted indinavir might be beneficial in the clinical setting.
Similar to indinavir, the exposure to ritonavir was reduced after reduction of indinavir dose. This finding was not anticipated, but is not surprising as indinavir has an inhibiting effect on the metabolism of ritonavir[20]. Accordingly, during treatment with the higher indinavir dose of 600 mg twice daily the metabolism of ritonavir possibly has a more pronounced inhibition than by the lower dose of 400 mg twice daily, resulting in increased ritonavir exposure. Interestingly, ritonavir levels in the 600 mg period resulted in a non-linear increase of indinavir levels after increase of indinavir dosage from 400 to 600 mg twice daily (data not shown). This also might be an explanation for the narrowing of indinavir C_{min} concentrations after dose reduction of indinavir. Higher ritonavir levels are associated with higher indinavir levels than expected (this is supported by the finding of significant correlations between C_{min} values in both dosage groups). Therefore, more inhibition of indinavir metabolism by ritonavir in the 600 mg group compared to the 400 mg group in general might lead to more variability in the 600 mg group. However, the clinical consequence of pharmacokinetic changes have to be derived from indinavir pharmacokinetics rather than from ritonavir, because low-dose ritonavir is meant only as a pharmacokinetic enhancer and will not contribute to the antiviral effect of this indinavir/ritonavir combination.
In contrast to antiviral efficacy, it is assumed that the development of indinavir-related toxicity depends on the height of plasma levels[19]. In particular, nephrotoxicity, which is caused by precipitation of indinavir-containing crystals in the urinary tract, seems to be dependent on the height of the plasma level[5,15,21]. Furthermore, there is anecdotal evidence that other indinavir-related side effects (nausea, vomiting, skin abnormalities, hyperbilirubinaemia) are associated with higher peak plasma levels of indinavir, and that these side effects disappear when plasma levels approach average population values. In general, indinavir plasma concentrations above 8 mg/L appear to be associated with severe side effects[1], although other thresholds have been defined also[7,14,15]. This hypothesis appears to be confirmed in our study. The overall incidence and median toxicity score of adverse events are lower in the 400 mg than in the 600 mg indinavir group. Although there was no clear statistical correlation to specific pharmacokinetic parameters of indinavir, which is difficult to draw, there is a clear association of indinavir dosage and severity of adverse events. The most limiting adverse event of indinavir is nephrotoxicity, clinically resulting in the development of flank pain, dysuria and kidney stones. In our study, one subject had to be withdrawn because of severe flank pain and dysuria, although a kidney stone was not found. This particular subject had tolerated the lower indinavir dose of 400 mg quite well with minor dysuria, but had to discontinue after switching to the higher indinavir dose of 600 mg. Of note, all subjects had to add an amount of 1.5 l water to their usual daily fluid intake[19,22]. Despite this measure, which was followed by this subject, severe flank pain could not be avoided. C_{max} and AUC values for indinavir were not significantly higher in this subject than in the other subjects, and the C_{min} indinavir concentration of this subject was well below the recently defined upper thresholds of 0.5 or 0.675

mg/L, which have been associated with higher indinavir toxicity[7,14].
It has to be taken into account that volunteers in this study were healthy volunteers not used to take medication regularly. It is not possible to transduce the results directly to HIV patients. On the contrary, compared to other studies with healthy volunteers the drop-out rate in this study is very low, thereby indicating the good tolerance of both indinavir/ritonavir combinations. Not all adverse events observed are necessarily linked to indinavir. Especially, perioral disturbances/oral discomfort and paresthesias are known to be specific to ritonavir. Unexpectedly, the incidence and severity of these adverse events was higher in the indinavir 600 mg group also. The elevated AUC of ritonavir in this group best explains this, although C_{max} values did not differ significantly between both dosage groups. However, it is known that these side effects can resolve after a longer period of time, whereas our subjects took each dosage only for 2 weeks.
In this study, there is no possibility to assess whether a reduced dose of indinavir will result in a reduced incidence or severity of long-term side effects such as lipodystrophy/lipoatrophy and associated changes. Interestingly, we found a significant change in fasting cholesterol and triglyceride levels, thereby indicating a metabolic influence of indinavir/ritonavir already after the 2-week period. In general, these side effects are considered to be long-term side effects also. Despite hyperbilirubinaemia there was no other relevant change in laboratory parameters, especially liver transaminases.
Theoretically, the optimal way to use indinavir is to keep trough levels above the threshold for HIV suppression and to avoid high peak levels. The results of our study show that this goal can by achieved by dose reduction of indinavir/ritonavir combination as compared to the common dose of 800 mg indinavir twice daily boosted by low-dose ritonavir. However, with the dose of 600 mg indinavir twice daily some patients will still suffer from indinavir-related toxicity, whereas lowering the indinavir dose to 400 mg bears the risk of insufficient indinavir plasma levels. Therefore, it seems to be mandatory to perform TDM in the individual patient to guide antiretroviral therapy of the indinavir/ritonavir twice daily combination. The clinical effectiveness of this approach has to be evaluated in further prospective clinical trials.

Acknowledgements

We thank the healthy volunteers for their participation in this study. We thank the technicians of the Department of Clinical Pharmacy at the University of Nijmegen for analysis of the plasma and urine samples. This work was supported by a grant from the HW & J Hector Stiftung, Weinheim.

References

1. **Burger, D. M., P. W. H. Hugen, R. E. Aarnoutse, J. P. Dieleman, J. M. Prins, T. v. d. Poll, J. H. t. Veen, J. W. Mulder, P. L. Meenhorst, W. J. Blok, J. T. M. Meer van der, P. Reiss, and J. M. A. Lange**. 2001. A retrospective, cohort-based survey of patients using twice daily indinavir + ritonavir combinations: pharmacokinetics, safety and efficacy. JAIDS **26**:218-224.
2. **Heeswijk van, R. P. G., A. I. Veldkamp, J. W. Mulder, P. L. Meenhorst, J. M. A. Lange, J. H. Beijnen, and R. M. W. Hoetelmans**. 2002. Combination of protease inhibitors for the treatment of HIV-1-infected patients: a review of pharmacokinetics and clinical experience. Antiviral Therapy **6**:201-229.
3. **Lichterfeld, M., H. D. Nischalke, F. Bergmann, W. Wiesel, A. Rieke, A. Theisen, G. Fatkenheuer, M. Oette, H. Carls, S. Fenske, M. Nadler, H. Knechten, J. C. Wasmuth, and J. K. Rockstroh**. 2002. Long-term efficacy and safety of ritonavir/indinavir at 400/400 mg twice a day in combination with two nucleoside reverse transcriptase inhibitors as first line antiretroviral therapy. HIV Med. **3**:37-43.
4. **Voigt, E., A. Wickesberg, J. C. Wasmuth, P. Gute, L. Locher, B. Salzberger, A. Wohrmann, A. Adam, L. Weitner, and J. K. Rockstroh**. 2002. First-line ritonavir/indinavir 100/800 mg twice daily plus nucleoside reverse transcriptase inhibitors in a German multicentre study: 48-week results. HIV Med. **3**:277-282.
5. **Arnaiz, J. A., J. Mallolas, D. Podzamczer, J. Gerstoft, J. D. Lundgren, P. Cahn, G. Fatkenheuer, A. D'Arminio-Monforte, A. Casiro, P. Reiss, D. M. Burger, M. Stek, and J. M. Gatell**. 2003. Continued indinavir versus switching to indinavir/ritonavir in HIV-infected patients with suppressed viral load. AIDS **17**:831-840.
6. **Lamotte, C., Peytavin, G., Perre, P., Chavanet, P., Truchis, d. P., Gilquin, J., Winter, C., Katlama, C., and Farinotti, R**. Increasing adverse events with indinavir dosages and plasma concentrations in four different ritonavir-indinavir containing regimens in HIV-infected patients. 2nd International Workshop on Clinical Pharmacology of HIV Therapy, April 2-4, 2001. Noordwijk, the Netherlands [abstract 4.1]. 2001.
7. **Lamotte, C., Peytavin, G., Perre, P., Chavanet, P., Truchis, d. P., Gilquin, J., Winter, C., Katlama, C., and Farinotti, R**. Increasing adverse events with indinavir dosages and plasma concentrations in four different ritonavir-indinavir containing regimens in HIV-infected patients. 8th Conference on Retrovirus and Opportunistic Infections, Chicago, Ill, February 4-8, 2001, abstract 738. 2001.
8. **Ghosn, J., C. Lamotte, H. Ait-Mohand, M. Wirden, R. Agher, L. Schneider, F. Bricaire, C. Duvivier, V. Calvez, G. Peytavin, and C. Katlama**. 2003. Efficacy of a twice-daily antiretroviral regimen containing 100 mg ritonavir/400 mg indinavir in HIV-infected patients. AIDS **17**:209-214.
9. **Aarnoutse, R. E., J. C. Wasmuth, G. Fatkenheuer, K. Schneider, K. Schmitz, T. M. de Boo, P. Reiss, Y. A. Hekster, D. M. Burger, and J. K. Rockstroh**. 2003. Administration of indinavir and low-dose ritonavir (800/100 mg twice daily) with food reduces nephrotoxic peak plasma levels of indinavir. Antivir.Ther. **8**:309-314.
10. **Hugen, P. W. H., C. P. W. G. M. Verwey-van Wissen, D. M. Burger, E. W. Wuis, P. P. Koopmans, and Y. A. Hekster**. 1999. Simultaneous determination of the HIV-protease inhibitors indinavir, nelfinavir, saquinavir and ritonavir in human plasma by reversed-phase high-performance liquid chromatography. Journal of Chromatography B Biomed Sci Appl **727**:139-149.
11. **Burger, D. M., d. M. Graaff, E. W. Wuis, P. P. Koopmans, and Y. A. Hekster**. 1997. Determination of indinavir, an HIV-protease inhibitor, in human plasma by reversed-phase high-performance liquid chromatography. J Chromatogr B **703**:235-241.
12. **Aarnoutse, R. E., K. J. Grintjes, D. S. Telgt, M. Stek, Jr., P. W. Hugen, P. Reiss, P. P. Koopmans, Y. A. Hekster, and D. M. Burger**. 2002. The influence of efavirenz on the pharmacokinetics of a twice-daily combination of indinavir and low-dose ritonavir in healthy volunteers. Clin Pharmacol Ther. **71**:57-67.

13. **Gibaldi, M.** 1991. Compartmental and noncompartmental pharmacokinetics, p. 14-23. *In*: Biopharmaceutics and clinical pharmacokinetics. Lea & Febiger, Philadelphia, London.
14. **Solas, C., S. Basso, I. Poizot-Martin, I. Ravaux, H. Gallais, J. A. Gastaut, A. Durand, and B. Lacarelle**. 2002. High Indinavir Cmin Is Associated With Higher Toxicity in Patients on Indinavir-Ritonavir 800/100 mg Twice-Daily Regimen. J Acquir Immune Defic Syndr **29**:374-377.
15. **Dieleman, J. P., I. C. Gyssens, v. d. M. E. Ende, d. S. Marie, and D. M. Burger**. 1999. Urological complaints in relation to indinavir plasma concentrations in HIV-infected patients. AIDS **13**:473-478.
16. **Rockstroh, J. K., F. Bergmann, W. Wiesel, A. Rieke, A. Theisen, G. Fatkenheuer, M. Oette, H. Carls, S. Fenske, M. Nadler, and H. Knechten**. 2000. Efficacy and safety of twice daily first-line ritonavir/indinavir plus double nucleoside combination therapy in HIV-infected individuals. AIDS **14**:1181-1185.
17. **Acosta, E. P., T. N. Kakuda, R. C. Brundage, P. L. Anderson, and C. V. Fletcher**. 2000. Pharmacodynamics of human immunodeficiency virus type 1 protease inhibitors. Clin.Infect.Dis. **30 Suppl 2**:S151-S159.
18. **Burger, D. M., R. M. W. Hoetelmans, P. W. H. Hugen, J. W. Mulder, P. L. Meenhorst, P. P. Koopmans, K. Brinkman, M. Keuter, W. Dolmans, and Y. A. Hekster**. 1998. Low plasma concentrations of indinavir are related to virological treatment failure in HIV-1 infected patients on indinavir-containing triple therapy. Antiviral Therapy **3**:215-220.
19. **Plosker, G. L. and S. Noble**. 1999. Indinavir: a review of its use in the management of HIV infection. DRUGS **58**:1165-1203.
20. **Hsu, A., G. R. Granneman, and R. J. Bertz**. 1998. Ritonavir. Clinical pharmacokinetics and interactions with other anti-HIV agents. Clinical Pharmacokinetics **35**:275-291.
21. **Casado, J. L., A. Moreno, R. Sabido, P. Marti-Belda, A. Antela, F. Dronda, M. J. Perez-Elias, and S. Moreno**. 2000. A clinical study of the combination of 100 mg ritonavir plus 800 mg indinavir as salvage therapy: influence of increased plasma drug levels in the rate of response. HIV Clin.Trials **1**:13-19.
22. **Famularo, G., S. Di Toro, S. Moretti, and C. De Simone**. 2000. Symptomatic crystalluria associated with indinavir. Ann.Pharmacother. **34**:1414-1418.

Part III

Nelfinavir

Chapter 6

Effect of efavirenz treatment on the pharmacokinetics of nelfinavir boosted by ritonavir in healthy volunteers

C.J.L. la Porte[1,2], M.J.A. de Graaff-Teulen[1,2], E.P.H. Colbers[3], D.S. Voncken[3], S. Marco Ibanez[4], P.P. Koopmans[2,5], Y.A. Hekster[1,2], D.M. Burger[1,2]

1 Department of Clinical Pharmacy, University Medical Centre Nijmegen, The Netherlands.
2 Nijmegen University Centre for Infectious diseases, Nijmegen, The Netherlands.
3 Farma Research BV, Nijmegen, The Netherlands.
4 F. Hoffman-La Roche Ltd., Basel, Switzerland.
5 Department of General Medicine, University Medical Centre Nijmegen, The Netherlands.

British Journal of Clinical Pharmacology. 2004; 58: 632-640

Abstract

Aims: A once daily (QD) nucleoside-sparing regimen can prevent mitochondrial toxicity, overcome viral resistance and improve compliance. In the present study the effect of efavirenz on the pharmacokinetics and tolerability of once daily nelfinavir/ritonavir was evaluated in healthy subjects.

Methods: This was a multiple-dose, open-label, single-group, two-period study in 24 healthy subjects. Each received from days 1–10 (period 1): 1875 mg nelfinavir plus 200 mg ritonavir QD with a 300-kcal snack. During days 11–20 (period 2) efavirenz 600 mg QD was added to the regimen. Blood samples were collected up to 24 h after dosing on days 10 (period 1) and 20 (period 2). High-performance liquid chromatography methods were used for the determination of the concentrations of all compounds. The main pharmacokinetic parameters were calculated using noncompartmental methods.

Results: All subjects completed the study. After the first period mean nelfinavir $AUC_{0\text{-}24\,h}$, C_{max} and C_{24} were 49.6 h.mg/L, 5.0 mg/L and 0.37 mg/L, and the sum of nelfinavir plus its active metabolite M8 C_{24} was 0.83 mg/L. The relative bioavailability, expressed as a geometric mean ratio (90% confidence interval) for nelfinavir 0-24 h, C_{max} and C_{24} of period 2 compared with period 1 was: 1.30 (1.21, 1.40), 1.29 (1.19, 1.40) and 1.48 (1.32, 1.66). The sum of nelfinavir and M8 C_{24} in period 2 was 0.99 mg/L, an increase of 19%. No serious adverse events occurred.

Conclusions: The studied regimens were well tolerated. Nelfinavir/ritonavir given together with efavirenz resulted in a 48% higher mean C_{24} for nelfinavir, and the sum of nelfinavir and M8 C_{24}s was 0.99 mg/L. Efavirenz exposure in this study was similar to that reported previously, and therefore can be used effectively in combination with ritonavir and nelfinavir.

Introduction

Since the introduction of protease inhibitors (PIs) in the mid 1990s for the treatment of HIV infection, life expectancy has increased substantially[1,2]. Nevertheless, treatment with highly active antiretroviral therapy (HAART) can still be improved, as efficacy is not 100%. Major concerns in treatment with HAART are the occurrence of adverse events[3], failure of compliance[4] and development of viral resistance[3]. To improve the treatment of HIV-infected persons these problems need to be overcome. A nucleoside-sparing regimen can prevent nucleoside reverse transcriptase inhibitor (NRTI)-induced mitochondrial toxicity, prevent NRTI (cross-) resistance and/or lead to an increase in the susceptibility of HIV to non-nucleoside reverse transcriptase inhibitors (NNRTIs)[5]. To improve compliance, and thus virological outcome[4], simplification of antiretroviral dosing regimens seems to be effective[6,7].

We have previously demonstrated that nelfinavir in combination with ritonavir can be given once daily, at optimal doses of 2000 mg and 200 mg, respectively[8]. As a potent inhibitor of the cytochrome P450 (CYP) enzyme CYP3A4[9], ritonavir is used in these circumstances to boost plasma nelfinavir concentrations to achieve a durable therapeutic response. CYP3A4 is mainly responsible for the metabolism of nelfinavir[10]. However, the formation of the virologically active[11] metabolite nelfinavir-hydroxy-t-butylamide (designated M8) is primarily dependent on CYP2C19[12]. M8 is subsequently metabolized by CYP3A4 into inactive metabolites[12]. Therefore, the combination of nelfinavir with ritonavir will result in both increased nelfinavir and M8 concentrations[13,14].

In the current study nelfinavir was given in form of new 625-mg tablets instead of 250-mg tablets. The new formulation was choosen for two reasons. It is expected to be marketed within a relative short period and enables the dosage of 1875 mg QD to be taken as three tablets only, which is more convenient for the patients. Ritonavir was dosed at 200 mg QD and efavirenz at 600 mg QD, once daily in the evening, to avoid potential neurological adverse events[15]. Nelfinavir should be given during the day and with food[16]. In the present study nelfinavir, ritonavir and efavirenz were all taken at bedtime (23.00 h) together with a snack of around 300 kcal.

The primary objective of this study was to characterize the pharmacokinetics of nelfinavir boosted by ritonavir when administered once daily with efavirenz in healthy subjects. The secondary objective was to determine the influence of nelfinavir when combined with ritonavir on the pharmacokinetics of efavirenz. Tolerability of the once daily regimen was also studied, as well as the influence of giving nelfinavir in the evening as opposed to the morning.

Methods

Study design

This was a multiple-dose, open-label, single-group, two-period study in 24 healthy subjects (12 males). Subjects received the following treatments: period 1 (days 1–10), 1875 mg nelfinavir plus 200 mg ritonavir to be taken once daily at 23.00 h with a light snack of around 300 kcal; period 2 (days 11–20), 1875 mg nelfinavir plus 200 mg ritonavir and 600 mg efavirenz to be taken once daily at 23.00 h with a light snack (around 300 kcal). Blood samples were collected throughout a 24-h period on days 10 and 20 following a light meal of 315 kcal. This consisted of one slice of bread with butter and cheese, or peanut butter, or two slices of sausage, together with a glass of semi skimmed milk (3.75% fat). All other meals eaten on the pharmacokinetic evaluation days were standardized (breakfast 485 kcal, lunch 656 kcal and dinner 1231 kcal). Medication was swallowed with 200 ml of noncarbonated water.

Subject selection

All subjects had to be in generally good health, appropriate for their age as established by medical history, physical examination, electrocardiography, blood pressure, heart rate, and the results of biochemistry, haematology and urinalysis performed within 3 weeks before the first dose. Subjects had to be between 18 and 65 years old. Body mass index had to be in the range of 18–30 kg/m^2. Subjects were not allowed to smoke more than 10 cigarettes, two cigars or two pipes per day, for at least 3 months prior to the study. The protocol was explained comprehensively to all subjects, and written informed consent was obtained prior to screening. Exclusion criteria included a febrile illness within 3 days before the first dose, exposure to any drug, except for paracetamol, hormonal contraceptives and loperamid, participation in another drug trial and/or donation of blood within 60 days before the study, and hypersensitivity to nelfinavir, ritonavir or efavirenz. In addition, counselling and confirmation of using adequate contraception were required for all females of child-bearing age. Pregnant or breast-feeding subjects were excluded. The study was approved by the Regional Ethics Review Board, located in Nijmegen, The Netherlands.

Blood sampling

Blood samples of 5 ml were collected in heparinized hard plastic tubes at the following time points: predose and at 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10, 12, 16, 20, and 24 h after drug intake on days 10 and 20, and at 9 h postdose on days 1, 4, 7, 14 and 17. Blood samples were centrifuged at 2500 g for 10 min at 4 °C. Plasma was stored at –18 °C within 2 h after collection.

Clinical assessment

Blood samples after fasting for serum biochemistry [including glucose, total bilirubin, direct bilirubin (in case total bilirubin was above normal range), aspartate amino transferase (ASAT), alanine amino transperase (ALAT), gamma glutamyl transferase

(GGT), alkaline phosphatase, creatinine kinase, amylase, total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglycerides, creatinine and potassium], haematology and a urine sample for urinalysis were taken at days 1, 4, 7, 11, 14, 17 and 21. In females of childbearing age an instant β-hCG urine pregnancy test was performed at each visit. A urine drug screen for tetrahydrocannabinol (THC), morphine, cocaine and amphetamines was performed on day 1 and at the start of study days 10 and 20, using the Instacheck™ Multi-Drug Screen panel (Forefront Diagnostics, San Diego, CA, USA). Blood pressure and heart rate were monitored throughout the study. Subjects were monitored for adverse events by the medical and nursing staff at the trial centre. Subjects also reported any adverse events in response to general questioning. For each adverse event the following information was recorded: onset and resolution date and time, intensity, relationship to trial medication, action taken, and outcome. All adverse events occurring between the first intake of the trial medication and 30 days after the end of the trial were reported. Adverse events were classified as unrelated, doubtful, possible and probable, and were assessed for intensity according to AIDS Clinical Trial Group (ACTG) classifications: mild (symptoms do not interfere with daily activities), moderate (symptoms interfere with daily activities), severe (symptoms markedly interrupt daily activities) or serious. The latter were defined as those that at any dose resulted in death, were life threatening, required inpatient hospitalization or prolongation of existing hospitalization, resulted in a persistent or significant disability/incapacity or were congenital anomalies/birth defects.

Drug analysis

A validated high-performance liquid chromatography (HPLC) method with ultraviolet detection was used for the determination of nelfinavir, M8 and ritonavir concentrations in plasma as published previously[17]. The lower limit of quantification was 0.04 mg/L for nelfinavir and ritonavir, and 0.10 mg/L for M8. For nelfinavir, accuracy ranged from 96% to 100% depending on the concentration. Intraday and interday precision ranged from 2.1% to 7.5% and from 0.4% to 3.5%, respectively. For ritonavir, accuracy ranged from 102% to 108%, and intraday and interday precision from 2.0% to 8.1% and 0% to 2.4%, respectively. For M8, accuracy ranged from 93% to 108%, and intraday and interday precision from 2.8% to 4.3% and 2.0% to 3.0%, respectively. A HPLC method with ultraviolet detection was also used for the analysis of efavirenz[18]. The lower limit of quantification was 0.20 mg/L. Accuracy ranged from 99% to 101%, and intraday and interday precision from 1.8% to 2.6% and from 1.1% to 2.8%, respectively.

Pharmacokinetic analysis

The following pharmacokinetic parameters were calculated using noncompartmental analysis (Excel version 2000, Microsoft Corporation 1985–1999): $AUC_{0-24\,h}$ (h.mg/L), the area under the plasma concentration– time curve; C_{max} (mg/L), the maximum plasma drug concentration; C_{24} (mg/L), the plasma drug concentration at 24 h after drug dosing; T_{max} (h), the time to reach maximum plasma drug concentration; $T_{1/2}$

(h), apparent elimination half-life; CL/*F* (l/h), apparent clearance; Vd/*F* (l), apparent volume of distribution. CL/*F* and Vd/*F* were not calculated for M8. If ritonavir plasma concentrations at the end of the 24-h dosing interval were lower than the quantification limit of 0.04 mg/L, they were estimated from the last quantifiable plasma concentration and the elimination constant.

Statistical analysis

For AUC_{0-24h}, C_{max} and C_{24} results were presented for treatment period 1 and treatment period 2 together with the ratios period 2 : period 1. The geometric mean and min–max range corresponding to mean and min–max range in the logarithmically transformed domain were given for both periods. The parametric point estimate (ratio estimate) and 90% confidence intervals were calculated for the ratio period 2 : period 1. Treatments were considered bioequivalent if the respective 90% classical confidence intervals for the AUC_{0-24h}, C_{24} and C_{max} ratios were included within the range of 0.80–1.25[19]. T_{max}, $T_{1/2}$, CL/*F* and Vd/*F* were considered to be secondary characteristics, the analysis of which was explorative. T_{max} data were not log-transformed and were compared between treatment periods using a Wilcoxon signed ranks test. Pharmacokinetic parameters for efavirenz were compared with historical controls derived from a previously published study in healthy subjects[18].

Data on adverse events were classified according to system organ class using MedDRA V 4.1 coding (Med-DRA MSSO, Reston, VA, USA). Frequencies and percentages of occurred adverse events were tabulated. Laboratory values were graded according to toxicity scales. For ALAT, ASAT and GGT Grade 1 was defined as 1.25–2.5 times upper limit of normal (ULN), Grade 2 was 2.6–5.0 times ULN. For amylase Grade 1 was 1.1–1.3 times ULN and Grade 2 was 1.4–2.0 times ULN. For total cholesterol Grade 2 was 6.19– 7.77 mmol/L and Grade 3 was 7.77–10.35 mmol/L. Grade 1 cholesterol was not used as it overlapped with the laboratory normal ranges. For triglycerides Grade 2 was 4.52–8.47 mmol/L and Grade 3 was 8.48– 13.55 mmol/L. For creatinine and total bilirubin Grade 6.42–8.91 mmol/L. For creatine phosphokinase Grade 1 was 1.1–2.0 times ULN, Grade 2 was 2.1–4.0 times ULN and Grade 3 was 4.1–6.0 times ULN. For HDL- and LDL-cholesterol no toxicity grades were defined. In addition, for each laboratory parameter the median change from the baseline value to the highest observed value was calculated as well as the median change from the baseline value to the last observed value in the study.

Results

Twenty-four subjects (12 males and 12 females) completed the study. All subjects were Caucasian except for one Black male. The mean age of the subjects was 45 years, mean weight 72 kg, and mean height 1.74 m. Pharmacokinetic results for nelfinavir, its active metabolite M8, ritonavir and efavirenz are presented in Table 1.

Table 1. Pharmacokinetic data for nelfinavir, M8, ritonavir and efavirenz (n=24)

	Geometric Mean (Range)		Relative Bioavailability[&] GMR (90% CI)	*P*-value[#]
	Period 1[†]	Period 2[‡]		
Nelfinavir				
AUC_{0-24}	49.6 (22.6-94.6)	64.3 (30.0-106.6)	1.30 (1.21-1.40)	<0.01
C_{max}	5.0 (2.4-8.2)	6.4 (3.5-10.7)	1.29 (1.19-1.40)	<0.01
C_{24}	0.37 (0.12-1.33)	0.55 (0.14-1.26)	1.48 (1.32-1.66)	<0.01
T_{max}*	4.0 (2.5-6.0)	4.0 (2.0-6.0)		0.64
$T_{1/2}$	5.4 (4.0-8.4)	5.7 (4.1-8.0)		0.02
Cl/*F*	38.1 (20.9-83.5)	29.6 (17.5-66.0)		<0.01
Vd/*F*	296.6 (181.4-590.2)	242.9 (164.3-461.9)		<0.01
M8				
AUC_{0-24}	39.0 (26.0-68.2)	36.1 (20.3-75.2)	0.93 (0.87-1.00)	0.09
C_{max}	3.4 (2.0-5.8)	3.0 (1.7-5.3)	0.87 (0.81-0.92)	<0.01
C_{24}	0.46 (0.22-1.16)	0.44 (0.17-1.31)	0.96 (0.85-1.09)	0.59
T_{max}*	5.0 (4.0-6.0)	6.0 (4.0-8.1)		0.01
$T_{1/2}$	6.4 (4.5-8.9)	6.3 (4.7-9.1)		0.39
Ritonavir				
AUC_{0-24}	17.5 (7.8-31.8)	14.0 (4.7-28.4)	0.80 (0.72-0.88)	<0.01
C_{max}	2.4 (1.2-5.1)	1.8 (0.59-3.2)	0.76 (0.67-0.85)	<0.01
C_{24}	0.04 (0.00[$]-0.14)	0.04 (0.01[$]-0.10)	0.88 (0.73-1.06)	0.24
T_{max}*	4.0 (1.5-8.0)	3.5 (1.5-8.0)		0.96
$T_{1/2}$	3.3 (2.2-4.1)	3.5 (2.4-4.7)		0.02
Cl/*F*	11.4 (6.3-25.5)	14.3 (7.1-41.9)		<0.01
Vd/*F*	54.3 (29.2-100.8)	72.1 (31.0-231.5)		<0.01
Efavirenz				
AUC_{0-24}		64.0 (37.9-156.9)		
C_{max}		4.7 (2.9-8.7)		
C_{24}		2.0 (1.1-5.5)		
T_{max}		2.5 (1.0-10.0)		
$T_{1/2}$		30.4 (14.5-73.8)		
Cl/*F*		8.3 (2.9-14.0)		
Vd/*F*		365.3 (157.9-864.3)		

GMR, Geometric Mean Ratio; CI, Confidence interval; AUC_{0-24}, (h.mg/L) area under the concentration-time curve; C_{max}, (mg/L) highest observed plasma concentration; C_{24}, (mg/L) trough concentration at 24 hours; T_{max}, (h) sampling time for C_{max}; $T_{1/2}$, (h) elimination half-life; Cl/*F*, (L/h) apparent clearance; Vd/*F*, (L) volume of distribution

* Median and range; Wilcoxon-signed rank test

[†] Combination of 1875 mg nelfinavir and 200 mg ritonavir

[‡] Combination of 1875 mg nelfinavir and 200 mg ritonavir and 600 mg efavirenz

[&] Relative bioavailability of period 2 (test) over period 1(reference)

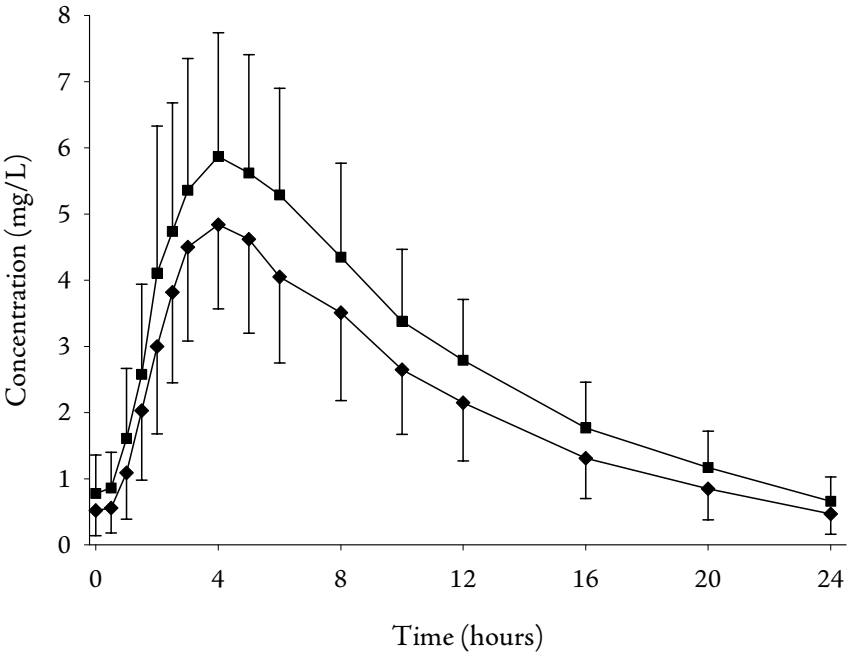
[#] *P*-Value based on a paired samples *t*-test with 95% confidence

[$] If ritonavir plasma concentrations at the end of the 24-hour dosing interval were lower than the quantification limit of 0.04 mg/L, the actual concentrations were calculated on the basis of the last quantifiable plasma concentration and the elimination constant.

Plasma concentration versus time plots are presented in Figure 1 for nelfinavir and in Figure 2 for the sum of nelfinavir and M8. An increase in AUC_{0-24h} (+ 30%), C_{max} (+ 29%) and C_{24} (+ 48%) for nelfinavir was seen when combined with efavirenz in period 2. As a result, these parameters were not bioequivalent over periods 1 and 2. Nelfinavir C_{24} increased in 21 subjects, and decreased in three (by –38%, –12% and –5%).

In contrast to the increased nelfinavir parameters, AUC_{0-24h} , C_{max} and C_{24} of the active metabolite M8 remained bioequivalent after the addition of efavirenz to the regimen. Decreases were observed in ritonavir AUC_{0-24h} (–20%), C_{max} (–24%) and C_{24} (–12%) after the addition of efavirenz to the regimen, resulting in a lack of bioequivalence over periods 1 and 2.

Figure 1. Nelfinavir concentration-time profiles for study days 10 and 20

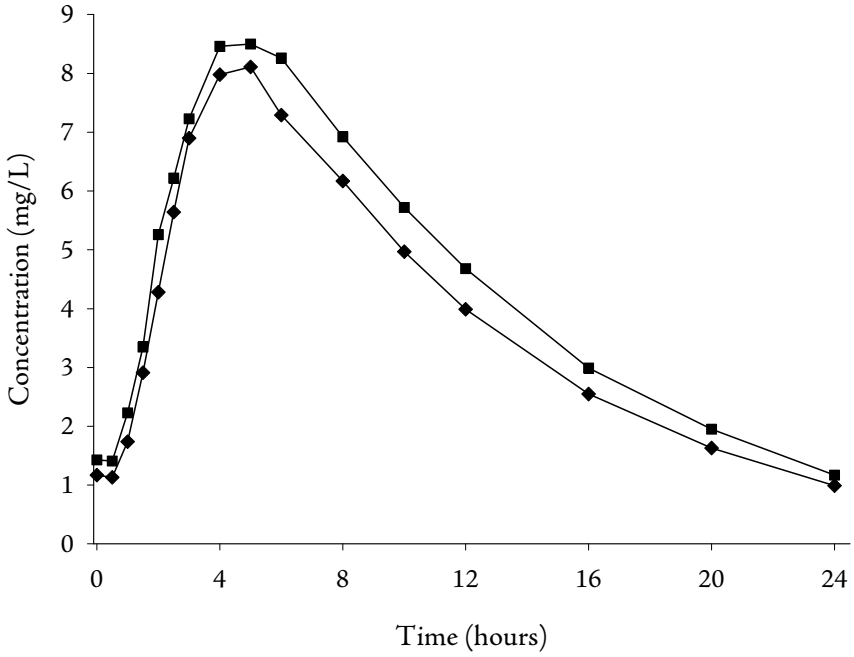


Data of 24 subjects, concentration-time profiles are mean values with standard deviation in error bars. From this figure is becomes clear that nelfinavir plasma concentrations are increased after the addition of efavirens to the regimen on day 20 in comparison to day 10.

—◆— Study day 10 (combination of 1875 mg nelfinavir and 200 mg ritonavir)

—■— Study day 20 (combination of 1875 mg nelfinavir, 200 mg ritonavir and 600 mg efavirenz)

Figure 2. Nelfinavir plus M8 concentration-time profiles for study days 10 and 20



Data of 24 subjects, concentration-time profiles are mean values. From this figure it becomes clear that M8 substantially contributes to the trough concentrations, but also to maximal concentrations on both study days 10 and 20.

—◆— Study day 10 (combination of 1875 mg nelfinavir and 200 mg ritonavir)

—■— Study day 20 (combination of 1875 mg nelfinavir, 200 mg ritonavir and 600 mg efavirenz)

All 24 subjects reported one or more adverse events out of a total of 225. The most frequently reported were diarrhoea (75.0%), dizziness (58.3%) and nausea (37.5%). Table 2 lists all adverse events that were suffered by two or more subjects and which were possibly or probably related to the study medication. Six of the 225 reported adverse events were unrelated, and were therefore not included in further analysis. One hundred and twenty-one adverse events were reported during treatment with nelfinavir/ritonavir (period 1), versus 98 when nelfinavir/ritonavir was given together with efavirenz (period 2). No serious adverse events were reported during either treatment period. One adverse event of lower abdominal pain was classified as being severe, and it occurred during period 1. The majority (85%) of all adverse events were mild. The causal relationship to study medication was reported to be doubtful for 29%, possible for 41% and probable for 30% of adverse events.

Table 2. Summary of adverse events occurring in two or more subjects throughout the study, considered possibly or probably related to study drug by the investigator

System Organ Class[#]	Description[$]	Whole Study[&] frequency and percentage		Period 1* frequency and percentage		Period 2[+] frequency and percentage	
Gastrointestinal disorders	Abdominal discomfort	2	8.3 %	2	8.3 %	0	0 %
	Diarrhoea	18	75.0 %	10	41.7 %	8	33.3 %
	Dyspepsia	2	8.3 %	0	0 %	2	8.3 %
	Flatulence	5	20.8 %	4	16.7 %	1	4.2 %
	Loose stools	5	20.8 %	4	16.7 %	1	4.2 %
	Nausea	9	37.5 %	8	33.3 %	1	4.2 %
	Stomatitis	2	8.3 %	1	4.2 %	1	4.2 %
General disorders and administration site conditions	Fatigue	7	29.2 %	5	20.8 %	2	8.3 %
Metabolism and nutrition	Anorexia	3	12.5 %	1	4.2 %	2	8.3 %
disorders	Hypercholesterolaemia	3	12.5 %	1	4.2 %	2	8.3 %
Musculoskeletal and	Myalgia	5	20.8 %	4	16.7 %	1	4.2 %
connective tissue disorders	Sensation of heaviness	2	8.3 %	2	8.3 %	0	0 %
Nervous system disorders	Disturbance in attention	2	8.3 %	0	0 %	2	8.3 %
	Dizziness	14	58.3 %	4	16.7 %	10	41.7 %
	Headache	4	16.7 %	3	12.5 %	1	4.2 %
	Paraesthesia	4	16.7 %	2	8.3 %	2	8.3 %
	Paraesthesia circumoral	2	8.3 %	1	4.2 %	1	4.2 %
	Somnolence	5	20.8 %	2	8.3 %	3	12.5 %
Psychiatric disorders	Abnormal dreams	5	20.8 %	1	4.2 %	4	16.7 %
	Agitation	2	8.3 %	0	0 %	2	8.3 %
	Apathy	2	8.3 %	1	4.2 %	1	4.2 %
	Insomnia	3	12.5 %	1	4.2 %	2	8.3 %
Skin and subcutaneous tissue disorders	Sweating increased	3	12.5 %	0	0 %	3	12.5 %

[#] Adverse events were classified according to system organ class with MedDRA V4.1.
[$] Description of adverse events according to MedDRA V4.1 preferred terms.
[&] Adverse events of both study period 1 and 2 together tabulated as frequencies and percentages (calculated as frequency divided by number of subjects (24) times 100).
* Adverse events of study period 1 tabulated as frequencies and percentages (calculated as frequency divided by number of subjects (24) times 100).
[+] Adverse events of study period 2 tabulated as frequencies and percentages (calculated as frequency divided by number of subjects (24) times 100).

The occurrence of adverse events did not lead to temporary or permanent discontinuation of trial medication, nor to dose modifications. Five percent of the adverse events were treated with concomitant therapy. Although diarrhoea was reported frequently, the use of loperamide for its relief was indicated in only one subject during period 1 and in another subject during period 2. Gastrointestinal disorders were observed more frequently during study period 1 than during period 2. Dizziness (41.7%) and psychiatric disorders such as abnormal dreaming (16.7%) and agitation (8.3%) were reported more frequently in period 2.

Laboratory abnormalities were recorded in all subjects. The results of laboratory safety analysis of biochemistry parameters are summarized in Table 3. Total cholesterol resulted in Grade 2 values in six subjects and Grade 3 values in four subjects. However, seven of these 10 subjects had Grade 2 toxicity values for cholesterol at screening. For triglycerides one subject showed Grade 2 toxicity and one subject Grade 3 toxicity. Both subjects had normal triglyceride values at screening. For haematology and urinalysis no clinically significant abnormalities were found.

Table 3. Laboratory measurements

	Normal ranges males (females)	Number (Grade) of subjects with toxicity[&]	Change to maximum median (range)[#]	Change to final median (range)[$]
Alanine amino transferase (U/L)	0-50 (0-40)	3 (I)	2 (0 - 70)	-2.5 (-23 - 63)
Aspartate amino transferase (U/L)	0-40	2 (I)	6.5 (0 - 28)	1 (-8 - 27)
Alkaline phosphatase (U/L)	0-120	-	5 (0 - 34)	-6.5 (-26 - 34)
Gamma glutamyl transferase (U/L)	0-50 (0-35)	1 (II)	5 (0 - 69)	3.5 (-14 - 52)
Amylase (U/L)	0-53	1 (I), 1 (II)	5.5 (0 - 63)	-2 (-13 - 9)
Total Cholesterol (mmol/L)	3.9-6.5	6 (II), 4 (III)	0.95 (0.1 - 3.2)	0.65 (-0.5 - 2.1)
HDL cholesterol (mmol/L)	0.9-1.7 (1.2-2.3)	Nd	0.1 (0 – 0.3)	-0.2 (-0.6 – 0.2)
LDL cholesterol (mmol/L)	3.5-4.5	Nd	0.9 (0.1 - 3.2)	0.55 (-0.2 - 2.5)
Triglycerides (mmol/L)	0.8-2.0	1 (II), 1 (III)	1.05 (0 – 6.7)	0.5 (-1.9 – 1.7)
Creatinine (μmol/L)	60-120 (53-100)	2 (I)	4.5 (0 - 26)	-4 (-18 - 8)
Total bilirubine (μmol/L)	3-17	1 (I)	6 (2 - 10)	1 (-4 - 5)
Glucose (mmol/L)	4.0-6.0	1 (I)	0.1 (0 - 1.9)	-0.3 (-1.4 - 1.5)
Creatine phosphokinase (U/L)	0-200 (0-170)	4 (I), 2 (II), 1 (III)	21 (0 - 614)	-23 (-157 - 84)

Nd = not determined

[#]Median and range of the individual changes of 24 subjects from baseline (day 1) to maximum values. If day 1 was the maximum the result was 0.

[$]Median and range of the individual changes of 24 subjects from baseline (day 1) to last observed values. If day 1 was the maximum the result was a negative value.

[&]Number of subjects with toxicity graded I, Grade 1; II, Grade 2; III, Grade 3.

Discussion

In the current study the combination of nelfinavir/ritonavir plus efavirenz dosed once daily was tested in comparison with the same combination without efavirenz. The results obtained in 24 healthy subjects showed an increased nelfinavir AUC_{0-24h} of 30%, C_{max} of 29% and a C_{24} of 48% after addition of efavirenz. Exposure to M8, the active metabolite of nelfinavir, was unaffected. Ritonavir concentrations decreased after addition of efavirenz to the regimen, but its boosting effects were still present. Efavirenz is known to inhibit CYP2C19[15], which catalyses the formation of M8, the active metabolite of nelfinavir[10]. For this reason one would expect the coadministration of nelfinavir and efavirenz to result in lower M8 concentrations, combined with higher nelfinavir exposure. In accordance with this, it has been reported that the combination of nelfinavir 750 mg three times daily and efavirenz 600 mg QD leads to a 20% increase in nelfinavir AUC and a 37% decrease in M8 AUC[20]. Somewhat contrasting data come from a study in patients receiving a dual NRTI regimen with either efavirenz 600 mg QD, or nelfinavir 1250 mg twice daily (BID), or the combination of efavirenz and nelfinavir[21]. In the latter study nelfinavir AUC_{0-12}, C_{max} and C_{min} were lowered by 37%, 21% and 65%, respectively, after the addition of efavirenz for 32 weeks. However, this study was an efficacy and not a bioequivalence study. After the initial 4 weeks of treatment in 40 patients no significant intra-individual differences in pharmacokinetics were seen, except for C_{min}, which was significantly lower after 32 weeks of treatment in 26 patients. No significant differences were noted in M8 exposure. A major difference with the current study, apart from nelfinavir dose, was the presence of ritonavir in our regimen. Ritonavir is a potent inhibitor of CYP3A4[9], the enzyme responsible for further metabolism of M8 and of nelfinavir itself[10,12]. The inhibition of M8 metabolism by ritonavir probably compensated for the decreased formation of M8 as a result of CYP2C19 inhibition by efavirenz. The alternative metabolism of nelfinavir, mediated by CYP3A4[10], is also inhibited by ritonavir. This could explain the observed higher nelfinavir exposure combined with a bioequivalent M8 exposure over the different regimens in the current study. For nelfinavir a minimal trough concentration of 0.80 mg/L has been proposed[22]. In the current study nelfinavir given with ritonavir but without efavirenz resulted in a C_{24} of 0.37 mg/L, increasing to 0.55 mg/L when efavirenz was added. Both these values are too low to ensure viral suppression. If nelfinavir is dosed at 1250 mg BID, plasma concentrations of M8 are 30% of those of nelfinavir itself[23]. Thus, the minimal trough concentration for nelfinavir and M8 together should be 1.0 mg/L. In the current study the sum of nelfinavir and M8 resulted in a C_{24} of 0.83 mg/L without efavirenz, which increased to 0.99 mg/L when efavirenz was added.

The observed decrease in ritonavir AUC_{0-24h} of −20%, C_{max} of −24% and C_{24} of −12% in the current study was unexpected, since a previous study found an increase in ritonavir exposure following efavirenz coadministration[24]. In the latter study ritonavir was dosed at 500 mg BID, which, on a daily basis, is five times the dose used in the current study. Efavirenz is reported to have both inhibitory and inducing effects on

CYP3A4[25], and ritonavir depends mainly on CYP3A4 for its metabolism[9]. These data suggest that efavirenz coadministration may accelerate the metabolism of ritonavir administered at a low dose, which has also been reported previously[18]. Although concentrations of ritonavir decreased, it was still capable of boosting nelfinavir metabolism. It is unknown precisely what ritonavir exposure is necessary to benefit from its boosting effects.

Nelfinavir was not expected to affect the pharmacokinetics of efavirenz[26]. Ritonavir at a dose of 500 mg has been shown to inhibit efavirenz metabolism, resulting in a 21% increase in AUC[24]. However, it is unclear whether the same effect would occur when ritonavir is used at the lower dose of 200 mg once daily. In a study of multiple doses of indinavir/ritonavir 800/100 mg BID in combination with efavirenz 600 mg, the AUC_{0-24h}, C_{min} and $T_{1/2}$ of the latter were 56.2 h.mg/L, 1.6 mg/L and 35.1 h[18] after intake in the fasting. In the present study these data were 64.0 h.mg/L, 2.0 mg/L and 30.4 h. Although the comparison of efavirenz pharmacokinetics with historical controls did not reveal relevant differences, in the current study efavirenz exposure was somewhat higher. This could be the result of the intake of efavirenz with food in the present study, which has been reported to give a 17% increase in bioavailability[15].

In a previous multiple-dose study, where nelfinavir/ritonavir 2000/200 mg was given once daily to eight healthy subjects, either with a 610-kcal breakfast or with a 271-kcal breakfast[8], nelfinavir AUC, C_{max} and C_{min} were 57.6 h.mg/L, 6.3 mg/L and 0.59 mg/L, M8 C_{min} was 0.53 mg/L following the 271-kcal breakfast. Nelfinavir exposure in this study was higher than that observed in our study after a 315-kcal snack in the evening. The lower nelfinavir concentrations in our study can partly be explained by a 6.25% lower dose. However, it is not possible to prove whether the remaining part of the observed difference results from the evening versus the morning intake, and/or the difference in coadministered food. If the combination of nelfinavir/ritonavir is to be used with non-interacting NRTIs instead of efavirenz, it is preferable to administer the drugs in the morning with a 610-kcal breakfast, which has been reported to result in higher nelfinavir exposure[8].

In total, 225 adverse events were reported by the 24 healthy subjects in this study. However, the majority were mild in nature and none met the criteria for a serious adverse event. None led to withdrawal from the study, indicating that the medication was tolerated by the subjects. The most frequently reported adverse drug reaction was diarrhoea, which is a known side-effect of both nelfinavir and ritonavir, and occurred in 75% of the subjects. This seems to be less than the previously reported value of 100% after treatment with nelfinavir/ ritonavir 2000/200 mg once daily[8]. The summary of product characteristics of nelfinavir states that 97.7% of mild to moderate severe diarrhoea occurs in patients treated with nelfinavir 750 mg thrice daily, without ritonavir[16]. It seems that the new formulation given at this dosage leads to less diarrhoea than the 250-mg tablets, notwithstanding the comedication with ritonavir. Treatment of diarrhoea with loperamid was only indicated in two subjects, which underlines the mild nature of this adverse event. More gastrointestinal disorders were reported during the first period of the study (nelfinavir plus ritonavir) than during the second period.

In the second period of the study, when efavirenz was introduced, dizziness, abnormal dreams and agitation occurred more frequently than in study period 1. These are known adverse events for efavirenz.
Increases in total cholesterol, leading to Grade 2 values in six subjects and Grade 3 values in four subjects, were observed. However, seven of these subjects suffered from Grade 2 values at screening, indicating that only three subjects showed increased values following drug treatment.
In conclusion, the studied regimens taken with a minimum amount of food at bedtime were tolerated well and thus considered safe to use in patients. Nelfinavir/ritonavir given together with efavirenz resulted in a 48% higher mean C_{24} concentration for nelfinavir, and the sum of nelfinavir and M8 C_{24} concentrations was 0.99 mg/L. From this study it became clear that not all subjects met the cut-off C_{min} of 1.0 mg/L for nelfinavir and M8 together, which should be approached with great care. Efavirenz exposure in this study was similar to that reported previously, and it can therefore be used effectively in combination with ritonavir and nelfinavir.

Acknowledgements

The authors thank the volunteers for their participation in this study. The laboratory technicians of the Department of Clinical Pharmacy of the University Medical Centre Nijmegen are kindly thanked for analysing all plasma samples. The study nurses at Farma Research BV are acknowledged for their assistance in performing this study. This study was funded by a grant from F. Hoffman-La Roche Ltd, Basel, Switzerland.

References

1. **Hogg, R. S., K. V. Heath, B. Yip, K. J. Craib, M. V. O'Shaughnessy, M. T. Schechter, and J. S. Montaner**. 1998. Improved survival among HIV-infected individuals following initiation of antiretroviral therapy. JAMA **279**:450-454.
2. **Palella, F. J. J., K. M. Delaney, A. C. Moorman, M. O. Loveless, J. Fuhrer, J. E. Satten, D. E. Aschman, and S. D. Holmberg**. 1998. Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. New England Journal of Medicine **338**:853-860.
3. **Harrington, M. and C. C. Carpenter**. 2000. Hit HIV-1 hard, but only when necessary. Lancet **355**:2147-2152.
4. **Paterson, D. L., S. Swindells, J. Mohr, M. Brester, E. N. Vergis, C. Squier, M. M. Wagener, and N. Singh**. 2000. Adherence to protease inhibitor therapy and outcomes in patients with HIV infection. Annals of Internal Medicine **133**:21-30.
5. **Joly, V., D. Descamps, and P. Yeni**. 2002. NNRTI plus PI combinations in the perspective of nucleoside-sparing or nucleoside-failing antiretroviral regimens. AIDS Rev. **4**:128-139.
6. **Mehta, S., R. D. Moore, and N. M. H. Graham**. 1997. Potential factors affecting adherence with HIV therapy. AIDS **11**:1665-1670.

7. **Stone, V. E.** 2001. Strategies for optimizing adherence to highly active antiretroviral therapy: lessons from research and clinical practice. Clin.Infect.Dis. **33**:865-872.
8. **Aarnoutse, R. E., J. A. Droste, J. J. Van Oosterhout, P. P. Koopmans, M. Popescu, P. Reiss, Y. A. Hekster, and D. M. Burger**. 2003. Pharmacokinetics, food intake requirements and tolerability of once-daily combinations of nelfinavir and low-dose ritonavir in healthy volunteers. Br.J.Clin. Pharmacol. **55**:115-125.
9. **Hsu, A., G. R. Granneman, and R. J. Bertz**. 1998. Ritonavir. Clinical pharmacokinetics and interactions with other anti-HIV agents. Clinical Pharmacokinetics **35**:275-291.
10. **Bardsley-Elliot, A. and G. L. Plosker**. 2000. Nelfinavir: an update on its use in HIV infection. DRUGS **59**:581-620.
11. **Zhang, K. E., E. Wu, A. K. Patick, B. Kerr, M. Zorbas, A. Lankford, T. Koba-yashi, Y. Maeda, B. Shetty, and S. Webber**. 2001. Circulating metabolites of the human immunodeficiency virus protease inhibitor nelfinavir in humans: structural identification, levels in plasma, and antiviral activities. Antimicrobial agents and chemotherapy **45**:1086-1093.
12. **Lillibridge, J. H., Lee, C. A., Pithavala, Y. K., Daniels, R. G., Samuel, T. M., Wu, E. Y., Zhang, K. E., Mazabel, E. L., Zhang, M., and Kerr, B. M**. 1998. The Role of Polymorphic CYP 2C19 in the Metabolism of Nelfinavir Mesylate. 12 th AAPS conference, abstract 3035
13. **Flexner, C**. Steady-state pharmacokinetic interactions between ritonavir (RTV), nelfinavir (NFV) and the nelfinavir active metabolite M8 (AG1402). 1998. 12th World Aids Conference, 28 June-3 July 1998, Geneva, Switzerland, abstract 42265 .
14. **Kurowski, M., B. Kaeser, A. Sawyer, M. Popescu, and A. Mrozikiewicz**. 2002. Low-dose ritonavir moderately enhances nelfinavir exposure. Clinical Pharmacology and Therapeutics **72**:123-132.
15. Stocrin® Summary of Product Characteristics. 2002. Merck Sharp & Dohme Limited, Hertfordshire, United kingdom.
16. Viracept® Summary of Product Characteristics. 2001. Roche Registration Limited, Hertfordshire, United Kingdom.
17. **Droste, J. A. H., C. P. W. G. M. Verweij-van Wissen, and Burger D.M.** 2003. Simultaneous Determination of the HIV Drugs Indinavir, Amprenavir, Saquinavir, Ritonavir, Lopinavir, Nelfinavir, the Nelfinavir hydroxymetabolite M8 and Nevirapine in Human Plasma by Reversed Phase High Performance Liquid Chromatography. Therapeutic Drug Monitoring **25**:393-399.
18. **Aarnoutse, R. E., K. J. Grintjes, D. S. Telgt, M. Stek, Jr., P. W. Hugen, P. Reiss, P. P. Koopmans, Y. A. Hekster, and D. M. Burger**. 2002. The influence of efavirenz on the pharmacokinetics of a twice-daily combination of indinavir and low-dose ritonavir in healthy volunteers. Clin Pharmacol Ther. **71**:57-67.
19. **Williams, R. L., M. L. Chen, and W. W. Hauck**. 2002. Equivalence approaches. Clin.Pharmacol. Ther. **72**:229-237.
20. **Fiske, W. D., Benedek, I. H., White, S. J., Pepperess, K. A., Joseph, J. L., and Kornhauser, D. M**. Pharmacokinetic Interaction between Efavirenz (EFV) and Nelfinavir Mesylate (NFV) in Healthy Volunteers. 5th Conference on retroviruses and opportunistic infections, February 1998, Chicago. Abstract no.349.
21. **Smith, P. F., Robbins, G., Shafer, R., Wu, H., Yu, S., Hirsch, M., Merigan, T., Morse, G. D., and ACTG 384 Study Team**. Effect of efavirenz on the pharmacokinetics of nelfinavir and M8 in naive, HIV-infected patients receiving long-term HAART therapy. 10th Conference on Retroviruses and Opportunistic Infections, Boston, 2003, abstract 148.
22. **Anonymous**. 2003. Optimising TDM in HIV clinical care. A practical guide to performing therapeutic drug monitoring (TDM) for antiretroviral agents. Available: http://www.HIVpharmacology.com. Last visited at May 24, 2004.
23. **Baede-van Dijk, P. A., P. W. H. Hugen, C. P. W. G. M. Verwey-van Wissen, P. P. Koopmans, D. M. Burger, and Y. A. Hekster**. 2001. Analysis of variation in plasma concentrations of nelfinavir and its active metabolite M8 in HIV-positive patients. AIDS **15**:991-998.

24. **Fiske, W. D., Benedek, I. H., Joseph, J. L., Dennis, S., O'Dea, R., Hsu, A., and Kornhauser, D. M**. 1998. Pharmacokinetics of efavirenz (EFV) and ritonavir (RIT) after multiple oral doses in healthy volunteers. 12th World Aids Conference, 28 June-3 July 1998, Geneva, Switzerland, Abstract no 42269.
25. **Smith, P. F., R. DiCenzo, and G. D. Morse**. 2001. Clinical pharmacokinetics of non-nucleoside reverse transcriptase inhibitors. Clin.Pharmacokinet. **40**:893-905.
26. **Villani, P., M. B. Regazzi, F. Castelli, P. Viale, C. Torti, E. Seminari, and R. Maserati**. 1999. Pharmacokinetics of efavirenz (EFV) alone and in combination therapy with nelfinavir (NFV) in HIV-1 infected patients. Br.J.Clin.Pharmacol. **48**:712-715.

Chapter 7

Is nelfinavir 1500 mg BID an effective intervention for patients on nelfinavir 1250 mg BID who have low nelfinavir exposure?

C.J.L. la Porte[1,2], D.M. Burger[1,2]

1 Department of Clinical Pharmacy, University Medical Centre, Nijmegen, The Netherlands.
2 Nijmegen University Centre for Infectious diseases, Nijmegen, The Netherlands.

Submitted

Abstract

Low nelfinavir plasma concentrations, resulting in concentration ratios (CR) <0.90, have been associated with failure of therapy. Nelfinavir CR <0.90 were observed in 56 patients receiving 1250 mg BID. In 38 dose was adjusted to 1500 mg BID, in 18 no adjustment was done. Dose adjustment to 1500 mg BID resulted in a CR >0.90 in only 45% of patients. For patients with an initial CR <0.52 this was 7%. In conclusion, dose adjustments were not always successful.

Introduction

Failing nelfinavir therapy can largely be explained by low plasma levels. Patients with nelfinavir 1250 mg BID containing HAART have a 3 times higher risk for virological failure when the CR is <0.90[1]. Therapeutic drug monitoring (TDM) can help to detect patients with such low plasma concentrations[2]. Nevertheless, to prevent patients with a CR <0.90 from failing their therapy an intervention is necessary. The chosen intervention should lead to a CR >0.90. It was previously shown that TDM improves nelfinavir treatment response, in patients with CR <0.90[3]. For patients treated with nelfinavir 1250 mg BID normally the first intervention is to ensure the intake with food. The second intervention is increasing nelfinavir dosage from 1250 mg BID to 1500 mg BID with food. We evaluated the pharmacokinetic effect of increasing nelfinavir dosage from 1250 mg to 1500 mg BID in patients with a CR between 0.52 and 0.90, and in patients with a CR <0.52.

Methods

This was a retrospective survey from our database of patients receiving 1250 mg BID nelfinavir. All patients were 18 years or older. Both males and females were included in the study. It was ensured that the patients took nelfinavir with enough food, and without other drugs known to interfere with nelfinavir metabolism. The database contained information on nelfinavir dosage and plasma nelfinavir concentrations, including time after intake of medicine. CR were calculated by dividing plasma concentrations by population data at the same time after intake of medication[4].

Patients receiving 1250 mg nelfinavir BID with an initial nelfinavir CR <0.90 were selected. After this selection patients were divided in 2 groups according to whether dose adjustments were made or not. Group 1 consisted of patients receiving a dose adjustment to 1500 mg nelfinavir BID. Group 2 was the control group consisting of patients who did not receive dosage adjustment. The second nelfinavir CR, obtained after dosage adjustment in group 1 or no intervention in group 2 was compared to the first CR. Additionally subgroup analyses were performed in groups 1 and 2 for patients with a nelfinavir CR <0.52, and a CR between 0.52 and 0.90.

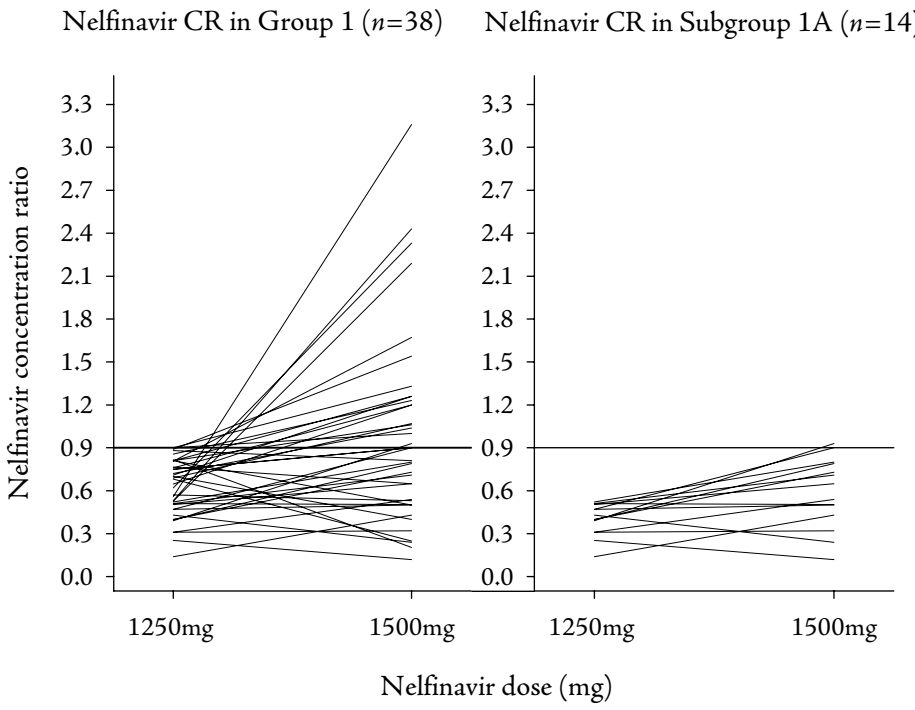
The Mann Whitney test was performed to compare the CRs from groups 1 and 2 from the first sample at baseline. The two-samples proportion test was used to test success rates between (sub)groups. *P*-values <0.05 were considered significant.

Plasma nelfinavir concentrations were measured using a high performance liquid chromatography method that has been published previously[5].

Results

In total 56 patients (38 in group 1 and 18 in group 2) were included in the analysis. Mean CR in group 1 and 2 at baseline was 0.61 and 0.64, respectively, the difference was not statistically significant (P=0.81). In group 1 seventeen patients (45%) had a CR >0.90 after dosage adjustment. In group 2 four patients (22%) had a CR >0.90 in the second sample. This difference between groups 1 and 2 was not significant with P=0.10. The subgroup analysis of group 1 showed that out of 14 patients (subgroup 1A) with an initial CR <0.52, one patient (7%) had a CR >0.90 after dosage adjustment. In subgroup 1B (24 patients) with an initial CR between 0.52 and 0.90, 16 patients (67%) had a CR >0.90 after dose adjustment. The difference between subgroups 1A and 1B was significant (7% versus 67 %, P<0.001).

Figure 1. Nelfinavir concentration ratio versus nelfinavir dose in group 1



Each line connects the two CRs, before and after dose adjustment, of each patient. The horizontal line represents the 0.90 cut off value for the CR.

For group 2 this subgroup analysis learned that out of five patients in subgroup 2A with an initial CR <0.52, two (40%) had a CR >0.90 in the second sample. From the 13 patients in subgroup 2B with an initial CR between 0.52 and 0.90, two (13%) had a CR >0.90 in the second sample.
The difference in success rates between subgroups 1B and 2B was significant (67% versus 15%, $P<0.01$), indicating that the observed higher success rate in subgroup 1B resulted from the dose adjustment.
In Figure 1 the nelfinavir CRs of individual patients in group 1 before and after the dose adjustment are displayed. The left graph shows group 1 as a total. In 28 out of 38 (74%) patients the CR increases after the dose adjustment, in the remaining 10 the second CR is lower than the first. The right graph (subgroup 1A) shows three patients with a lower CR in the second sample compared to the first. Eleven patients have an increased CR after the dose adjustment, however only one patient reaches a CR >0.90.

Conclusion

A nelfinavir dose adjustment from 1250 mg BID to 1500 mg BID in patients with an initial CR < 0.90 was effective in 45%, which is consistent with previously reported data[3]. For patients receiving nelfinavir 1250 mg BID with a CR <0.52 dose adjustment to 1500 mg nelfinavir BID was not an effective intervention, as the dose adjustment was effective in only 1 out of 14 patients. The same intervention was effective in 67% of patients with a CR between 0.52 and 0.90.
The effect of additional dose adjustments in patients with a CR <0.52 was not studied. Nelfinavir doses up to 1750 mg BID might help prevent patients from plasma concentrations that are low. Addition of ritonavir to the regimen is another option to prevent patients from low nelfinavir plasma concentrations. Ritonavir is an inhibitor of the Cytochrome P450 isoenzyme 3A4 and inhibits the metabolism of nelfinavir and its active metabolite M8[6,7], leading to higher plasma concentrations of both nelfinavir and M8. In the Athena study dose adjustment to 1750 mg nelfinavir BID or the addition of ritonavir was effective in 4 out of 7 patients with a CR <0.90[3], however it is not reported how many of these patients had a CR <0.52.
With the future marketing of the 625 mg tablet dose adjustment to 1875 mg BID will be easy, however, it is not said that the same effects are to be expected.
Additional research is needed to define interventions for patients with a CR <0.52, leading to a CR >0.90. In the meanwhile, TDM can help physicians to evaluate the effect of dose adjustments in clinical practice.

References

1. **Burger, D. M., P. W. Hugen, R. E. Aarnoutse, R. M. Hoetelmans, M. Jambroes, P. T. Nieuwkerk, G. Schreij, M. M. Schneider, M. E. Van Der Ende, and J. M. Lange**. 2003. Treatment failure of nelfinavir-containing triple therapy can largely be explained by low nelfinavir plasma concentrations. Ther.Drug Monit. **25**:73-80.
2. **Aarnoutse, R. E., J. M. Schapiro, C. A. Boucher, Y. A. Hekster, and D. M. Burger**. 2003. Therapeutic drug monitoring: an aid to optimising response to antiretroviral drugs? DRUGS **63**:741-753.
3. **Burger, D., P. Hugen, P. Reiss, I. Gyssens, M. Schneider, F. Kroon, G. Schreij, K. Brinkman, C. Richter, J. Prins, R. Aarnoutse, J. Lange, and for the ATHENA Cohort Study Group**. 2003. Therapeutic drug monitoring of nelfinavir and indinavir in treatment-naive HIV-1-infected individuals. AIDS **17**:1157-1165.
4. **Baede-van Dijk, P. A., P. W. H. Hugen, C. P. W. G. M. Verwey-van Wissen, P. P. Koopmans, D. M. Burger, and Y. A. Hekster**. 2001. Analysis of variation in plasma concentrations of nelfinavir and its active metabolite M8 in HIV-positive patients. AIDS **15**:991-998.
5. **Hugen, P. W. H., C. P. W. G. M. Verwey-van Wissen, D. M. Burger, E. W. Wuis, P. P. Koopmans, and Y. A. Hekster**. 1999. Simultaneous determination of the HIV-protease inhibitors indinavir, nelfinavir, saquinavir and ritonavir in human plasma by reversed-phase high-performance liquid chromatography. Journal of Chromatography B Biomed Sci Appl **727**:139-149.
6. **Kurowski, M., B. Kaeser, A. Sawyer, M. Popescu, and A. Mrozikiewicz**. 2002. Low-dose ritonavir moderately enhances nelfinavir exposure. Clinical Pharmacology and Therapeutics **72**:123-132.
7. **Flexner, C**. Steady-state pharmacokinetic interactions between ritonavir (RTV), nelfinavir (NFV) and the nelfinavir active metabolite M8 (AG1402). 12th World Aids Conference, 28 June-3 July 1998, Geneva, Switzerland, abstract 42265 . 2002.

Part IV

Non-nucleosides

Chapter 8

Predicting factors in interpatient variability of nevirapine pharmacokinetics

C.J.L. la Porte[1,2], M.E. van der Ende[3], I. Gyssens[3], W. Miesen[4], H. Sprenger[4], P.P. Koopmans[1,2], Y.A. Hekster[1,2], D.M. Burger[1,2]

1 University Medical Centre, Nijmegen, The Netherlands.
2 Nijmegen University Centre for Infectious diseases, Nijmegen, The Netherlands.
3 Erasmus Medical Centre, Rotterdam, The Netherlands.
4 University Hospital, Groningen, The Netherlands.

In preparation

Abstract

Introduction: Interpatient variability of nevirapine pharmacokinetics is a reason to perform Therapeutic drug monitoring (TDM) for this drug. The literature suggests gender and ethnicity as possible causes for interpatient variability of pharmacokinetics. In this study the association of ethnicity, gender and other demographic factors with nevirapine plasma concentrations were evaluated.
Methods: Data were retrospectively collected from routine TDM samples for nevirapine. The first nevirapine plasma level of each patient that was measured in the year 2002 was used for data collection. Only patients that used nevirapine 200 mg BID were included.
Results: For nevirapine data were collected of 89 females and 215 males. Average plasma nevirapine concentrations were statistically higher in Caucasian females, compared to Caucasian males (6.30 versus 5.61 mg/L, P=0.029). Race and time after intake of nevirapine were significantly associated with nevirapine plasma levels, following a stepwise multiple regression model.
Conclusion: Higher nevirapine levels were seen in non-Caucasian versus Caucasian patients, and higher average nevirapine levels were observed in female Caucasians compared to male Caucasians. Physicians should be alert for a higher risk for toxicity when treating females or non-Caucasian patients with nevirapine.

Introduction

Several studies describing a relationship between nevirapine plasma concentrations and antiviral effect or toxicity have been published. Data from these studies show a large interpatient variability for nevirapine[1-4]. This interpatient variability might lead to unexpected high plasma levels in certain groups of patients, and low plasma levels in others. To prevent patients from unwanted under- or over-exposure to nevirapine, knowledge of factors causing this variability is necessary.

A review article on the influence of gender on pharmacokinetics indicated that a great number of drugs show pharmacokinetic differences between females and males[5]. Gender related pharmacokinetic differences, presented as higher plasma levels in female patients, have been reported for the antiretroviral drugs indinavir[6] and lopinavir[7]. These differences may have their implications on treatment outcome with regard to virological failure in males due to subtherapeutic plasma levels on one hand and toxicity in females due to toxic plasma levels on the other hand. Pharmacokinetic gender differences have been reported for a number of drugs with a metabolism that is mediated through CYP3A[5]. Metabolism of nevirapine is mediated by CYP3A4 and to a lesser extent by CYP2B6[8]. So far the only data that has been published showing pharmacokinetic differences resulted from a population pharmacokinetic study reporting a 25% lower clearance of nevirapine in females[9]. For that reason pharmacokinetic gender differences can be expected for nevirapine.

Ethnicity has been described as a factor related to differences in pharmacokinetics of drugs metabolized by CYP3A[10-13]. Therefore it is not excluded that ethnicity can influence nevirapine pharmacokinetics, although recently a study described the absence of an association between race and nevirapine pharmacokinetics[14]. In such cohort studies unequal distribution of gender and race can result in very small subgroups that are not powered to prove significance for differences between subgroups.

We performed a retrospective cohort study to evaluate the influence of gender, race and other demographic parameters on the pharmacokinetics of nevirapine.

Methods

Study population

Data were retrospectively collected from routine TDM samples for nevirapine. The first nevirapine plasma level of each patient, both females and males, that was measured in the year 2002 was used for data collection. This was done to avoid bias from patients being repeatedly sampled for reasons of subtherapy/virological failure or toxicity. Only patients that used the recommended dose of nevirapine (200 mg BID) were included. Data was collected from the form that accompanied the sample. Collected data concerned: gender, race, age, length, body weight, body mass index (BMI) (BMI is equal to weight [in kilograms]/height^2 [in square meters]), indication

for TDM, concomitant medication, plasma level and time of blood draw after intake of medication. Indication for TDM could be: control, suspicion of interaction, suspicion of non-compliance, suspicion of subtherapy and suspicion of intoxication. In the case of a suspicion of non-compliance data were excluded. The reason for this exclusion was that these samples could show low plasma levels, without having a pharmacokinetic background for that. All data were captured in a Microsoft EXCEL 2000 database. The HIV Monitoring Foundation (SHM) follows all HIV-infected patients in the Netherlands as a national cohort and patients have given informed consent. This cohort protocol has been approved by all institutional review boards of the 22 Dutch treatment centers for HIV-infected patients.

Bioanalytical methods

Nevirapine was analyzed in plasma using a previously described high performance liquid chromatographic (HPLC) methods with ultraviolet detection[15]. The accuracy of this assay ranges from 91.5 to 102.6%. The intraday and interday precision ranges from 1.3% to 3.9% and from 1.9% to 3.0%, respectively. Preceding analysis plasma was separated from the blood sample and stored at a temperature lower than 20 degrees Celsius below zero.

Statistical analysis

Differences in population characteristics and plasma nevirapine concentrations between subgroups based on gender and race were analyzed using an independent samples t-test and an analysis of variance (ANOVA), respectively. To determine variables that influence plasma nevirapine concentrations a stepwise multiple regression model was used. A *P*-value <0.05 was considered significant in all analyses.

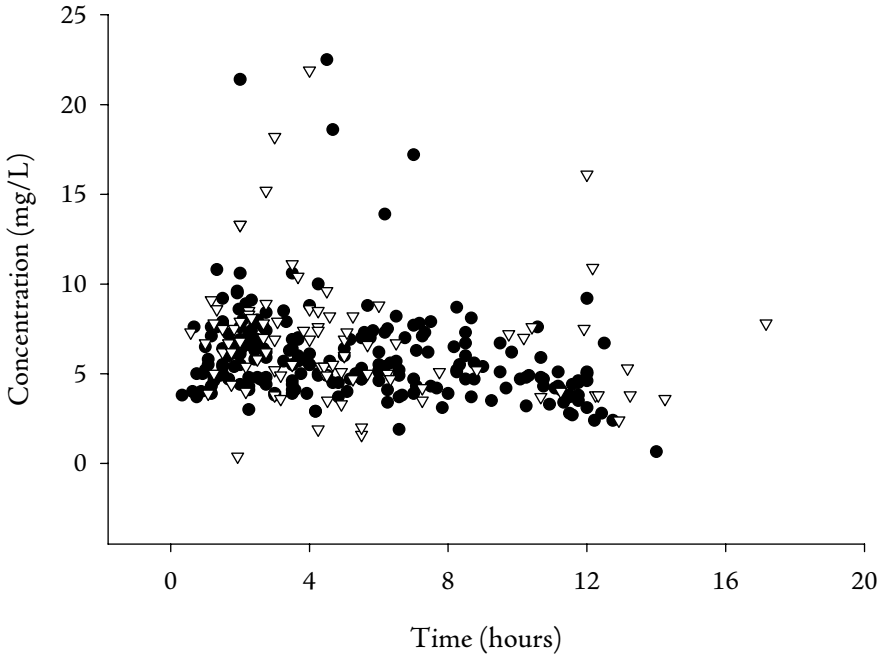
Results

Patient population

Data were collected of 304 patients; 89 females and 215 males. The distribution of males and females was different in the 3 races. Race was Caucasian in 207 patients (171 males and 36 females), Black in 88 patients (40 males and 48 females) and Asiatic in 9 patients (4 males and 5 females). Detailed information on demographic variables can be found in Table 1. Mean plasma nevirapine levels were higher in females than in males, 6.78 mg/L and 5.95 mg/L, respectively (P=0.042). Figure 1 is a reflection of all available plasma levels, grouped by gender.

In Caucasians, Blacks and Asiatic plasma nevirapine levels were 5.73 mg/L, 6.98 mg/L and 9.09 mg/L, respectively. The difference between plasma levels of Caucasians and Blacks was statistically significant with P=0.027, however the difference between Asiatics and Caucasians or Blacks was not statistically significant. In more detail, average plasma nevirapine concentrations were statistically higher in Caucasian

Figure 1. Nevirapine plasma levels of 304 HIV-infected patients



females, compared to Caucasian males (6.30 versus 5.61 mg/L P=0.029). No such differences were seen between males and females of the Black and Asiatic races. No statistically significant differences were observed between subgroups, based on gender and race, with regard to: reason for TDM, concomitant medication and time of blood draw after intake of nevirapine.

Factors influencing plasma nevirapine concentrations

The variables age, weight, length, BMI, time after intake, gender and race were evaluated for association to nevirapine plasma concentration in a univariate regression model. A stepwise multiple regression model was performed to identify variables with influence on plasma nevirapine levels. It appeared that race and time after last intake were the only two variables that were significantly associated with nevirapine exposure ($P \leq$ 0.001). The Asiatic race was relatively underrepresented in our data set, and therefore might lead to bias in the analysis. To eliminate this possible influence, additional multiple regression analyses were performed. In the first additional analysis Asiatic race was excluded, in the second the Asiatic race was regrouped together with Blacks as non-Caucasian. In these two additional analyses race and time after last intake were again associated with plasma nevirapine concentrations ($P \leq$ 0.001).

Table 1. Demographic data

		All	caucasian	black	asiatic
Number[a]	A	304	207 (68.1%)	88 (28.9%)	9 (3.0%)
	M	215 (70.7%)	171 (79.5%)	40 (18.6%)	4 (1.9%)
	F	89 (29.3%)	36 (40.1%)	48 (53.9%)	5 (5.6%)
Level (mg/L)	A	6.19±2.93	5.73±1.73	6.98±4.25	9.09±5.43
	M	5.95±2.68	5.61±1.67	6.98±4.24	10.05±8.69
	F	6.78±3.41	6.30±1.91	6.98±4.31	8.32±0.82
Time (h)	A	5.3±3.6	5.4±3.7	5.1±3.1	5.3±5.4
	M	5.4±3.5	5.3±3.6	5.6±3.0	5.2±4.3
	F	5.1±3.7	5.5±4.1	4.8±3.1	5.4±6.7
Age (y)	A	41.5±9.9	44.0±9.3	35.6±8.7	41.8±13.3
	M	43.9±9.2	44.8±9.2	40.2±8.7	39.5±3.7
	F	35.7±9.4	40.0±8.7	31.8±6.7	43.6±18.3
Weight (kg)	A	75.5±13.9	76.6±13.2	74.0±15.3	65.2±10.2
	M	78.4±12.1	79.1±11.9	76.0±12.5	71.9±9.4
	F	68.6±15.5	64.7±12.5	72.3±17.2	59.9±7.7
Length (m)	A	1.75±0.10	1.78±0.09	1.68±0.10	1.62±0.08
	M	1.79±0.07	1.80±0.06	1.76±0.08	1.71±0.02
	F	1.64±0.07	1.66±0.08	1.62±0.06	1.56±0.02
BMI (kg/m^2)	A	24.7±4.1	24.2±3.6	26.1±5.0	24.0±2.2
	M	24.3±3.2	24.3±3.3	24.2±2.8	23.1±0.9
	F	25.6±5.5	23.4±4.4	27.5±5.8	24.5±2.7

A = all patients, M = males, F = females
Data are means ± standard deviation, unless stated otherwise
[a] Data given as frequencies and percentages

The multiple regression model was also repeated for the separate subgroups of females and males. This was done to further investigate the association between gender and nevirapine levels as found in the univariate regression analysis. It appeared that in both males and females the variables race and time after intake were predictive for nevirapine plasma levels.

Discussion

In this retrospective study we evaluated the influence of several demographic factors on the pharmacokinetics of the antiretroviral agent nevirapine. In total 304 HIV-1 positive patients using nevirapine 200 mg BID were included. Plasma nevirapine concentrations were significantly higher in non-Caucasians versus Caucasians, and in female versus male Caucasians. Race and time after intake of nevirapine are significantly associated with nevirapine plasma levels, following a multiple regression model. It may be evident that plasma nevirapine concentrations decrease with increasing time after intake. In our data time after intake of nevirapine was similar in subgroups based on gender and ethnicity, so there is no contribution of this variable to the observed differences.

Recently it was published that race was not significantly related to clearance of nevirapine in a population pharmacokinetic model[14]. Although not significant, a trend towards lower clearance of nevirapine was observed for Blacks and Asiatics in this model, compared to Caucasians. Clearance was 6.4% and 12.6% (P=0.14) lower for Blacks and Asiatics, respectively. Based on these data, de Maat *et al*. conclude that race is not a factor to take into consideration when treating patients with nevirapine. Our data do show a significant relationship between race and nevirapine plasma levels, which might be the result of a larger group of non-Caucasian patients. The observations from our study advocate the use of TDM in certain groups of patients to prevent them from high nevirapine plasma concentrations.

Body composition has been mentioned in the literature as a possible explanation for variability in pharmacokinetics[16]. In our study the BMI was not associated with plasma nevirapine concentrations, although a statistical significant higher BMI was seen in Black women compared to Caucasian women or Black males (P<0.01). This observation however, was not accompanied by a significant difference in the average nevirapine levels in these three groups.

Differences were also present between age, body weight and length in several subgroups, nevertheless these variables were not associated with nevirapine concentrations.

The information collected on co-medications used and the indication for TDM did not show any differences between subgroups. Therefore it was not expected that these variables were predictive for the occurrence of higher plasma levels in the subgroups.

Although the current data collection did not show a difference in the use of comedications between females and males and/or racial groups, there might be an underestimation of the use of oral contraceptives. For the data collection we were depending on the completeness and accuracy of the sampling forms. For that reason it cannot be excluded that the use of oral contraceptives had no influence on plasma levels of nevirapine. However it has been shown that single doses of ethinyl estradiol and norethindrone did not affect nevirapine pharmacokinetics[17].

Differences in hormonal status of females compared to males might also influence pharmacokinetics of nevirapine, and within the group of females the menopausal status might be a factor of influence as well[18]. As for the differences in hormonal status

between females and males the current data collection has no additional information. With regard to the possibility of subgroups of pre- and postmenopausal females within the group of females, the used database did not contain information on the menopausal status of the females. However, based on the reported ages of the females with an average of 35.7 years, the number of postmenopausal females will be very limited. Therefore it is not likely that the menopausal status of the females results in subgroups with different pharmacokinetics.

In pregnant females a number of physiologic changes can alter different pharmacokinetic processes, however the net total effect is often small[19]. Data from some small studies did not show differences in plasma nevirapine concentrations between pregnant and non-pregnant females[20]. The database used in the current study contained no information on pregnancy. However, the use of nevirapine in pregnant females is less likely, as the antiretroviral drug of first choice in pregnancy in The Netherlands is nelfinavir. For these reasons pregnancy is not likely to interfere with our data.

Our study is limited by the absence of clinical data in the database, especially with regard to the occurrence of toxicity. This makes it impossible to correlate the occurrence of adverse events to the plasma nevirapine concentrations. For this reason the objective of our study was limited to pharmacokinetics only. In the literature however, a higher incidence of nevirapine related toxicity such as rash in females as compared to males has been described[21-23]. The clinical observations from these studies support our pharmacokinetic findings in the current study.

In conclusion, from our data, race and time after intake are predictors for plasma nevirapine concentrations. After correction for time after intake this results in higher mean plasma nevirapine concentrations in Blacks and Asiatics in comparison to Caucasians. Apart from that a significant higher average nevirapine level was observed in female Caucasians compared to male Caucasians. These higher nevirapine levels might lead to more nevirapine related toxicity in these subgroups. Therefore, physicians treating non-Caucasian patients or Caucasian females with nevirapine should use TDM to individualize dosages and prevent toxicity.

Acknowledgements

Minny Meeuwissen, Tessa Rooyaards and Piet van der Meulen are kindly thanked for their assistance in the collection of the data needed for this study.

References

1. **Gonzalez de Requena, D., M. Nunez, I. Jimenez-Nacher, and V. Soriano**. 2002. Liver toxicity caused by nevirapine. AIDS **16**:290-291.
2. **Havlir, D., S. H. Cheeseman, M. McLaughlin, R. Murphy, A. Erice, S. A. Spector, T. C. Greenough, J. L. Sullivan, D. Hall, M. Myers, and .** 1995. High-dose nevirapine: safety, pharmacokinetics, and antiviral effect in patients with human immunodeficiency virus infection. J Infect.Dis. **171**:537-545.
3. **Veldkamp, A. I., G. J. Weverling, J. M. A. Lange, J. S. G. Montaner, P. Reiss, D. A. Cooper, S. Vella, D. Hall, J. H. Beijnen, and R. M. W. Hoetelmans**. 2001. High expossure to nevirapine in plasma is associated with an improved virological response in HIV-1-infected individuals. AIDS **15**:1089-1095.
4. **Vries-Sluijs, T. E., J. P. Dieleman, D. Arts, A. D. Huitema, J. H. Beijnen, M. Schutten, and M. E. Van Der Ende**. 2003. Low nevirapine plasma concentrations predict virological failure in an unselected HIV-1-infected population. Clin.Pharmacokinet. **42**:599-605.
5. **Schwartz, J. B.** 2003. The influence of sex on pharmacokinetics. Clin.Pharmacokinet. **42**:107-121.
6. **Burger, D. M., M. C. Siebers, P. W. Hugen, R. E. Aarnoutse, Y. A. Hekster, and P. P. Koopmans**. 2002. Pharmacokinetic variability caused by gender: do women have higher indinavir exposure than men? J Acquir Immune Defic Syndr **29**:101.
7. **Burger D.M., Muller R.J., van de Leur M.R., and la Porte C.J.L**. Lopinavir plasma levels are significantly higher in female than in male HIV-1 infected patients. 3rd International Workshop on Clinical Pharmacology of HIV Therapy, April 11-13, 2002, Washington DC, USA, abstract 6.5. 2002.
8. **Erickson, D. A., G. Mather, W. F. Trager, R. H. Levy, and J. J. Keirns**. 1999. Characterization of the in vitro biotransformation of the HIV-1 reverse transcriptase inhibitor nevirapine by human hepatic cytochromes P-450. Drug Metab Dispos. **27**:1488-1495.
9. **Zhou, X. J., L. B. Sheiner, R. T. D'Aquila, M. D. Hughes, M. S. Hirsch, M. A. Fischl, V. A. Johnson, M. Myers, and J. P. Sommadossi**. 1999. Population pharmacokinetics of nevirapine, zidovudine, and didanosine in human immunodeficiency virus-infected patients. The National Institute of Allergy and Infectious Diseases AIDS Clinical Trials Group Protocol 241 Investigators. Antimicrob.Agents Chemother. **43**:121-128.
10. **Sowunmi, A., T. J. Rashid, O. O. Akinyinka, and A. G. Renwick**. 1995. Ethnic differences in nifedipine kinetics: comparisons between Nigerians, Caucasians and South Asians. Br.J.Clin. Pharmacol. **40**:489-493.
11. **Wandel, C., J. S. Witte, J. M. Hall, C. M. Stein, A. J. Wood, and G. R. Wilkinson**. 2000. CYP3A activity in African American and European American men: population differences and functional effect of the CYP3A4*1B5'-promoter region polymorphism. Clin.Pharmacol.Ther. **68**:82-91.
12. **Kinirons, M. T., C. C. Lang, H. B. He, K. Ghebreselasie, S. Shay, D. W. Robin, and A. J. Wood**. 1996. Triazolam pharmacokinetics and pharmacodynamics in Caucasians and Southern Asians: ethnicity and CYP3A activity. Br.J.Clin.Pharmacol. **41**:69-72.
13. **Mancinelli, L. M., L. Frassetto, L. C. Floren, D. Dressler, S. Carrier, I. Bekersky, L. Z. Benet, and U. Christians**. 2001. The pharmacokinetics and metabolic disposition of tacrolimus: a comparison across ethnic groups. Clin.Pharmacol.Ther. **69**:24-31.
14. **De Maat, M. M., J. F. Nellen, A. D. Huitema, F. W. Wit, J. W. Mulder, J. M. Prins, and J. H. Beijnen**. 2004. Race Is Not Associated with Nevirapine Pharmacokinetics. Ther.Drug Monit. **26**:456-458.
15. **Hollanders, R. M. W., E. W. J. van Ewijk-Beneken Kolmer, D. M. Burger, E. W. Wuis, P. P. Koopmans, and Y. A. Hekster**. 2000. Determination of nevirapine, an HIV-1 non-nucleoside reverse transcriptase inhibitor, in human plasma by reversed-phase high-performance liquid chromatography. Journal of Chromatography B Biomed Sci Appl **744**:65-71.
16. **Meibohm, B., I. Beierle, and H. Derendorf**. 2002. How important are gender differences in pharmacokinetics? Clin.Pharmacokinet. **41**:329-342.

17. **Mildvan, D., R. Yarrish, A. Marshak, H. W. Hutman, M. McDonough, M. Lamson, and P. Robinson**. 2002. Pharmacokinetic interaction between nevirapine and ethinyl estradiol/norethindrone when administered concurrently to HIV-infected women. J.Acquir.Immune.Defic.Syndr. **29**:471-477.
18. **Tanaka, E.** 1999. Gender-related differences in pharmacokinetics and their clinical significance. J.Clin.Pharm.Ther. **24**:339-346.
19. **Loebstein, R., A. Lalkin, and G. Koren**. 1997. Pharmacokinetic changes during pregnancy and their clinical relevance. Clin.Pharmacokinet. **33**:328-343.
20. **Mirochnick, M., D. F. Clarke, and A. Dorenbaum**. 2000. Nevirapine: pharmacokinetic considerations in children and pregnant women. Clin.Pharmacokinet. **39**:281-293.
21. **Bersoff-Matcha, S. J., W. C. Miller, J. A. Aberg, H. C. van Der, H. J. Hamrick Jr, W. G. Powderly, and L. M. Mundy**. 2001. Sex differences in nevirapine rash. Clin.Infect.Dis. **32**:124-129.
22. **Mazhude, C., S. Jones, S. Murad, C. Taylor, and P. Easterbrook**. 2002. Female sex but not ethnicity is a strong predictor of non-nucleoside reverse transcriptase inhibitor-induced rash. AIDS **16**:1566-1568.
23. **Antinori, A., F. Baldini, E. Girardi, A. Cingolani, M. Zaccarelli, S. Di Giambenedetto, A. Barracchini, P. De Longis, R. Murri, V. Tozzi, A. Ammassari, M. G. Rizzo, G. Ippolito, and A. De Luca**. 2001. Female sex and the use of anti-allergic agents increase the risk of developing cutaneous rash associated with nevirapine therapy. AIDS **15**:1579-1581.

Chapter 9

Interpatient variability in the pharmacokinetics of the HIV non-nucleoside reverse transcriptase inhibitor efavirenz: the effect of sex, race and genetics

D.M. Burger[1,2], I. van der Heiden[3], C.J.L. la Porte[1,2], M.E. van der Ende[3], P. Groeneveld[4], C. Richter[5], P.P. Koopmans[1,2], F.P. Kroon[6], H. Sprenger[7], J. Lindemans[3,] P. Schenk[3], R. van Schaik[3]

1 University Medical Center Nijmegen, The Netherlands.
2 Nijmegen University Center for infectious diseases, The Netherlands.
3 Erasmus Medical Center Rotterdam, The Netherlands.
4 Isala Hospital Zwolle, The Netherlands.
5 Rijnstate Hospital Arnhem, The Netherlands.
6 Leiden University Medical Center, The Netherlands.
7 University Hospital Groningen, The Netherlands.

Submitted

Abstract

Background: The pharmacokinetics of efavirenz is characterized by large interpatient variability. In a preliminary analysis of patients from our Therapeutic drug monitoring (TDM) service, significantly higher efavirenz plasma levels were observed in females.
Methods: All samples that were analysed for efavirenz in our TDM service in 2002 and 2003 were reviewed. Information on sex, age, body weight, length, race, hormonal contraceptive use, and time between sampling and last intake was recorded. DNA was isolated from plasma of these patients, and PCR-restriction fragment length polymorphism analysis was performed to detect the cytochrome P450 2B6 (CYP2B6) C1459T single nucleotide polymorphism (SNP) which is associated with low CYP2B6 activity.
Results: A total of 255 patients were included in this analysis. The median efavirenz plasma level was 2.50 (interquartile range: 1.85–3.55) mg/L. Out of these 255 patients, 8 (3.1%) were considered to have a subtherapeutic efavirenz plasma level (< 1.0 mg/L) and 48 (18.9%) a toxic efavirenz level (> 4.0 mg/L). Sex, time after last intake, and race were the only factors that were significantly associated with the efavirenz plasma level in a multivariate analysis. No influence was observed for body weight, hormonal contraceptive use, and the presence of the CYP2B6 C1459T SNP (*5 and *7 allele).
Conclusions: Sex and race are important factors in determining the interpatient variability in efavirenz plasma levels. This effect is not caused by low CYP2B6 activity as determined by SNP analysis. Physicians should primarily be alert for signs of efavirenz-induced toxicity in females and non-Caucasian patients.

Introduction

The HIV non-nucleoside reverse transcriptase inhibitor (RTI) efavirenz is recommended as one the preferred agents, combined with two nucleoside RTIs, as initial treatment of patients with HIV infection[1]. Several studies have demonstrated the potent antiviral activity of efavirenz in this combination, leading to >80% of patients with an HIV-1 viral load below the detection limit of 50 copies/mL[2-4].
The pharmacokinetics of efavirenz can be characterized by extensive protein binding (>99%), hepatic clearance through cytochrome P450 (CYP) 2B6 and 3A4 isozymes, and a long elimination half-life (40–55h)[5]. Interpatient variability in efavirenz pharmacokinetics is significant, as has repeatedly been demonstrated[6-8]. The clinical relevance of this interpatient variability is the translation into variable response in HIV-infected patients: patients with low exposure to efavirenz have an increased risk of virological failure[6,7,9-11], while on the other hand those with high exposure suffer from efavirenz-induced side effects, mostly related to the central nervous system [9,12]. Based on this information, a therapeutic range for efavirenz of 1.0–4.0 mg/L has been recommended[13]. Given the presence of a concentration-effect relationship for efavirenz and the large interpatient variability in its pharmacokinetic parameters, one would benefit from more data on factors that influence the pharmacokinetic behaviour of this agent. As a result, treatment with efavirenz could be more individualized, and treatment failure, either due to insufficient virological response or (CNS-) toxicity, can be prevented. In a preliminary analysis of patients from our Therapeutic drug monitoring (TDM) service, we found that female sex was a significant risk factor for the development of toxic efavirenz plasma levels[14]. In this study, we have extended our study group and investigated potential factors associated with abnormal efavirenz plasma levels in female patients.

Methods

Patients

Patients were selected on the basis of a plasma sample that was submitted to our national TDM service for efavirenz from six different sites in 2002 and 2003. Only the first sample from a patient was included to avoid potential bias from repeated sampling. Patients used the standard dose for efavirenz of 600 mg once daily. Subjects for whom a sample was submitted with a suspicion of nonadherence (indicated by the physician on the application form) and samples with an undetectable efavirenz plasma level (< 0.2 mg/L) were excluded from the analysis. Also, samples withdrawn more than 24h after the last dose intake were omitted.
Demographic data were extracted from the application form or the patient's medical records. All HIV-infected patients in The Netherlands are followed as a national cohort by the HIV Monitoring Foundation (SHM) and patients have given informed

consent. This cohort protocol has been approved by all institutional review boards of the 22 Dutch treatment centers for HIV-infected patients.

Efavirenz plasma levels

Efavirenz plasma levels were determined by a validated high-performance liquid chromatographic assay, as previously prescribed[15]. Accuracy of this assay ranges from 99.0 to 100.5%; maximum intra- and interday precision are 2.6% and 2.8%, respectively. Efavirenz plasma levels were defined as either subtherapeutic (< 1.0 mg/L), therapeutic (1.0–4.0 mg/L), or toxic (> 4.0 mg/L)[13].

Genetic analysis

The CYP2B6 C1459T single nucleotide polymorphism (SNP) was selected for genotyping following the previously reported reduced protein expression in human liver samples containing this SNP[16]. Our hypothesis was that this SNP could be related to increased exposure of the CYP2B6 substrate efavirenz. DNA was isolated from plasma of all patients and PCR-restriction fragment length polymorphism analysis was performed to detect the C1459T variant of the CYP2B6 gene, which is part of the CYP2B6*5 and CYP2B6*7 haplotypes, and corresponds to the Arg487Cys amino acid change of the CYP2B6 isozyme. The method employed was a modification of the assay previously described by Lang *et al.*[14] using forward primer 5'-CTGTTGCAGTGGACATTTG-3' and reverse primer 5'-ATCTCACTCCTGCACTCAC-3'. The absence or presence of the nucleotide change C1459T results in either wild-type CC (WT), heterozygous variant CT, or homozygous variant TT.

Statistical analysis

Differences in efavirenz plasma levels between subgroups were compared by analysis of variance. Univariate and multivariate regression were applied to identify factors related to efavirenz plasma levels. Test results with a *P*-value < 0.05 were considered statistically significant.

Results

Patients

A total of 255 patients were selected from the six different study sites. Patient demographics are listed in Table 1. The median efavirenz plasma level was 2.50 mg/L with an interquartile range from 1.85 to 3.55 mg/L. The distribution of efavirenz plasma levels during the 24h dose interval is depicted in Figure 1. Out of these 255 patients, 8 (3.1%) were considered to have a subtherapeutic efavirenz plasma level (< 1.0 mg/L) and 48 (18.9%) to have a toxic efavirenz level (> 4.0 mg/L). Consequently, the remaining 199 subjects (78.0%) had an efavirenz plasma level within the therapeutic range (1.0–4.0 mg/L).

Table 1. Demographics

Parameter (unit)	% of patients	Median	Interquartile range
Age (yr)		40	33-48
Gender			
Male	74.1%		
Female	25.9%		
Weight (kg)		72	63-80
Length (cm)		176	168-181
Body surface area (m^2)		1.85	1.73-2.00
Time after intake (h)		13	12-16
EFV plasma level (mg/L)		2.5	1.7-3.7
Race			
Caucasian	63.5%		
Black	32.5%		
Asian	3.9%		
CYP2B6 genotype at position 1459	N=228		
C/C	82.9%		
C/T	14.5%		
T/T	2.6%		

Demographic factors influencing efavirenz exposure

All demographic factors were entered in a univariate regression model for a potential relationship with the efavirenz plasma level. Subsequently, factors that were significantly associated with efavirenz exposure were stepwise added in a multivariate analysis. Results are depicted in Table 2. It appeared that sex, time after last intake, and race were the only factors that were significantly associated with the efavirenz plasma level. The effect of sex and race are also presented in Figure 2. The average (± standard deviation (SD)) efavirenz plasma level in female patients was 4.0 (±3.2) mg/L versus 2.8 (± 1.7) mg/L in male patients ($P < 0.001$). Three different ethnic groups were present in our study population: Asians (n=10), blacks (n=84) and Caucasians (n=161). The average (± SD) efavirenz plasma levels in these ethnic groups were 3.3 (± 1.6), 3.8 (± 3.0) and 2.8 (± 1.6) mg/L, respectively (P=0.003).

An additional analysis of the influence of body weight was conducted. As expected, female patients had a lower average body weight than male patients (65.0 versus 75.0 kg); the same was true for non-Caucasians versus Caucasians: 68.1 versus 76.4 kg. Thus, a lower body weight in female and non-Caucasian patients could be an explanation for the association between sex and race with efavirenz plasma levels. In a multivariate analysis, however, body weight was no longer associated with higher efavirenz plasma levels when corrected for sex, time after intake, and race (P=0.355).

Figure 1. Efavirenz plasma levels of 255 patients

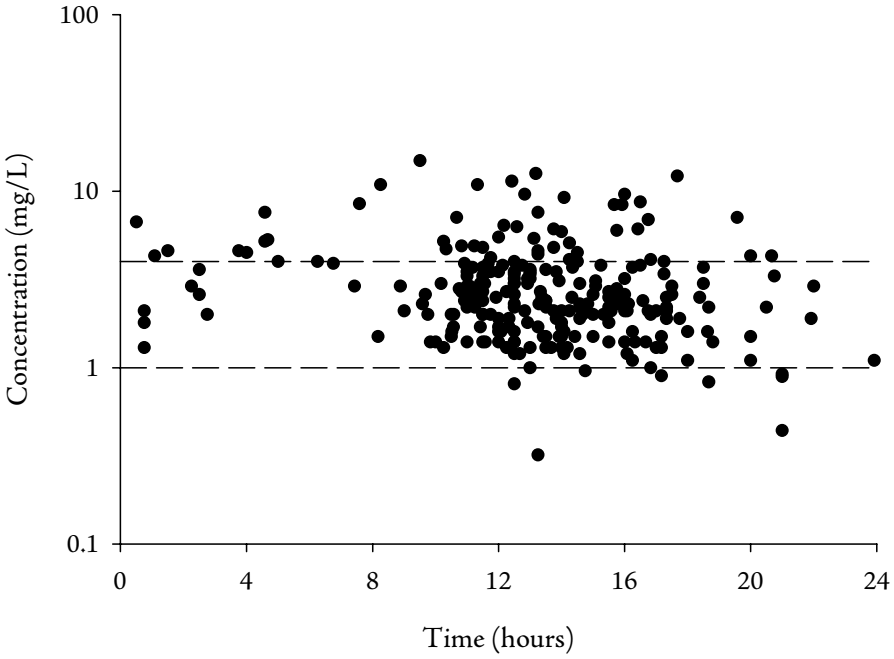
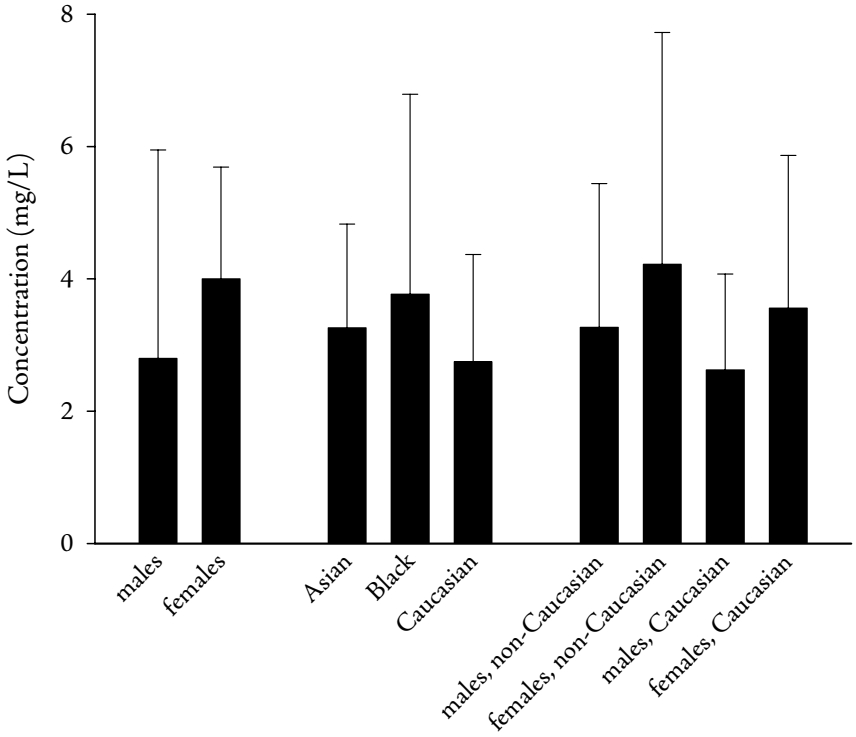


Table 2. Univariate and multivariate regression analysis

Parameter	Univariate			Multivariate		
	R	F	*P*-value	R	F	*P*-value
Age	0.093	2.22	0.137			
Sex	0.238	15.18	<0.001	0.287	17.65	<0.001
Body weight	0.217	12.15	0.001			
Body surface area	0.218	10.84	0.001			
Length	0.163	6.07	0.015			
Time after intake of medication	0.158	6.45	0.012	0.350[#]	13.65[#]	< 0.001[#]
Race	0.183	8.80	0.003	0.389[##]	11.56[##]	< 0.001[##]
CYP2B6 Genotype	0.132	3.99	0.047			

[#]: in addition to gender; [##]: in addition to gender and time after intake of medication. R = regression coefficient; F = Fischer's exact test value.

Figure 2. Mean efavirenz plasma level (+ standard deviation) in the various subgroups



Another possible explanation for the observed effect of female sex on efavirenz plasma levels is the use of hormonal contraceptives in (part of) the female subjects. We were able to track information on hormonal contraceptive use in 39 of the 66 female patients in our cohort. Eight of these 39 women used some kind of hormonal contraceptives, but hormonal contraceptive use was not associated with higher efavirenz plasma levels. In fact, an opposite trend was found towards higher efavirenz plasma levels in females who reported that they did *not* use hormonal contraceptives versus those who did: mean (± SD) values were 5.0 (± 3.7) versus 2.7 ± (1.1) mg/L, respectively (P=0.10).

Pharmacogenetic analysis

To investigate a possible genetic background for the observed differences in efavirenz plasma levels between different ethnic groups, we have evaluated the C1459T polymorphism of CYP2B6 in 228 samples where DNA could be amplified. A large majority of the patients (82.9%) could be identified as wild type (CC), while heterozygous (CT) and homozygous (TT) variants could be found in 14.5 and 2.6% of the patients, respectively (Table 1). A significant difference in the frequency of variant alleles (CT and TT combined) was observed between Caucasians (21.6%),

Blacks (8.2%) and Asians (0%) (P=0.033). This genetic polymorphism, however, could not be linked to differences in efavirenz plasma levels: median values (+ IQR) were 2.6 (1.8–3.7) mg/L, 2.1 (1.5–2.7) mg/L, and 2.1 (1.5–2.8) mg/L for CC, CT, and TT genotypes, respectively (P=0.074).

Discussion

Our study is the largest interpatient comparison of efavirenz pharmacokinetics reported so far. The most important observations are the consistently higher plasma efavirenz levels in female patients as well as in non-Caucasian patients. In a post-hoc subgroup analysis, female non-Caucasian patients appeared to have a 60% higher efavirenz plasma level than male Caucasian patients (Figure 2). Thus, when treating patients with efavirenz, physicians should be aware of a higher risk for efavirenz-induced toxicity in females and non-Caucasian patients, as higher exposure to efavirenz has been linked to an increased risk of toxicity[9,12].

Our data confirm the large interpatient variability in efavirenz plasma levels. Still, 78% of patients had an efavirenz plasma level within the therapeutic range of 1.0–4.0 mg/L, which is comparable to observations in other, smaller cohort studies[6-9,11]. This indicates that the observed interpatient variability in efavirenz exposure does not lead to unwanted effects as long as they remain within this relatively narrow range. For the remaining 22% of the patients in this cohort, it appears that efavirenz treatment needs optimisation.

Remarkably, far less patients had subtherapeutic plasma levels of efavirenz (< 1.0 mg/L) when compared to toxic levels (> 4.0 mg/L). This may have been caused by our exclusion criterion for non-adherent patients (based on a suspicion by the physician and/or the presence of an undetectable efavirenz level in the TDM sample). Including these samples would have confounded our investigations for factors associated with efavirenz plasma levels. Another explanation for the higher proportion of toxic efavirenz plasma levels may be a selection bias why physicians have sent a TDM sample for efavirenz. TDM of efavirenz is recommended in the Netherlands for all patients at week 4 and 24 after starting treatment with this agent, and when there is a suspicion of intoxication, suboptimal therapy, drug-drug interaction, or non-adherence. Nevertheless, given the significant number of patients with toxic efavirenz plasma levels, it is important to investigate potential causative factors.

As reported earlier in a preliminary analysis of a smaller cohort in our TDM service, female patients had a significantly higher efavirenz plasma level than male patients (Figure 2). The consequence might be an increased risk for efavirenz-induced toxicity in female patients. Several cohort studies have demonstrated a 1.5–1.7 fold higher risk for adverse drug reactions in female patients using antiretroviral agents (reviewed by[17]), although details for those using efavirenz are not available. Recently, Spire *et al.* presented a cross-sectional study that identified characteristics associated with

an increased risk for discontinuation of efavirenz use[18]. Female patients had a 2.2 times (95% confidence interval: 1.2–3.8) higher risk for discontinuation of efavirenz than males.

In addition, an effect of race became apparent in our analyses, although this can partly be explained by a higher proportion of females among non-Caucasian (46.8%) versus Caucasian patients (13.7%). Nevertheless, the effect of race was significant in a multivariate analysis when corrected for sex and time after intake (Table 2). It can also be observed in the post-hoc analysis presented in Figure 2 where non-Caucasian females and males displayed higher efavirenz plasma levels than in their respective Caucasian counter partners. Pfister *et al.* also observed a lower hepatic clearance rate of efavirenz in a combined group of African-Americans/Hispanics as compared to white non-Hispanics[6].

We analysed several factors that could be the (partial) explanation for these sex and racial effects. First, differences in body weight, length, or body composition are present between females and males, and to some extent also between races. However, body weight (P=0.355), length (P=0.673) and body surface area (P=0.471) were not related to efavirenz plasma levels in the multivariate analysis when corrected for sex, race and time after intake of medication. Second, the use of hormonal contraceptives by a subgroup of female patients may be related to inhibition of efavirenz metabolism, as reported earlier for another CYP2B6 substrate, bupropion[19]. However, we could not confirm this association and even observed a trend toward lower efavirenz exposure in females using hormonal contraceptives. In addition, the Summary of Product Characteristics of Stocrin/Sustiva® describes that no effect of a single dose of ethinyl oestradiol was observed on the steady-state pharmacokinetics of efavirenz[20].

Third, we performed a pharmacogenetic analysis of the CYP2B6 C1459T polymorphism as a possible explanation of sex and/or racial effects on exposure to the CYP2B6 substrate efavirenz. By amplification of DNA from plasma we were able to detect the CYP2B6 C1459T SNP in 17.1% of the subjects. This is comparable to what other groups have found, although the incidence of this SNP varies among races[16,21-24]. Although this SNP has been associated with low CYP2B6 protein expression, and thus low activity, in human liver samples[16], we did not observe a relationship between this genotype and efavirenz plasma levels. Preliminary data from other groups suggest a relationship for the G516T SNP in CYP2B6 which is present in haplotypes CYP2B6*6 and CYP2B6*7[21,25]. This G516T SNP was more frequently detected in African-Americans than in Caucasians in the ACTG study (20% versus 3%)[25], which is in agreement with a higher risk for toxic efavirenz plasma levels in this subgroup. No information is currently available on any sex effects observed in the AACTG study. In addition, it cannot be excluded that SNPs in CYP3A4 may play a role in efavirenz pharmacokinetics although the metabolism of efavirenz is predominantly handled by 2B6[26].

Finally, differences in co-medication between subjects may cause variability in exposure to efavirenz. However, information on co-medication is usually not provided on application forms for TDM samples. In addition, inhibitors of CYP450 enzymes such

as ritonavir, ketoconazole, and clarithromycin demonstrate none or minimal effects on efavirenz plasma levels[5,20], underscoring the limited contribution of CYP3A4 on efavirenz clearance.

In conclusion, this analysis of our TDM service database has demonstrated a significant effect of sex and race on efavirenz plasma levels, which was independent of differences in body composition, hormonal contraceptive use, or the C1459T SNP in CYP2B6. Physicians should be aware of an increased risk for efavirenz-induced toxicity in females and non-Caucasian patients. Further research, especially in developing countries where females dominate the HIV epidemic, is needed to determine potential factors influencing efavirenz exposure. In this way, treatment with this highly potent drug can be further optimised.

Acknowledgements

We would like to thank the following persons who helped with data collection: Bert Zomer, Minny Meeuwissen, Iman Padmos, Anja van den Berg, Nienke Langebeek, Piet van der Meulen, and Willemien Dorama.

References

1. Panel on clinical practices for treatment of HIV infection, convened by the Department of Health and Human Services DHHS. Guidelines for the use of antiretroviral agents in HIV-1 infected adults and adolescents, March 23 2004. 2004.
2. **Staszewski, S., J. Morales-Ramirez, K. T. Tashima, A. Rachlis, D. Skiest, J. Stanford, R. Stryker, P. Johnson, D. F. Labriola, D. Farina, D. J. Manion, and N. M. Ruiz**. 1999. Efavirenz plus zidovudine and lamivudine, efavirenz plus indinavir, and indinavir plus zidovudine and lamivudine in the treatment of HIV-1 infection in adults. Study 006 Team. N.Engl.J.Med. **341**:1865-1873.
3. **Van Leth, F., P. Phanuphak, K. Ruxrungtham, E. Baraldi, S. Miller, B. Gazzard, P. Cahn, U. G. Lalloo, d. W. van, I, D. R. Malan, M. A. Johnson, B. R. Santos, F. Mulcahy, R. Wood, G. C. Levi, G. Reboredo, K. Squires, I. Cassetti, D. Petit, F. Raffi, C. Katlama, R. L. Murphy, A. Horban, J. P. Dam, E. Hassink, R. van Leeuwen, P. Robinson, F. W. Wit, and J. M. Lange**. 2004. Comparison of first-line antiretroviral therapy with regimens including nevirapine, efavirenz, or both drugs, plus stavudine and lamivudine: a randomised open-label trial, the 2NN Study. Lancet **363**:1253-1263.
4. **Gulick, R. M., H. J. Ribaudo, C. M. Shikuma, S. Lustgarten, K. E. Squires, W. A. Meyer, III, E. P. Acosta, B. R. Schackman, C. D. Pilcher, R. L. Murphy, W. E. Maher, M. D. Witt, R. C. Reichman, S. Snyder, K. L. Klingman, and D. R. Kuritzkes**. 2004. Triple-nucleoside regimens versus efavirenz-containing regimens for the initial treatment of HIV-1 infection. N.Engl.J.Med. **350**:1850-1861.
5. **Smith, P. F., R. DiCenzo, and G. D. Morse**. 2001. Clinical pharmacokinetics of non-nucleoside reverse transcriptase inhibitors. Clin.Pharmacokinet. **40**:893-905.

6. **Pfister, M., L. Labbe, S. M. Hammer, J. Mellors, K. K. Bennett, S. Rosenkranz, and L. B. Sheiner**. 2003. Population pharmacokinetics and pharmacodynamics of efavirenz, nelfinavir, and indinavir: Adult AIDS Clinical Trial Group Study 398. Antimicrob.Agents Chemother. **47**:130-137.
7. **Brundage, R. C., F. H. Yong, T. Fenton, S. A. Spector, S. E. Starr, and C. V. Fletcher**. 2004. Intrapatient variability of efavirenz concentrations as a predictor of virologic response to antiretroviral therapy. Antimicrob.Agents Chemother. **48**:979-984.
8. **Csajka, C., C. Marzolini, K. Fattinger, L. A. Decosterd, J. Fellay, A. Telenti, J. Biollaz, and T. Buclin**. 2003. Population pharmacokinetics and effects of efavirenz in patients with human immunodeficiency virus infection. Clin.Pharmacol.Ther. **73**:20-30.
9. **Marzolini, C., A. Telenti, L. A. Decosterd, G. Greub, J. Biollaz, and T. Buclin**. 2001. Efavirenz plasma levels can predict treatment failure and central nervous system side effects in HIV-1-infected patients. AIDS **15**:71-75.
10. **Joshi, A. S., Barrett, J. S., Fiske, W. D., Pieniaszek, H. J., Ludden, T. M., Bacheler, L. T., and Ruiz, N. M**. Population pharmacokinetics of efavirenz in phase II studies and relationship with efficacy. 39th ICAAC, september 26-29, 1999, San Francisco, California, USA, abstract 1201. 2000.
11. **Stahle, L., L. Moberg, J. O. Svensson, and A. Sonnerborg**. 2004. Efavirenz plasma concentrations in HIV-infected patients: inter- and intraindividual variability and clinical effects. Ther.Drug Monit. **26**:267-270.
12. **Nunez, M., d. R. Gonzalez, L. Gallego, I. Jimenez-Nacher, J. Gonzalez-Lahoz, and V. Soriano**. 2001. Higher efavirenz plasma levels correlate with development of insomnia. J Acquir Immune Defic Syndr **28**:399-400.
13. **Anonymous**. 2003. Optimising TDM in HIV clinical care. A practical guide to performing therapeutic drug monitoring (TDM) for antiretroviral agents. Available: http://www.HIVpharmacology.com. Last visited at May 24, 2004.
14. **Burger, D. M., La Porte, C., Van der Ende, M., Miesen, J., and Koopmans, P**. Gender-related differences in efavirenz pharmacokinetics. 4th International Workshop on Clinical Phamacology of HIV Therapy.March 27-29, 2003, Cannes, France . 2003.
15. **Aarnoutse, R. E., K. J. Grintjes, D. S. Telgt, M. Stek, Jr., P. W. Hugen, P. Reiss, P. P. Koopmans, Y. A. Hekster, and D. M. Burger**. 2002. The influence of efavirenz on the pharmacokinetics of a twice-daily combination of indinavir and low-dose ritonavir in healthy volunteers. Clin Pharmacol Ther. **71**:57-67.
16. **Lang, T., K. Klein, J. Fischer, A. K. Nussler, P. Neuhaus, U. Hofmann, M. Eichelbaum, M. Schwab, and U. M. Zanger**. 2001. Extensive genetic polymorphism in the human CYP2B6 gene with impact on expression and function in human liver. Pharmacogenetics **11**:399-415.
17. **Gandhi, M., F. Aweeka, R. M. Greenblatt, and T. F. Blaschke**. 2004. Sex differences in pharmacokinetics and pharmacodynamics. Annu.Rev.Pharmacol.Toxicol. **44**:499-523.
18. **Spire, B., P. Carrieri, M. A. Garzot, M. L'henaff, T. Group, and Y. Obadia**. 2004. Factors associated with Efavirenz discontinuation in a large community-based sample of patients. AIDS Care **16**:558-564.
19. **Palovaara, S., O. Pelkonen, J. Uusitalo, S. Lundgren, and K. Laine**. 2003. Inhibition of cytochrome P450 2B6 activity by hormone replacement therapy and oral contraceptive as measured by bupropion hydroxylation. Clin.Pharmacol.Ther. **74**:326-333.
20. Stocrin® Summary of Product Characteristics. Merck Sharp & Dohme Limited, Hertfordshire, United kingdom . 2002.
21. **Tsuchiya, K., H. Gatanaga, N. Tachikawa, K. Teruya, Y. Kikuchi, M. Yoshino, T. Kuwahara, T. Shirasaka, S. Kimura, and S. Oka**. 2004. Homozygous CYP2B6 *6 (Q172H and K262R) correlates with high plasma efavirenz concentrations in HIV-1 patients treated with standard efavirenz-containing regimens. Biochem.Biophys.Res.Commun. **319**:1322-1326.

22. **Lamba, V., J. Lamba, K. Yasuda, S. Strom, J. Davila, M. L. Hancock, J. D. Fackenthal, P. K. Rogan, B. Ring, S. A. Wrighton, and E. G. Schuetz**. 2003. Hepatic CYP2B6 expression: gender and ethnic differences and relationship to CYP2B6 genotype and CAR (constitutive androstane receptor) expression. J.Pharmacol.Exp.Ther. **307**:906-922.
23. **Kirchheiner, J., C. Klein, I. Meineke, J. Sasse, U. M. Zanger, T. E. Murdter, I. Roots, and J. Brockmoller**. 2003. Bupropion and 4-OH-bupropion pharmacokinetics in relation to genetic polymorphisms in CYP2B6. Pharmacogenetics **13**:619-626.
24. **Ingelman-Sundberg, M.** 2004. Human drug metabolising cytochrome P450 enzymes: properties and polymorphisms. Naunyn Schmiedebergs Arch.Pharmacol. **369**:89-104.
25. **Haas, D. W., H. J. Ribaudo, R. B. Kim, I. Tierney, G. R. Wilkinson, R. Gulick, D. Clifford, T. Hulgan, and E. Acosta**. 2004. A common CYP2B6 variant is associated with efavirenz pharmacokinetics and central nervous system side effects: AACTG study NWCS214. 11th Conference on Retroviruses and Opportunistic Infections.San Francisco, CA.Feb 8-11, 2004 (abstract 133).
26. **Ward, B. A., J. C. Gorski, D. R. Jones, S. D. Hall, D. A. Flockhart, and Z. Desta**. 2003. The cytochrome P450 2B6 (CYP2B6) is the main catalyst of efavirenz primary and secondary metabolism: implication for HIV/AIDS therapy and utility of efavirenz as a substrate marker of CYP2B6 catalytic activity. J.Pharmacol.Exp.Ther. **306**:287-300.

General discussion

General Discussion

This thesis was written to gain more information on those drug interactions that play a role in the clinical pharmacology of antiretroviral agents. Dose optimizations of several antiretroviral regimens were studied to generate evidence for the implications of these dose adjustments in clinical practice. Studies on gender, race and pharmacogenetics learned us about their roles in variability of drug exposure between patients. This general discussion will connect the presented studies with their background, being antiretroviral treatment of HIV-infected patients. Additionally, attention will be paid to the roles of research ethics and research quality.

Drug interactions

Drug interactions of various backgrounds were studied in **Chapters 1, 2, 4 and 6.** Numerous studies on drug interactions involving antiretroviral agents have been published so far, and subsequently many reviews have summarized all these data. Nevertheless there are still new subjects where additional research needs to be done.
The treatment of HIV-infected patients, that suffer from tuberculosis as well, is very complicated. As long as rifampin and rifabutin are part of the first-line treatment of tuberculosis, multiple potent drug interactions will occur with HAART[1]. This has been described in **Chapter 1**, where two different options for the combination of lopinavir/ritonavir with rifampin were studied. Tuberculosis is seen more often in HIV-infected persons in the developed countries than it was before[2]. We have to realize that in the developing countries the incidence of tuberculosis among HIV-infected persons is dramatically higher[3-5]. When more combinations of tuberculostatic and antiretroviral agents are studied, more insight is gained in the treatment of this group of patients that suffer from this double infection.
Multiple pre-treated patients are another group of patients where drug interactions can be expected more frequently. These patients in general will use more medications for the treatment of HIV infection or concomitant disease than patients that just started HAART while still being in a relative good health condition. In **Chapter 2** a combination of two protease inhibitors, both boosted by ritonavir was studied in multiple pre-treated patients. Including the backbone therapy used by these patients at least five different agents were used to control HIV-infection. Apart from HAART most of these patients had impressive lists of concomitant drugs for the treatment of other diseases. For the medical team surrounding a patient it is very challenging to evaluate the occurrence of drug interactions, possibly leading to inactive therapy or extra toxicity.
In **Chapter 4** the influence of food on the plasma concentrations of indinavir/ritonavir and didanosine was studied. Food-drug interactions are common in the treatment with antiretroviral agents. Some of the antiretrovirals need to be taken with food

to ensure complete absorption[6,7]. Others have to be taken on an empty stomach to avoid a long passage time with risk of decomposition of the drug by the acid from the stomach as a result[8]. The different requirements to food intake of the different drugs of the HAART regime contributes to more complex regimens, which on their turn contribute to lack of compliance[9,10]. Therefore it is important to study the effects of food and be able to make balanced decisions with complexity and compliance on one side and antiviral activity and toxicity on the other.
A once daily regimen including nelfinavir boosted by ritonavir in combination with efavirenz was studied in **Chapter 6**. This combination of drugs leads to complex drug interactions, and therefore plasma concentrations of the different drugs of this regimen were hard to predict. The result of this study is a regimen that might find its place among other user-friendly once daily regimens.

Dose optimization

Dose optimizations were studied in **Chapters 1, 3, 5 and 7.** In individual patients dose optimization following therapeutic drug monitoring is a common intervention when drug concentrations are outside the therapeutic window. In this way therapeutic drug monitoring helps to individualize drug dosages[11].
In several situations, like the occurrence of drug interactions, inactivity or toxicity, or when dose frequency is altered, data collection on dose optimization in a study is indicated. The reason for this is to prevent individual patients from the iterative process of dose adjustments following therapeutic drug montoring.
Drug interactions resulting in altered pharmacokinetic profiles, compel for dose adjustment of the object drug. In **Chapter 1** two different adjusted doses of lopinavir/ritonavir were studied in combination with rifampin in healthy volunteers. The potent drug interaction between these drugs were already known, however no solution was available[12]. After the performance of this trial we can give advice with regard to plasma lopinavir concentrations and side effects that are expected.
Lower doses of indinavir were studied in healthy volunteers as described in **Chapter 5**. This study provided pharmacokinetic data for a dose reduction that was already adopted in the treatment of patients experiencing toxicity[13,14]. The regimens studied here are currently being evaluated in patients, to diminish toxicity without turning in antiviral activity. An additional advantage of these dose reductions from 800/100 mg indinavir/ritonavir BID to 600/100 mg BID or even 400/100 mg BID is the reduction of medication costs.
A dose adjustment from 1250 mg nelfinavir BID to 1500 mg BID is common in patients with low nelfinavir plasma levels. Nevertheless no data existed whether or not such a dose adjustment resulted in the desired effect. **Chapter 7** describes that this dose adjustment was successful in only a minority of patients. This illustrates that it is necessary to evaluate the effect of dose adjustments in clinical practice.

Another example of this observation was found in the development of a once daily regimen containing lopinavir/ritonavir 800/200 mg, studied in **Chapter 3**. In this study in patients, a lower threshold for lopinavir trough concentrations was defined to ensure antiviral activity. Subsequently, patients with plasma concentrations below this value were subjected to a dose increase. It was found that these dose adjustments were not likely to be successful.

Gender, race and pharmacogenetics

There are no two patients that are the same, which is important to realize in the search for differences between groups of patients. Two studies in this thesis, described in **Chapters 8 and 9**, were undertaken to explain interpatient variability in nevirapine and efavirenz plasma concentrations. In these studies common parameters like gender, race and genetics were studied to group patients with plasma concentrations different from the mean. In clinical practice data from these studies can help to evaluate individual patients. The occurrence of toxicity or the absence of activity can then sometimes be anticipated, which eases clinical management of patients.

Emergence of research questions

The study of drugs in human beings is an interesting part of drug development. After the initial phases of synthesizing new compounds with possible pharmacological effects *in-vitro* tests follow. Positive results in *in-vitro* tests will be followed by tests in animals and finally the new compound is tested in humans. When this sequence of studies results in satisfying data the new drug will be registered in a certain dose regimen for a certain indication.
Although this registration by the authorities is the result of a critical evaluation, questions may raise when the drug is used in practice in a patient population, that differs from the study group. These patient populations generally will differ from the study populations used to resemble data for registration, with regard to age, gender and co-morbidity and number of patients. When more patients are treated with the new drug, new properties will be discovered, that were unknown until then. These properties can be related to effectivity, drug interactions and adverse events.
The combinations of several drugs in the treatment of HIV-infected persons make the chance to encounter problems even higher. The studies presented in this thesis originated from problems and questions encountered during the treatment of HIV-infected persons, with already licensed drugs.

Objectives for clinical research

Questions derived from the treatment of patients with certain drugs are not yet fit for medical research. To be able to answer a certain question it is necessary to translate it into an objective for a study. It may be evident that this is a very crucial step in the early development of a new study. Performing a clinical study is an expensive and laborious process. It is very important to obtain as much information from the study as possible. On the other hand one should realize that answering multiple questions in one trial is challenging. Multiple objectives compel large study populations, sample sizes, which is sometimes hard to realize, for reasons of capacity and costs.

The calculated sample size is a result of the anticipated effect of the intervention studied and additional factors like precision of measurement and the desired precision of the answer. If the sample size is too small it will be impossible to answer the objectives with sufficient statistical power.

In the study protocol, which describes the course of the study, the objectives should clearly be described. The objectives are primary or secondary according to the importance of the objective and the burden of proof the researcher expects to find. The objectives of the study will clearly direct the design of the study. For example, studies like in **Chapters 2 and 3**, evaluating long term activity of an antiretroviral regimen will not succeed when the follow up is limited to 4 weeks. The objectives of the study will also direct the choice for a study population.

Study population

When the objectives are clearly defined time has come to select a proper study population. The study population included in clinical research can consist of patients or healthy volunteers. A well-defined set of inclusion and exclusion criteria will always be needed to select a subgroup as necessary.

If disease related parameters need to be studied like viral loads and CD4 cell counts or long-term adverse events, HIV-infected patients need to be studied. For these reasons patients were studied in **Chapters 2 and 3**. Data sets on larger groups of subjects as needed in **Chapters 8 and 9** lead to the choice for patients as well. It would have been very aggravating to expose these numbers of healthy volunteers to the use of drugs. Short-term data collections were needed on pharmacokinetics of one or more (interacting) agents in **Chapters 1, 4, 5 and 6**, which could be studied best in healthy volunteers. These studies technically could have been performed in patients as well, however we could not guarantee antiviral activity of the regimens studied.

Ethics in clinical research

"Medical research involving human subjects should only be conducted if the importance of the objective outweighs the inherent risks and burdens to the subject. This is especially important when the human subjects are healthy volunteers" [15].

This is a statement from the Declaration of Helsinki and illuminates an important issue in the development of a clinical study with regard to the patients or healthy volunteers. Healthy volunteers, unlike patients will not obtain medical benefit from the treatment offered in the study. We have to realize that study subjects are exposed to risks, for the potential benefit of other human beings. A risk-benefit assessment helps the researcher to find the optimum between the risks for the study subjects and the benefits for society.

Risk management in clinical research

Risks for participants to clinical research can be estimated by determining probability, magnitude and duration of harm. With knowledge of potential harms, risks must be minimized within the context of designing and conducting a valuable study. A way to minimize subject risks is the definition of a set of inclusion and exclusion criteria that will rule out subjects that are at increased risk for harm to be expected from the study[16]. Such measures will result in less drop out of study subjects, which is a positive effect for both study subjects and the study itself, as statistics will not be valuable if there are too few subjects left.

The study described in **Chapter 1** was a study in healthy volunteers. The subjects in this study were treated with lopinavir/ritonavir combined with rifampin in the second part of the study. The dosage of lopinavir/ritonavir was increased in the second part of the study. In total 12 of 32 subjects dropped out of the study due to adverse events and liver enzyme elevations. Although this high rate of adverse events occurred, risk management succeeded in this particular study. Study medication was stopped in those subjects that reached toxicity grades as pre-defined in the study protocol. After study medication was discontinued in these subjects' adverse events disappeared and liver enzymes normalized. In general, less adverse events or toxicities are accepted in a healthy volunteer study in comparison to the treatment of patients. This is due to the fact that healthy volunteers will not experience medical profit from the treatment. Patients suffering from adverse events can still experience medical profit, especially when there are no other treatment options left.

Another example of risk management in a healthy volunteer study is found in **Chapter 4**. It was found that the objectives of this study could be answered by a single dose study. This meant that the subjects had to takes single dosages on four consecutive study days only. It may be evident that this resulted in less medication related risk compared to a two week multiple dose study.

In the development and execution of a clinical trial five different complementary actors, responsible for the risk management of study subjects, can be distinguished[17]. Each of these five actors has its own role and challenge in this risk management. First, the investigators need to make responsible decisions while developing or executing the study. Detailed knowledge helps the investigators to balance risk and benefit in these processes. In an undesirable situation the investigator may have conflicts of interest of scientific or financial nature, which should be avoided whenever possible. Secondly, Institutional Review Boards (IRBs) will evaluate the scientific and ethical integrity of

the study protocol. It is important that the IRB has members that represent a broad spectrum of scientific specialisms. Thirdly, there are bodies other than IRBs ensuring regulatory compliance and responsible research. These bodies however fulfil their function with more distance from the study protocol and the researcher. Therefore these bodies evaluate the study protocols at a less detailed level, resulting in less power to recognize an unbalanced risk benefit ratio. Fourthly, research sponsors have a responsibility to evaluate and adjust the safety of the trial, but suffer from financial conflicts of interest. Fifthly, monitoring bodies and committees can assess the safety of the trial while being executed. These bodies should operate independently under recognized guidelines and have the power to improve safety of the trial or in extreme situations of harm to stop a trial prematurely.

Quality in clinical research

Good clinical practice (GCP) is important in the performance of clinical studies. As pointed out above it is in the interest of both the participants of a study and society that is requesting a study, that quality of clinical research is guaranteed. Quality in clinical research was an important reason to explore different ways of working in the different studies in this thesis. We learned from our cooperation with a clinical research organisation and study monitors. In the case of the study presented in **Chapter 1** the combined measures on quality control supported submission of the study report to the Food and Drug Administration (FDA).

The clinical research organisation

The healthy volunteer study presented in **Chapter 1** and later the study presented in **Chapter 6** were the first studies that we conducted in cooperation with a clinical research organisation (CRO). The CRO executed the practical part of these studies, including recruiting healthy volunteers, examining their medical status and admitting the healthy volunteers for drug administration and blood sampling. We developed the study protocols and measured the drug concentrations in blood samples obtained from the healthy volunteers in our own laboratory. This cooperation lead to substantial more professionalism in the conduct of those studies. Both these studies have numbers of participants that are larger than most of the studies we had performed before. At that time our own setting did not allow us to perform studies with 24 to 32 participants, as capacity was not big enough.

Apart from being able to execute the study in one group with up to 32 healthy volunteers we learned from the collaboration with the clinical research organisation. We prepared trial documents, including study protocol, case report form (CRF), study report and several other documents, in collaboration with the CRO. There is an important coherence between all these documents and it is important to recognize this coherence to be able to make them user-friendly. This coherence starts with the

uniform graphical design of the documents, but also includes the format of data entry in the different documents. Coherence also means that all documents have to be consistent with each other. Consistent documents help prevent errors possibly made by the different members of the team working on a study. Chain quality improves by such measures and therefore the results of the study are even more valuable.
After performing the mentioned studies with the CRO more studies followed in this cooperation. However, as total study costs are higher in such cooperation there will be need to perform studies in our own setting as well. Nevertheless we will incorporate the knowledge from this fruitful cooperation in future research.

Study monitoring and audit
A third party in performing a clinical study can help control quality. We have experience with third parties in the form of study monitors and auditors, both contributing to quality control in their own way.
A study monitor was appointed by the sponsor of the studies presented in **Chapters 1 and 6**. The study monitor is responsible for controlling data entry and visiting the study site. The study monitor raises questions comprising missing or invalid data from the case report forms.
An internal audit is performed by the CRO to control for deviations from the study protocol and standard operating procedures while performing the study. The auditor pays attention to the procedures followed and their execution in practice by the personnel. The auditor reports deviations when necessary followed by feedback to prevent repetition of the deviation.

Future aspects

We have to keep in mind that a good study design, followed by a perfectly executed trial and a robust statistical analysis alone will not result in meaningful data, if knowledge and research philosophy, with regard to the research area, are lacking. The studies presented in this thesis may lead to better antiretroviral treatment in patients. Nevertheless ongoing research is necessary, as new antiretroviral agents will become available with their own characteristics. This will be an ongoing process as long as there is no cure for the infection with HIV. Since the beginning of the HIV pandemic, research has brought major improvements in the life expectancy of HIV-infected patients. Although this development gives hope, more than 90% of HIV-infected persons in the world are living in developing countries and still have no access to treatment. We have to realize that for our studies to be meaningful, global access to care is urgent.

References

1. **Piscitelli, S. C. and K. D. Gallicano.** 2001. Interactions among drugs for HIV and opportunistic infections. N.Engl.J.Med. **344**:984-996.
2. **Centers for Disease Control and Prevention.** 1998. Prevention and treatment of tuberculosis among patients infected with human immunodeficiency virus: principles of therapy and revised recommendations. Morb.Mortal.Wkly.Rep. **47**(RR-20), 1-58.
3. **Bowen, E. F., P. S. Rice, N. T. Cooke, R. J. Whitfield, and C. F. Rayner.** 2000. HIV seroprevalence by anonymous testing in patients with Mycobacterium tuberculosis and in tuberculosis contacts. Lancet **356**:1488-1489.
4. **Msamanga, G. I. and W. W. Fawzi.** 1997. The double burden of HIV infection and tuberculosis in sub-Saharan Africa. N.Engl.J.Med. **337**:849-851.
5. **Schluger, N. W.** 1999. Issues in the treatment of active tuberculosis in human immunodeficiency virus-infected patients. Clinical Infectious Diseases **1999**:130-135.
6. Viracept® Summary of Product Characteristics. 2001. Roche Registration Limited, Hertfordshire, United Kingdom.
7. Kaletra® Summary of Product Characteristics. 2000. Abbott, Chicago, IL, USA.
8. Videx EC® Summary of Product Characteristics. 2003. Bristol-Myers Squibb B.V., Woerden, The Netherlands.
9. **Mehta, S., R. D. Moore, and N. M. H. Graham.** 1997. Potential factors affecting adherence with HIV therapy. AIDS **11**:1665-1670.
10. **Stone, V. E.** 2001. Strategies for optimizing adherence to highly active antiretroviral therapy: lessons from research and clinical practice. Clin.Infect.Dis. **33**:865-872.
11. **Aarnoutse, R. E., J. M. Schapiro, C. A. Boucher, Y. A. Hekster, and D. M. Burger.** 2003. Therapeutic drug monitoring: an aid to optimising response to antiretroviral drugs? DRUGS **63**:741-753.
12. **Bertz, R., A. Hsu, W. Lam, L. Williams, C. Renz, M. D. Karol, S. Dutta, A. Carr, Y. Zhang, Q. Wang, S. Schweitzer, C. Foit, A. Andre, B. Bernstein, G. Granneman, and E. Sun.** 2000. Pharmacokinetic interactions between lopinavir/ritonavir (ABT-378/r) and other non-HIV drugs. 5th International Congress on Drug Therapy in HIV Infection.Glasgow, UK, October 2000. Abstract P291.
13. **Lamotte, C., Peytavin, G., Perre, P., Chavanet, P., Truchis, d. P., Gilquin, J., Winter, C., Katlama, C., and Farinotti, R.** Increasing adverse events with indinavir dosages and plasma concentrations in four different ritonavir-indinavir containing regimens in HIV-infected patients. 8th Conference on Retrovirus and Opportunistic Infections, Chicago, Ill, February 4-8, 2001, abstract 738 . 2001.
14. **Ghosn, J., C. Lamotte, H. Ait-Mohand, M. Wirden, R. Agher, L. Schneider, F. Bricaire, C. Duvivier, V. Calvez, G. Peytavin, and C. Katlama.** 2003. Efficacy of a twice-daily antiretroviral regimen containing 100 mg ritonavir/400 mg indinavir in HIV-infected patients. AIDS **17**:209-214.
15. **World Medical Association Declaration of Helsinki:** ethical principles for medical research involving human subjects. 2000. JAMA **284**:3043-3045.
16. **Miller, F. G.** 2003. Ethical issues in research with healthy volunteers: risk-benefit assessment. Clin. Pharmacol.Ther. **74**:513-515.
17. **Stein, C. M.** 2003. Managing risk in healthy subjects participating in clinical research. Clin. Pharmacol.Ther. **74**:511-512.

Summary

In this thesis the clinical pharmacology of antiretroviral agents has been approached with a focus on drug interactions and dose optimization. Antiretroviral drugs are in use for the treatment of HIV-infected persons. Clinical pharmacology is the study of the relationship between concentration of the drugs in blood and their efficacy and toxicity. This thesis comprises research with the different antiretroviral drugs.

In **Part I** of the thesis three studies with lopinavir are presented. In **Chapter 1** blood concentrations of lopinavir after adjusted doses of lopinavir/ritonavir 800/200 mg or 400/400 mg twice daily with rifampin and after normal doses of lopinavir/ritonavir 400/100 mg twice daily alone were compared. The first choice tubercolustatic drug rifampin accelerates the breakdown, metabolism, of lopinavir. For this reason lopinavir combined with rifampin leads to plasma concentrations of lopinavir, which are too low to be antiretroviral effective. Lopinavir is always combined with ritonavir as ritonavir positively influences lopinavir plasma concentrations. It appeared that the higher doses of lopinavir/ritonavir may allow concomitant use of rifampin. Nevertheless plasma concentrations of lopinavir need to be checked in patients to assure effective therapy.

As described above, ritonavir is used to improve, to boost, plasma concentrations of lopinavir. This effect can be used for other protease inhibitors like saquinavir, indinavir and nelfinavir as well. In **Chapter 2** ritonavir was used to boost both lopinavir and saquinavir. Seven patients that failed several other antiretroviral cocktails were given lopinavir/ritonavir in a dose of 400/100 mg twice daily, together with 1000 mg saquinavir twice daily. In addition several other drugs were used. The plasma concentrations of lopinavir and saquinavir were comparable with literature data. This indicated that ritonavir was capable to boost plasma concentrations of lopinavir and saquinavir simultaneously. The tolerability of the regimen was good and efficacy was encouraging.

In the treatment of HIV-infected persons there is an increasing interest in antiretroviral cocktails that can be dosed once daily rather than twice daily. For lopinavir/ritonavir, only sparse data are available on plasma concentrations and the differences between patients after once daily administration. **Chapter 3** presents a study in 20 HIV-infected persons treated with lopinavir/ritonavir 800/200 mg once daily. Plasma concentrations of lopinavir as well as the effect of dose modifications in case of ineffective plasma concentrations were studied. Once daily administration of lopinavir/ritonavir resulted in plasma concentrations of lopinavir, which were on average similar compared to twice-daily administration, with the exception of a somewhat lower plasma concentration just before the next dose was given. Therapeutic drug monitoring may be helpful in identifying patients with lower-than-expected LPV exposure. However, dose modifications did not lead to C_{trough} levels above 1.0 mg/L in the majority of the patients in this study.

In **Part II** of this thesis two studies with the protease inhibitor indinavir are presented. In **Chapter 4** the combination of indinavir/ritonavir with didanosine enteric-coated (EC) was studied. Didanosine EC should be taken on an empty stomach, but the once

daily combination of indinavir/ritonavir can best be taken with food. Because these drugs are frequently included in 1 regimen, the food effects on the pharmacokinetics were evaluated. In this study, the regimen dosing didanosine EC 400 mg + indinavir/ritonavir 1200/400 mg once daily with breakfast indicated no decrease in the amount of absorption for either didanosine and indinavir and that this regimen could be administered with food. Reduced dosages of twice daily indinavir boosted by low-dose ritonavir were studied in **Chapter 5** to assess the pharmacokinetics and tolerability in healthy volunteers. Indinavir/ritonavir 400/100 mg twice daily resulted in significant lower indinavir exposure, with three out of 15 subjects revealing C_{min} values below the recommended threshold for wild-type virus of 0.10 mg/l. Tolerability, however, was lower in the 600 mg indinavir group. Therapeutic drug monitoring in the individual patient appears to be necessary to guarantee appropriate drug levels and simultaneously minimize toxicity.

Part III of the thesis presents two studies with the protease inhibitor nelfinavir. In **Chapter 6** the effect of efavirenz on the pharmacokinetics and tolerability of once daily nelfinavir/ritonavir was evaluated in healthy subjects. A once daily nucleoside-sparing regimen can prevent mitochondrial toxicity, overcome viral resistance and improve compliance. Nelfinavir/ritonavir given together with efavirenz resulted in a 48% higher mean C_{24} concentration for nelfinavir, and the sum of nelfinavir and M8 C_{24} concentrations was 0.99 mg/L. The studied regimens were well tolerated. Efavirenz exposure in this study was similar to that reported previously, and therefore can be used effectively in combination with ritonavir and nelfinavir. In **Chapter 7** a nelfinavir concentration ratio (CR) <0.90 was observed in 56 patients receiving 1250 mg twice daily. Such low nelfinavir plasma concentrations have been associated with failure of therapy. In 38 patients the dosage was adjusted to 1500 mg twice daily, in 18 patients no adjustment was done. Dose adjustment to 1500 mg twice daily resulted in a CR >0.90 in only 45% of patients. For patients with an initial CR <0.52 this was 7%. From this study it was concluded that dose adjustments were only successful in a minority of patients.

In **Part IV** non-nucleosides were the subject of research in two studies. In **Chapter 8** interpatient variability of nevirapine is subject of study. The literature suggests gender and ethnicity as possible causes for interpatient variability of pharmacokinetics. In this study the association of ethnicity, gender and other demographic factors with nevirapine plasma concentrations were evaluated. Higher nevirapine levels were seen in non-Caucasian versus Caucasian patients, and higher average nevirapine levels were observed in female Caucasians compared to male Caucasians. As a result physicians should be alert for a higher risk for toxicity when treating females or non-Caucasian patients with nevirapine. In **Chapter 9** the effects of gender and ethnicity on the pharmacokinetics of efavirenz were studied. The pharmacokinetics of efavirenz is characterized by large interpatient variability. In a preliminary analysis of patients from our Therapeutic drug monitoring service, significantly higher efavirenz plasma levels were observed in females. Sex and race are important factors in determining the interpatient variability in efavirenz plasma levels. This effect is not caused by

low CYP2B6 activity as determined by single nucleotide polymorphism analysis. Physicians should primarily be alert for signs of efavirenz-induced toxicity in females and non-Caucasian patients.

In the **General Discussion** a connection between the presented studies and their background, being antiretroviral treatment of HIV-infected patients, is made. Additionally, attention is paid to the roles of research ethics and research quality. The studies presented in this thesis may lead to better antiretroviral treatment in patients. Nevertheless ongoing research is necessary, as new antiretroviral agents will become available with their own characteristics. This will be an ongoing process as long as there is no cure for the infection with HIV. Since the beginning of the HIV-pandemic, research has brought major improvements in the life expectancy of HIV-infected patients. Although this development gives hope, more than 90% of HIV-infected persons in the world are living in developing countries and still have no access to treatment. We have to realize that for our studies to be meaningful, global access to care is urgent.

Samenvatting

In dit proefschrift wordt de klinische farmacologie van antiretrovirale geneesmiddelen benaderd met een focus op geneesmiddelinteracties en dosisoptimalisatie. Antiretrovirale geneesmiddelen worden gebruikt voor de behandeling van patiënten die met HIV zijn geïnfecteerd. Klinische farmacologie is de studie naar de verbanden tussen concentraties van geneesmiddelen in het bloed en hun effectiviteit en toxiciteit. Dit proefschrift omvat onderzoek met verschillende antiretrovirale geneesmiddelen.
In **Deel I** van het proefschrift worden drie studies met lopinavir gepresenteerd. In **Hoofdstuk 1** worden plasma concentraties van lopinavir na aangepaste doseringen lopinavir/ritonavir 800/200 mg of 400/400 mg tweemaal daags met rifampicine en na normale doseringen lopinavir/ritonavir 400/100 mg tweemaal daags zonder rifampicine vergeleken. Het eerste keus geneesmiddel voor de behandeling van tuberculose, rifampicine, versnelt de afbraak, het metabolisme, van lopinavir. Om deze reden leidt de combinatie van lopinavir en rifampicine tot plasma concentraties van lopinavir, die te laag zijn om effectief te zijn tegen HIV. Lopinavir wordt altijd gegeven in combinatie met ritonavir, omdat ritonavir de plasma concentraties van lopinavir positief beïnvloedt. Uit de studie bleek dat de hogere doseringen lopinavir/ritonavir het gebruik van rifampicine mogelijk maken. Desalniettemin zal het nodig zijn de lopinavir plasma concentraties te controleren om effectieve therapie te verzekeren. Zoals hierboven beschreven, wordt ritonavir gebruikt om concentraties van lopinavir te verbeteren; te boosten. Dit effect kan ook voor andere proteaseremmers zoals saquinavir, indinavir en nelfinavir gebruikt worden. In **Hoofdstuk 2** wordt ritonavir gebruikt om zowel lopinavir als saquinavir te boosten. Zeven HIV-patiënten die gefaald hadden op eerdere antiretrovirale cocktails kregen lopinavir/ritonavir in een dosering van 400/100 mg tweemaal per dag, samen met 1000 mg saquinavir tweemaal per dag. Verder werden nog diverse andere geneesmiddelen gebruikt. De plasma concentraties van lopinavir en saquinavir waren vergelijkbaar met data uit de literatuur. Dit gaf aan dat ritonavir in staat is om de plasma concentraties van lopinavir en saquinavir gelijktijdig te boosten. De medicatie werd goed verdragen en de effectiviteit was bemoedigend. Er is een groeiende belangstelling voor antiretrovirale cocktails die éénmaal daags ingenomen kunnen worden in plaats van tweemaal daags, vanwege het gemak voor de patient. Voor lopinavir/ritonavir zijn er beperkte data beschikbaar over de verschillen tussen patiënten na éénmaal daagse dosering. **Hoofdstuk 3** omvat een studie in 20 HIV-patiënten die behandeld werden met lopinavir/ritonavir 800/200 mg éénmaal daags. Zowel de lopinavir plasma concentraties als het effect van dosisaanpassingen in het geval van subtherapeutische plasma concentraties werden bestudeerd. Eenmaal daagse inname van lopinavir/ritonavir resulteerde in lopinavir plasma concentraties, die gemiddeld genomen vergelijkbaar waren met tweemaal daagse inname, uitgezonderd een iets lagere plasma concentratie op het moment net voor de volgende dosering. Therapeutic drug monitoring kan behulpzaam zijn bij het identificeren van patiënten met lopinavir plasma concentraties die lager zijn dan

verwacht. Desondanks leidde het aanpassen van de dosering in een meerderheid van de patiënten in deze studie niet tot een dalspiegel hoger dan 1,0 mg/l.

In **Deel II** van dit proefschrift worden twee studies met de proteaseremmer indinavir gepresenteerd. In **Hoofdstuk 4** wordt de combinatie van indinavir/ritonavir met didanosine enteric-coated (EC) bestudeerd. Didanosine EC moet op een nuchtere maag ingenomen worden, terwijl de éénmaal daags te doseren combinatie van indinavir met ritonavir bij voorkeur met voedsel moet worden ingenomen. Omdat deze geneesmiddelen frequent worden gecombineerd in een cocktail, werden de voedseleffecten op de farmacokinetiek geëvalueerd. Deze studie laat zien dat de combinatie van didanosine EC 400 mg éénmaal daags + indinavir/ritonavir 1200/400 mg éénmaal daags, gegeven met ontbijt, geen afname in de opname van didanosine en indinavir gaf. De medicatie kan dus met voedsel worden ingenomen. Lagere doseringen indinavir tweemaal daags samen met een lage dosering van het boosting ritonavir worden bestudeerd in **Hoofdstuk 5** om de farmacokinetiek en verdraagbaarheid in gezonde vrijwilligers te testen. Indinavir/ritonavir 400/100 mg tweemaal daags gaf een significant lagere indinavir blootstelling, waarbij 3 van de 15 deelnemers minimale plasmaconcentraties hadden die lager waren dan de aanbevolen grenswaarde van 0,10 mg/l. De verdraagbaarheid was echter lager na 600 mg indinavir. Therapeutic drug monitoring lijkt nodig te zijn voor de individuele patiënt om goede bloedspiegels te kunnen garanderen en tegelijkertijd toxiciteit te minimaliseren.

Deel III van het proefschrift presenteert twee studies met de proteaseremmer nelfinavir. In **Hoofdstuk 6** wordt het effect van efavirenz op de farmacokinetiek van éénmaal daags nelfinavir/ritonavir geëvalueerd in gezonde vrijwilligers. Een éénmaal daags nucleoside-sparend regime kan mitochondriale toxiciteit voorkomen, virale resistentie doorbreken en therapietrouw verhogen. Nelfinavir/ritonavir samen met efavirenz resulteerde in een 48% hogere gemiddelde nelfinavir bloedspiegel 24 uur na inname en de som van de nelfinavir en M8 24 uurs spiegels was 0,99 mg/l. De bestudeerde regimes werden goed verdragen. Efavirenz-blootstelling was in deze studie vergelijkbaar met wat eerder gerapporteerd was en efavirenz kan daarom effectief gebruikt worden in combinatie met ritonavir en nelfinavir. In **Hoofdstuk 7** wordt in 56 patiënten die 1250 mg nelfinavir tweemaal daags kregen een concentratie ratio (CR) <0.90 gezien. Dergelijk lage nelfinavir plasmaspiegels zijn geassocieerd met therapie-falen. Bij 38 patiënten werd de dosering aangepast naar tweemaal daags 1500 mg en bij 18 patiënten werd geen aanpassing gedaan. De dosisaanpassingen naar 1500 mg tweemaal daags resulteerden in een CR > 0.90 in slechts 45% van de patiënten. Voor patiënten met een initiële CR <0.52 was dit 7%. Uit deze studie kan worden geconcludeerd dat dosisaanpassingen slechts in een minderheid van patiënten succesvol is.

In **Deel IV** zijn de non-nucleosides het onderwerp van onderzoek in twee studies. In **Hoofdstuk 8** is de variabiliteit in farmacokinetiek van nevirapine tussen patiënten het onderwerp van het onderzoek. De literatuur laat geslacht en etniciteit zien als mogelijke oorzaken van variabiliteit. In deze studie werd de associatie tussen etniciteit, geslacht en andere demografische factoren onderzocht. Hogere nevirapine-spiegels werden

gezien in niet-caucasische patiënten vergeleken met caucasische patiënten en hogere spiegels werden gezien in vrouwelijke caucasische patiënten vergeleken met mannelijke caucasische patiënten. Om deze reden zouden artsen zich ervan bewust moeten zijn dat er een hoger risico op toxiciteit is wanneer vrouwen of niet caucasische patiënten met nevirapine behandeld worden. In **Hoofdstuk 9** worden de effecten van geslacht en etniciteit op de farmacokinetiek van efavirenz bestudeerd. De farmacokinetiek van efavirenz wordt gekenmerkt door een grote variabiliteit tussen patiënten. In een preliminaire analyse van patiënten uit onze Therapeutic drug monitoring service, werden significant hogere efavirenz plasmaspiegels gezien in vrouwen. Geslacht en ras zijn belangrijke factoren bij het bepalen van de variabiliteit van efavirenz tussen patiënten. Dit effect wordt niet veroorzaakt door lage CYP2B6 activiteit zoals bepaald met single nucleotide polymorfisme analyse. Artsen zouden oplettend moeten zijn voor tekenen van efavirenz geïnduceerde toxiciteit bij vrouwen en niet-caucasische patiënten.

In de **Algemene Discussie** wordt een verband gelegd tussen de gepresenteerde studies en hun achtergrond, namelijk de behandeling van HIV patiënten. Aanvullend wordt er aandacht besteed aan de rol van onderzoeksethiek en kwaliteit van onderzoek. De studies zoals gepresenteerd in dit proefschrift kunnen leiden tot een betere antiretrovirale behandeling van patiënten. Desalniettemin is verdergaand onderzoek nodig, omdat nieuwe antiretrovirale middelen, met hun eigen karakteristieken, beschikbaar zullen komen. Dit zal een voortdurend proces zijn zolang er geen echte genezing van HIV mogelijk is. Sinds het begin van de HIV-pandemie heeft onderzoek geleid tot grotere verbeteringen in de levensverwachtingen van HIV-patiënten. Alhoewel deze ontwikkeling hoop geeft, leeft meer dan 90% van de HIV-patiënten in ontwikkelingslanden, zonder toegang tot adequate behandeling. We moeten ons realiseren dat, om onze studies zinvol te laten zijn, wereldwijde toegang tot deze zorg urgent is.

Dankwoord

Tijdens de verdediging van het proefschrift, dat laatste uurtje doctorandus, sta je er ogenschijnlijk alleen voor. Dit staat echter in schril contrast met de werkelijkheid van de jaren werk daarvoor. Tijdens deze jaren heb ik intensief samengewerkt en -geleefd met een groot aantal mensen. Zonder hen was het onmogelijk geweest dit proefschrift te voltooien. Om die reden is hier het dankwoord voor al diegenen die hun steentje hebben bijgedragen, op welke wijze dan ook.

Prof. Dr. Y.A. Hekster, promotor, lieve Chiel, een aantal keer heb je heel verbaasd naar mij gekeken. De eerste keer was dat omdat ik U tegen je zei. Dat was duidelijk niet de bedoeling. Die benaderbaarheid is iets wat jij uitdraagt, jij bent gewoon in de buurt, om mee te denken, te ondersteunen of ideeën te geven. Ik dank je hartelijk voor alle steun en wijze lessen!

Dr. D.M. Burger, co-promotor, lieve David, je bent een man van goud, vanaf het begin van het onderzoek liet jij merken dat wij (destijds Alina en ik als onderzoeksgroentjes) er helemaal bijhoorden. Ik heb erg genoten van de onderzoeksjaren met de vele leerzame momenten. Bedankt ook voor je vertrouwen bij het uitvoeren van de diverse studies, dat gaf mij ook de mogelijkheid de dingen op mijn manier te doen. Ook prive bracht je nieuwe ervaringen in mijn leven. Met jou ging ik voor het eerst van mijn leven naar een voetbalwedstrijd in het bomvolle Goffert stadion. David, reuze bedankt!

Dr. P.P. Koopmans, co-promotor, beste Peter, bedankt dat je me zoveel leerde over de dokterskant van het HIV onderzoek. Het bediscussiëren van de onderzoeksprotocollen met jou was altijd erg leerzaam. De keren samen op reis waren ook steeds gezellig en culinair verantwoord, ik kijk met veel plezier op onze samenwerking terug.

Dit onderzoek was in het geheel niet mogelijk geweest zonder de medewerking van alle gezonde vrijwilligers en patiënten. Zij hebben menige ontbering moeten doorstaan om het verzamelen van alle data mogelijk te maken.

Mede-onderzoekers zijn erg belangrijk voor het welslagen van een onderzoek, omdat je samen aan serieuze dingen kunt werken, maar ook samen lol kunt maken. Beste Alina, inmiddels werk je ergens anders, maar we hebben een fantastische tijd gehad, samen werkend aan het onderzoek. Jij memoreerde in jouw dankwoord al onze samenwerking aan de universiteit, wellicht komt het in de toekomst nog eens zover. Beste Rob, naast het feit dat je een fijne en betrouwbare collega bent, is het met jou ook heerlijk discussiëren over theoretische achtergronden van het onderzoek. Het commentaar dat je eens leverde op één van mijn manuscripten was omvangrijker dan dat van de overige auteurs bij elkaar, hetgeen nogmaals je betrokkenheid onderstreept. Beste Jackie, het was fijn om met je samen te werken en ik wens je ook veel succes in jouw onderzoek. Beste Pieter en Charles, jullie besmuikte opmerkingen over het HIV-onderzoek kwamen natuurlijk voort uit jaloezie, dat geeft niet. Gelukkig heb ik niet naar jullie optimisme over het beschikbaar komen van een vaccin tegen HIV geluisterd. Helaas is dat nog niet beschikbaar en heb ik mooi onderzoek kunnen doen met die "lap-middelen". Pieter, succes met het afronden van je proefschrift! Rafaella

en Hanneke, ik heb weinig met jullie samengewerkt, maar als jullie net zoveel plezier beleven aan het onderzoek als ik dan komt het zeker goed.
De artsen Monique Keuter, Joep van Oosterhout, Denise Telgt, Annemarie Brouwer, Martin Boeree, Patrick Vrijlandt en later ook Sander van Assen en de HIV-verpleegkundigen Marjolein Bosch, Karin Grintjes en Bert Zomer en verder Minny Meeuwissen en Lanny Langendam wil ik danken voor de fijne samenwerking en de hulp bij de diverse studies.
De analisten van het laboratorium hebben met hun vele bloedspiegelmetingen de ruwe data voor het onderzoek verzameld, dank hiervoor. Daarnaast wil ik in het bijzonder Corrien, Noor en Marga bedanken voor het ontwikkelen van de diverse analysemethoden.
De studenten Neeltje Coolen en Martijn van der Leur wil ik bedanken voor hun bijdrage aan de onderzoeken met nevirapine en lopinavir.
Beste Remco, als hoofd van de Apotheek/Klinische farmacie heb je een zeer belangrijke rol gespeeld bij mijn onderzoek. Je trad daarbij niet noodzakelijkerwijs op de voorgrond, maar gaf mij wel de mogelijkheden om mij als onderzoeker nationaal en internationaal te ontwikkelen. Mijn oprechte dank hiervoor.
Beste Ellen, Mieke, Hans, Stephanie en Eveline, als collega-apothekers waren jullie steeds geïnteresseerd in mijn onderzoek, waarvoor ik jullie wil bedanken. De apothekers in opleiding tot ziekenhuisapotheker, Audrey, Nicole en Manon, bedankt voor jullie steun en interesse. Alle overige medewerkers van de apotheek wil ik ook bedanken voor hun interesse. In het bijzonder wil ik Marijcke bedanken voor de hulp bij het verzenden van het proefschrift (en nog zoveel andere dingen!), daarnaast ook Nellie van het bedrijfsburo voor haar altijd aanwezige vrolijkheid. Ook wil ik Ingrid bedanken voor haar geest(ige)elijke steun in de afrondende fase van het proefschrift. Lex en Jan Stegeman, bedankt voor de ondersteuning op het gebied van de computertechniek. Voor de financiele afwikkeling van diverse studies kon ik bij Jan op den Camp terecht, waarvoor dank.
Ook wil ik mijn dank uitspreken naar tal van mensen buiten de muren van de apotheek, die een rol speelden bij één of meerdere van de onderzoeken: Frank Kroon, Emile Schippers en Willemien Dorama uit het LUMC. Ineke van der Ende, Inge Gyssens, Iman Padmos en Marja Bendik uit het EMC. Herman Sprenger, Jeroen Miesen en Piet van der Meulen uit het UMCG. Annemarie Wensing uit het UMCU. Willem Blok uit het Walcheren Ziekenhuis en Robert Kauffmann en Anneke Maat uit het Leyenburg Ziekenhuis.
Zwei Kapitel dieser Dissertation sind in zusammenarbeit mit Jürgen Rockstroh, Jan-Christian Wasmuth und Katrin Schneider erschienen. Die Zusammearbeit war immer schön und hat den Kontakt in jeder Hinsicht gestärkt. Vielen Dank.
Een aantal studies uit dit proefschrift hebben we uitgevoerd samen met Farma Research B.V. in Nijmegen. Dit was een uitermate leerzame en ook plezierige samenwerking. Angela Colbers en Diana Voncken wil ik hiervoor hartelijk danken.
Cooperation with researchers from several drug companies lead to a number of studies presented in this thesis. Therefore I would like to thank Rick Bertz, Karen Wikstrom

and Kevin Niemi from Abbott, Chicago, USA, Michael Stek Jr. from Merck & Co, Whitehouse Station, USA and Santiago Marco Ibanez from F. Hoffman-La Roche, Basel, Switzerland.
De leden van de manuscriptcommissie wil ik danken voor de genomen moeite om mijn manuscript te beoordelen.
Lieve András en Manon, fantastisch dat jullie mijn paranimfen willen zijn, bedankt voor de inspanningen en ondersteuning.
Talloze vrienden en familieleden die hun betrokkenheid toonden wanneer we elkaar spraken. Bedankt voor deze interesse, het is altijd fijn je verhaal weer eens kwijt te kunnen.
András, Arnold, Bas, Marcel en Sicco, ik geniet altijd weer van het samenzijn met jullie, om wat te eten, te drinken, lol te trappen of alles tegelijk! Bedankt voor de vele gezellige en ontspannende uren. Marcel, waar hadden we het nou toch over??
Als je een meisje kiest krijg je de schoonfamilie er gratis bij wordt er dan gezegd. Ik kan zeggen dat ik het bijzonder getroffen heb met jullie! Pieter, Anneke, Roeland en Jolanda, bedankt voor jullie steun en gezelligheid.
Madelon, lieve zus, dank je voor het maken van zo'n mooie omslag voor dit boekje. Ik grapte steeds naar mijn collegas dat ik een witte kaft met zwarte letters wilde, maar dit is toch duizendmaal mooier. René, David en Aila, bedankt dat jullie Madelon de ruimte gaven dit te doen.
Lieve pa en ma, jullie steun en interesse, ook als het even wat minder goed gaat, zijn altijd weer hartverwarmend. Ik hoop dat we nog lang samen zijn.
Lieve Rozemarijn, dank voor je hulp om dit boekje er schitterend uit te laten zien. Je weet dat ik me nu ga storten op mijn volgende levenswerk, en jij weet ook wat dat is. Het zal wat minder wetenschappelijk worden en wat meer samen……ga je mee?

Charles la Porte
februari 2005

Curriculum vitae

Charles la Porte werd op zondagmiddag 3 mei 1975 geboren in Halsteren. Tijdens de middelbare schooltijd nam Charles vioollessen en speelde hij bij het muziekgezelschap HMR in Bergen op Zoom. In 1993 behaalde hij zijn diploma Gymnasium B aan het Juvenaat Heilig Hart in Bergen op Zoom. In september van datzelfde jaar begon hij met de studie Farmacie in Utrecht. Tijdens de studie bleef er ook aandacht voor het vioolspel. Daarnaast groeide uit de wens om een auto te rijden en studentikoze geldnood de interesse om aan auto's te sleutelen. Na de keuzevakken biofarmacie en bioanalyse deed hij een afstudeeronderzoek bij de vakgroep Bioanalyse aan de faculteit Farmacie van de Universiteit Utrecht, onder leiding van Dr. W.J.M. Underberg. Dit leidde tot het doctoraal Farmacie in 1999, gevolgd door de apothekersbul in 2000. Al tijdens het afstudeeronderzoek werd de interesse gewekt om promotieonderzoek te gaan doen. Daarom werd aansluitend gestart met een baan als apotheker-onderzoeker bij de Apotheek/Klinische Farmacie van het Universitair Medisch Centrum St Radboud te Nijmegen. Dit proefschrift is het resultaat van het verrichte onderzoekswerk.

List of publications

Publications in English

C.J.L. la Porte, C.P.W.G.M. Verweij-van Wissen, N. van Ewijk, R.E. Aarnoutse, P.P. Koopmans, P. Reiss, M. Stek Jr, Y.A. Hekster, and D.M. Burger. 2005. Pharmacokinetic interaction study of indinavir/ritonavir and the enteric-coated capsule formulation of didanosine in healthy volunteers. Journal of Clinical Pharmacology. 45: 211-218.

C.J.L. la Porte, E.P. Colbers, R. Bertz, D.S. Voncken, K. Wikstrom, M.J. Boeree, P.P. Koopmans, Y.A. Hekster, and D.M. Burger. 2004. Pharmacokinetics of adjusted-dose lopinavir-ritonavir combined with rifampin in healthy volunteers. Antimicrobial Agents and Chemotherapy. 48: 1553-1560.

C.J.L. la Porte, M.J. Graaff-Teulen, E.P. Colbers, D.S. Voncken, S.M. Ibanez, P.P. Koopmans, Y.A. Hekster, and D.M. Burger. 2004. Effect of efavirenz treatment on the pharmacokinetics of nelfinavir boosted by ritonavir in healthy volunteers. British Journal of Clinical Pharmacology. 58: 632-640.

J.C. Wasmuth, C.J.L. la Porte, K. Schneider, D.M. Burger, and J.K. Rockstroh. 2004. Comparison of two reduced-dose regimens of indinavir (600 mg vs 400 mg twice daily) and ritonavir (100 mg twice daily) in healthy volunteers (COREDIR). Antiviral Therapy. 9: 213-220.

C.J.L. la Porte, J.C. Wasmuth, K. Schneider, J.K. Rockstroh, and D.M. Burger. 2003. Lopinavir/ritonavir plus saquinavir in salvage therapy; pharmacokinetics, tolerability and efficacy. AIDS. 17: 1700-1702.

J.C. Waterval, C.J.L. la Porte, H.R. van 't Hof, J. Teeuwsen, A. Bult, H. Lingeman, and W.J. Underberg. 1998. Development and validation of transient isotachophoretic capillary zone electrophoresis for determination of peptides. Electrophoresis. 19: 3171-3177.

Publications in Dutch

D.M. Burger, M.J. van der Lee, J.A.H. Droste, R.F.A. L'homme, C.J.L. la Porte, en R.E. Aarnoutse, 2005. Met spiegels en vensters meer zicht. Pharmaceutisch Weekblad. 140: 22-25.

P.J.W.S. Vrijlandt, C.J.L. la Porte, D.M. Burger, P.P. Koopmans, en J.A.M. Dekens-Konter. Tenofovir (Viread®). 2003. Pharmaceutisch Weekblad. 138: 676-680.

D.M. Burger, C.J.L. la Porte, A.S. Bergshoeff, J.A.H. Droste, en R.E. Aarnoutse. 2002. Klinisch relevante interacties met anti-HIV middelen. Pharmaceutisch Weekblad. 137: 248-254.

C.J.L. la Porte, D. M. Burger, P. P. Koopmans, en Y. A. Hekster. 2001. Lopinavir + ritonavir (Kaletra®). Pharmaceutisch Weekblad. 136: 935-940.

Forthcoming publications

C.J.L. la Porte, E.F. Schippers, M.E. van der Ende, P.P. Koopmans, W.L. Blok, R.H. Kauffmann, F.P. Kroon, and D.M. Burger. Pharmacokinetics of once-daily lopinavir/ritonavir as part of a regimen also containing two nucleosides administered once daily: the influence of dose modifications. Submitted.

C.J.L. la Porte, and D.M. Burger. Is nelfinavir (NFV) 1500 mg BID an effective intervention for patients on NFV 1250 mg BID who have low NFV exposure? Submitted.

C.J.L. la Porte, M.E. van der Ende, I. Gyssens, W. Miesen, H. Sprenger, P.P. Koopmans, Y.A. Hekster, and D.M. Burger. Predicting factors in interpatient variability of nevirapine pharmacokinetics. In preparation.

D.M. Burger, I van der Heiden, C.J.L. la Porte, M.E van der Ende, P. Groeneveld, C. Richter, P.P. Koopmans, F.P. Kroon, H. Sprenger, J. Lindemans, P. Schenk, and R. van Schaik. Interpatient variability in the pharmacokinetics of the HIV non-nucleoside reverse transcriptase inhibitor efavirenz: the effect of sex, race, and genetics. Submitted.