Safety aspects of HIV-protease inhibitors

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Veiligheids aspecten van HIV-proteaseremmers

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Dieleman JP, Gyssens IC, van der Ende ME, de Marie S, Burger DM. Urological complaints in relation to indinavir plasma concentrations in HIV-infected patients. AIDS 1999;13:473-8.

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Dieleman JP, Sturkenboom MCJM, Jambroes M, Gyssens IC, Weverling GJ, ten Veen JH, Schrey G, Reiss P, Stricker BHCh, on behalf of the ATHENA-study group. Risk factors for urological symptoms in a cohort of users of HIV-protease inhibitor indinavir. The ATHENA-cohort. Arch Intern Med, accepted for publication

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Dieleman JP, Salahuddin S, Hsu YS, Burger DM, Gyssens IC, Sturkenboom MC, et al. Indinavir Crystallization Around the Loop of Henle: Experimental Evidence. J Acquir Immune Defic Syndr 2001;28:9-13.

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Dieleman JP, van Rossum AMC, Stricker BHCh, Sturkenboom MCJM, de Groot R, Telgt D, Blok W, Burger DM, Blijenberg BG, Zietse R, Gyssens IC. Persistent leukocyturia and loss of renal function in a prospectively monitored cohort of HIV-infected patients treated with indinavir. Submitted for publication

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Chapter 1

General introduction

HIV-infection and AIDS

In 1981, the first cases of Acquired Immune Deficiency Syndrome (AIDS) were discovered in gay men. Two years later, the human immunodeficiency virus (HIV) was isolated and recognized as the causative agent of AIDS. Uncontrolled infection with HIV imminently leads to AIDS and ultimately to death. Due to its long latency time until disease manifestation the virus can easily spread unnoticed. A high turn-over and rapid mutation rate lead to continuous viral changes, which have complicated the development of durable effective therapeutic interventions (1).

The natural course of HIV-infection

HIV is a ribonucleic acid (RNA)-containing retrovirus of the lentivirus-subfamily. It has an affinity for CD4 cell receptors, which are mainly present on helper T-lymphocytes of the immune system but also occur on other cells (2). Through these receptors and with the help of chemokine co-receptors (CCR5 and CXCR4) (3-6), viral RNA is released into the cell cytoplasm where it is converted into deoxyribonucleic acid (DNA) by virus-encoded reverse transcriptase (Figure 1).

The resulting proviral DNA is transferred into the cell nucleus and incorporated into the host genome (7). Hence, HIV-infected progeny is generated at each cell division and proviral RNA and viral precursor polyproteins are produced through regular cellular transcription and translation processes. HIV-encoded proteases subsequently cleave the polyprotein precursors, yielding mature core polypeptides and essential enzymes, which are assembled into new infectious HIV-virions (7-10) (Figure 1).

Eventually, infection leads to CD4 cell depletion and impaired immune function effectively leading to AIDS (11). The number of circulating HIV-RNA particles and CD4 positive lymphocytes are used for the monitoring of HIV-infection, representing the degree of viral activity on the one hand and the degree of immune deficiency on the other hand (12-15).

Antiretroviral treatment

The enzymes reverse transcriptase and protease are fundamental to the survival and multiplication of HIV and therefore constitute important targets for antiretroviral treatment. Reverse transcriptase inhibitors were the first agents used for the treatment of HIV-infection (16-18), but failed to significantly change the course of infection (19).

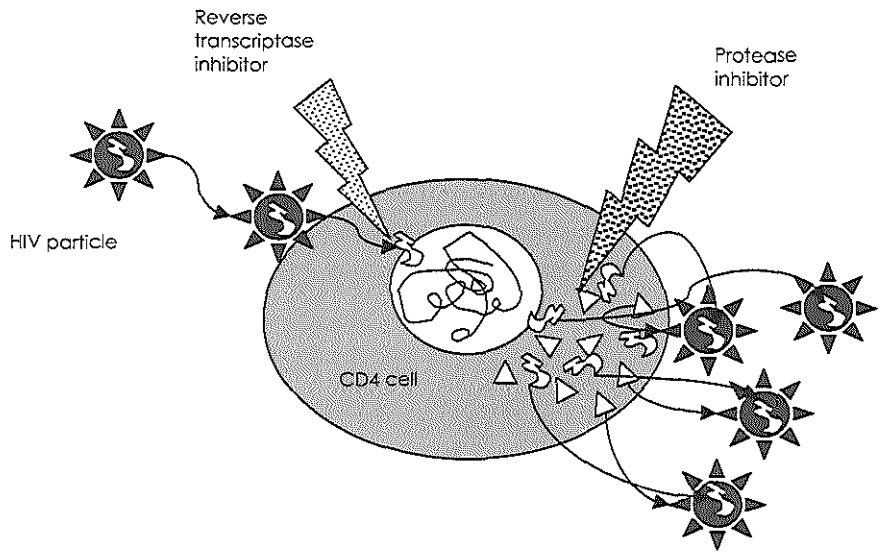

Figure 1 Life-cycle of HIV and action of antiretroviral agents

In 1996 the first HIV-protease inhibitors became available (20-25). Short term studies showed that the combination of reverse transcriptase inhibitors and HIV-protease inhibitors was able to suppress HIV-RNA to undetectable levels and improve the immune system (24, 25).

Optimal treatment of HIV-infection nowadays involves the chronic administration of multiple drugs, which attack the virus from different angles, also referred to as highly active antiretroviral therapy (HAART) (26-28) (Figure 1). HAART usually consists of two nucleoside analogue reverse transcriptase inhibitors (NRTIs) for optimal inhibition of HIV-encoded reverse transcriptase, and an HIV-protease inhibitor. The aim of this treatment is to intercept both the infection of new and the production of new infectious HIV-particles (Figure 1) (9, 29). HAART has shown to inflict a steep reduction in HIV-related morbidity and mortality in populations in which such treatment was made available (30-32). By the turn of the century the focus of care for HIV-infected patients in Western countries had shifted from treating opportunistic diseases and in-hospital care to providing antiretroviral treatment and monitoring HIV-infection in an outpatient clinic setting.

Issues in antiretroviral therapy

An important risk of HAART concerns the selective production of resistant viruses in case of insufficient viral suppression, which eventually may accumulate into treatment resistence and viral breakthrough (1, 9, 15, 33-36). Patients experiencing such treatment failure have

only a limited number of options for treatment switches due to the existence of cross-resistance between antiretroviral agents, especially between agents of the same class (28, 37-41).

Adequate drug concentrations have shown to be essential for an optimal treatment effect. Suboptimal plasma concentrations of HIV-protease inhibitors may fail to achieve sufficient viral suppression and hence facilitate viral resistance (42-44). To reduce the risk of low plasma concentrations, good treatment compliance should be maintained (45-47).

High plasma concentrations, on the other hand, increase the risk of adverse effects (44, 48). Adverse effects constitute a major limiting factor in antiretroviral treatment. Many patients experience gastro-intestinal problems and other adverse effects on a continuous basis. This may eventually influence the willingness and ability to maintain optimal compliance.

Finally, it is well recognized that current HAART regimens are unlikely to achieve complete elimination of HIV because of the existence of HIV-sanctuary sites and latent infected CD4 positive cells (49-53). Sustained viral suppression below the detection limit, therefore, remains the primary goal of HAART (54). Thus, the cornerstone of present HAART is optimal compliance. To facilitate this, improvement of treatment schedules and minimization of adverse effects can play a pivotal role.

HIV-protease inhibitors

HIV-protease inhibitors were introduced worldwide to the market after accelerated registration procedures. This happened under high pressure from the field, because treatment combinations with these agents were the first to show substantial efficacy against HIV-infection. At the time of introduction, however, knowledge regarding long-term effects and safety of this entirely new generation of drugs was sparse and insufficient.

Clinical information about the first HIV-protease inhibitors was based on clinical trials examining relatively small patient populations over a period of one to two years (55-60). These clinical trials were designed to show clinical benefit in terms of HIV-suppression in particular, mostly based on surrogate parameters. Clearly, the relatively small number of selected patients examined over relatively short periods of time (Table 1), was insufficient to uncover the full picture of the risks involved with the use of HIV-protease inhibitors. In addition, clinical trials provided little insight into the severity, risk factors and impact of adverse effects.

Table 1 Product license information on HIV-protease inhibitors

Compound	Saquinavir mesylate	Ritonavir	Indinavir sulphate	Nelfinavir mesylate
Alternative names	Ro 31-8959 Invirase®	ABT-538, Norvir®	MK-639, Crixivan®	AG1343, Viracept®
EMEA approval	4 Oct 1996	27 Aug 1996	4 Oct 1996	22 Jan 1998
Formulation	200 mg hard gel capsules or soft gel capsules	100 mg capsules; 80 mg/mL suspension	200 mg and 400 mg capsules	250 mg tablets
Clinical trials prior to registration	302 patients 24 weeks(55), 978 patients 16 weeks	84 patients 32 weeks(56), 1090 patients 24 weeks(117)	1165 patients 38 weeks(58), 97 patients 24 weeks	65 patients 28 days(118) 33 patients 24 weeks(119)

General characteristics of HIV-protease inhibitors

HIV-encoded protease displays structural similarity to human aspartic proteases, pepsin and renin (8, 10). This knowledge served as a basis for the computer-aided development of HIV-protease inhibitors (7, 61-65), which exert their antiretroviral effect through specific inhibition of viral protease (7, 9, 29, 66).

The first HIV-protease inhibitors which became available were saquinavir, ritonavir and indinavir. Three quarters of a year later, a fourth HIV-protease inhibitor nelfinavir was introduced. Only recently lopinavir and amprenavir have been added to the repertoire, these HIV-protease inhibitors will not be described here. Saquinavir, ritonavir, indinavir and nelfinavir are chemically related compounds (Figure 2). The most important characteristics in the context of this thesis are summarized in table 2. Like many other drugs, HIV-protease inhibitors are given in standard dose frequency schedules. Depending on the type of HIV-protease inhibitor, presence (saquinavir, ritonavir, nelfinavir) (25, 67-70) or absence (indinavir) (71-74) of food around the time of ingestion improves absorption. Once absorbed, HIV-protease inhibitors are subjected to extensive first-pass metabolism (75, 76). The net bio-availability varies from 4% for saquinavir to 60-80% for other HIV-protease inhibitors.

In plasma, HIV-protease inhibitors are highly protein bound (60% for indinavir, >95% for other HIV-protease inhibitors) as a result of which renal clearance through glomerular filtration is generally low. The primary metabolic pathway for HIV-protease inhibitors is oxidation through the liver cytochrome P450 system isoenzyme 3A4 (77-80). None of the resulting metabolites, except for one metabolite of ritonavir and nelfinavir, has antiretroviral activity of any significance (81-83). The short half-life of HIV-protease inhibitors demands frequent dosing regimens ranging from two (ritonavir) to three times daily (other HIV-

protease inhibitors). Typically, plasma concentrations of HIV-protease inhibitors have a high peak (Cmax) and steep decline to a low trough level.

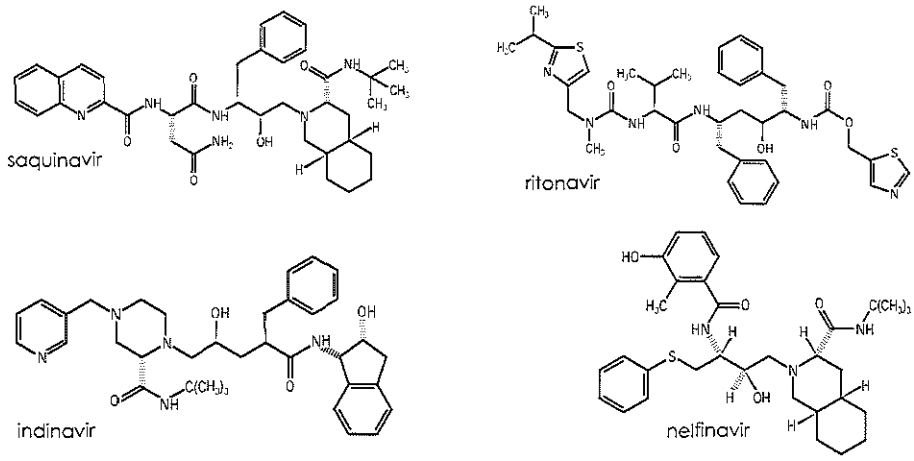

Figure 2 Crystal structure of HIV-protease inhibitors saquinavir, ritonavir, indinavir and nelfinavir (kindly provided by: WINAp Geneesmiddelinformatie, Informatorium Medicamentorum, 's Gravenhage: Koninklijke Nederlandse Maatschappij ter Bevordering der Pharmacie, 2002)

Table 2 Physico-chemical and pharmacokinetic properties and adverse reactions of HIV-protease inhibitors

Compound	Saquinavir mesylate	Ritonavir	Indinavir sulphate	Nelfinavir mesylate
Chemical characteristics	Lipophilic, weak base	Lipophilic, weak base	Hydrophilic, weak base	Lipophilic, weak acid
Protein binding	>98%	98-99%	60%	≥98%
Urine excretion	1%	11%	20%	1-2%
Metabolic route	CYP3A4	CYP3A4/2C9/2C19/2D6, N-glucuronidation	CYP3A4, N-glucuronidation	CYP3A4, (CYP2C19/2D6)
Active metabolites	None	Isopropylthiazole oxidation metabolite (M2)	None	hydroxy-t-butylamide metabolite (M8)
Bio-availability	4%	66-75%	60-65%	>75%
Half life	1-2 hours	3.2 hours	1.8 hours	3.5-5 hours
Time to Cmax	3.8 hours	4 hours	0.8 hour	2-4 hours
Recommended dosing	1200 mg tid with food	Dose escalation day 1-9, 600 mg bid with food	800 mg tid fasting (low gastric pH)	750 mg tid or 1250 mg bid with food
Adverse effects	Diarrhoea, nausea, gastro-intestinal discomfort, CPK ↑	Nausea, vomiting, diarrhoea, circumoral paraesthesia, asthenia, taste perversion, liver enzyme ↑, triglyceride ↑	Nephrolithiasis*, bilirubin ↑	Diarrhoea

* patients are recommended to drink at least 1.5 L extra fluid per day

Due to their dependence on cytochrome P450, HIV-protease inhibitors are susceptible to interactions with other agents that influence this system (84-87) (Table 3). Stimulation of cytochrome P450 leads to a faster clearance, to lower plasma concentrations and therefore to reduced treatment effects, whereas inhibition may lead to adverse effects as a consequence of reduced clearance and elevated plasma concentrations (88, 89). The potential for drug interaction is especially daunting for patients with a more advanced stage of HIV-infection or concurrent diseases, who are more likely to concomitantly use interfering drugs. In order to avoid interactions, the full medication-history of patients should be known. In the Netherlands such information is available through community pharmacies, who keep electronic records of all drug dispensings. Alternatively, the possibility of HIV-protease inhibitor plasma concentration monitoring (83, 90-98) has facilitated the safe use of drug combinations.

Apart from being metabolized by cytochrome P450 enzymes, HIV-protease inhibitors may influence this system themselves (Table 3). Especially ritonavir is known for its strong inhibition of isoenzyme 3A4 (99, 100). This property of ritonavir is used to improve the pharmacokinetic profiles of other HIV-protease inhibitors. By slowing down the metabolism of one HIV-protease inhibitor with a minor dose of ritonavir, dosing frequencies can be reduced to twice daily and even once daily instead of three times daily (99, 101-106).

Characteristics of saquinavir

Saquinavir is a generally well-tolerated HIV-protease inhibitor. It is delivered in 200 mg hard gelatine capsules which have 4% bio-availability if taken together with food (67). The defined daily dose used to be 600 mg three times daily, but has been increased to 1200 mg three times daily in the past years. Presently, a soft gelatine capsule formulation is available, which is claimed to have similar bio-equivalence but higher bio-availability, hence allowing lower dosages (107). Both the hard gel and soft gel capsules are commercially available.

After first being used as a single HIV-protease inhibitor in HAART, saquinavir is now mainly used in combination with ritonavir or nelfinavir, to improve its bio-availability by reducing clearance (106, 108, 109). Treatment with a combination of saquinavir and ritonavir is usually administered in dosages of 400 mg twice daily of each compound.

Characteristics of ritonavir

Ritonavir is available in both liquid formulation (80 mg/mL) and 100 mg capsule formulation, of which the capsules are the most widely used. Although ritonavir has a favorable dosing schedule of 600 mg twice daily as opposed to three times daily, its gastro-intestinal adverse effects, triglyceride elevations and extensive drug interactions (70) limit widespread use. Slow induction of the cytochrome P450 system by ritonavir demands dose escalation from

300 mg twice daily to 600 mg twice daily over a period of nine days, in order to prevent severe adverse effects at the start of treatment.

Ritonavir has become especially popular as a booster of other HIV-protease inhibitor regimens, because of its strong inhibitory effect on cytochrome P450 isoenzyme 3A4. Apart from its effect on cytochrome P450, ritonavir has an inhibitory effect on p-glycoprotein, which acts as a filter for the blood brain barrier and potentially other barriers to sanctuary sites for HIV (110). Inhibition of p-glycoprotein may lead to increased access of drugs to these sites. Thus, the addition of ritonavir to an HIV-protease inhibitor increases the cerebrospinal fluid concentrations of this drug leading to increased antiviral activity (111).

Characteristics of indinavir

Indinavir is a widely used potent HIV-protease inhibitor, available in a 200 and a 400 mg capsule formulation. Its formal dosing regimen is 800 mg three times daily to be taken two hours after a meal or with a low fat meal since absorption is dependent on a low gastric pH. Diet restrictions are a major draw back of this HIV-protease inhibitor. These restrictions, however, are no longer necessary if indinavir is given in combination with ritonavir (99). Moreover, ritonavir boosted indinavir regimens can be given twice daily or even once daily instead of three times daily (101, 102, 105, 112, 113).

Indinavir is the only HIV-protease inhibitor with substantial renal clearance (20%), although the primary clearance route is the liver (80%) (72, 80). Due to poor solubility in aqueous solutions of high pH, such as urine, a unique complication of indinavir treatment is formation of renal crystals and stones due to indivavir crystallisation. This adverse effect requires patients to ensure sufficient fluid intake (114). Renal complications are the main treatment-limiting adverse effect of indinavir.

Characteristics of nelfinavir

Nelfinavir was the last of four HIV-protease inhibitors to be registered for treatment of HIV-infection as part of HAART. It is marketed as a tablet formulation containing 250 mg of nelfinavir. The recommended dose frequency is 750 mg three times daily (69), but dosing schedules of 1250 mg twice daily are being applied as well. Like other HIV-protease inhibitors, cytochrome P450 isoenzyme 3A4 is the primary metabolic route of nelfinavir (86, 100). Oxidative bio-transformation of nelfinavir forms an active metabolite, which is equally effective as the parent molecule and represents an appreciable proportion (25%) of the circulating plasma concentration (81-83). Like ritonavir, nelfinavir may reduce plasma concentrations of other drugs because of cytochrome P450 enzyme induction.

Table 3 Characteristics HIV-protease inhibitors: potential and known interactions

Compound	Saquinavir mesylate	Ritonavir	Indinavir sulphate	Nelfinavir mesylate
Agents increasing clearance	Nevirapine, rifabutin, Rifampin, carbamazepine*, dexamethasone*, phenobarbital*, phenytoin*	Rifampin	Fluconazole, nevirapine, Rifabutin, Rifampin, carbamazepine*, dexamethasone*, phenobarbital*, phenytoin*	Rifabutin, Rifampin
Agents slowing down clearance	Delavirdine, ketoconazole, ranitidine, ritonavir, indinavir, nelfinavir	-	Clarithromycin, delavirdine, Ketoconazole, nelfinavir ritonavir, itraconazole*	Indinavir, Ritonavir
Agents of which clearance is increased	-	ethinyl estradiol, meperidine, methadone, theophylline	-	Ethinyl estradiol
Agents of which clearance is slowed down	Astemizole*, calcium-channel blockers*, clindamycin*, cisapride*, dapsone*, midazolam*, quinidine*, sildenafil*, terfenadine*, triazolam*	Clarithromycin, desipramine, ergotamine, indinavir, ketoconazole, rifabutin, saquinavir, sildenafil, warfarin, alprazolam*, amiodarone*, astemizole*, calcium-channel blockers*, carbamazepine*, ciclosporin*, cisapride*, clorazepate*, dexamethasone*, diazepam*, encainide*, eythromycine*, flecainide*, fluoxetine*, flurazepam*, itraconazole*, loratadine*, mefloquine*, midazolam*, paroxetine*, pethidine*, pimozide*, piroxicam*, prednisolone*, propafenone*, propoxyphene*, quinidine*, sertraline*, terfenadine*, trazodone*, triazolam*, zoldipem*	Clarithromycin, rifabutin, saquinavir, astemizole*, terfenadine*, cisapride*, pimozide*, quinidine*, alprazolam*, triazolam*, midazolam*, sildenafil*	indinavir, rifabutin, saquinavir, terfenadine, astemizol*, calcium-channel blockers*, cisapride*, HMG CoA reducatase inhibtors*, midazolam*, pimozide*, sildenafil*, tracrolimus*, triazolam*

* theoretical interaction

Since nelfinavir was marketed later than saquinavir, ritonavir and indinavir, it was initially used predominantly as second-line or rescue treatment. The most important treatment limiting adverse effect is diarrhoea (60, 115, 116).

Post-marketing surveillance

The HIV-protease inhibitors were introduced abruptly under special circumstances, whereas they represented a completely novel type of medicines. The usual delays of approximately six months for accepting new agents in the Dutch medicine reimbursement system were overcome by a special financial allowance from the health authorities. Hence, the HIV-protease inhibitors were available free of charge immediately after formal registration by the health authorities, which allowed for immediate widespread use among HIV-infected persons. The special circumstances surrounding the introduction of the HIV-protease inhibitors demanded the implementation of post-marketing surveillance more than usual.

Clinical trial populations, on which the available data for registration are based, represent highly selective patient groups. Strict inclusion and exclusion criteria generate relatively healthy (exclusion of active disease, pregnant women, children and abnormal laboratory values) and motivated groups (written informed consent). The number of patients studied is usually too low to detect events with relatively low incidences. For example, clinical trials with HIV-protease inhibitors for registration purposes generally involved less than 1000 patients (55, 56, 58, 117-119). Assuming normal distribution and 95% confidence, these trials were able to detect events with a cumulative incidence of at least 4 in 1000 patients (0.4%) within the observation period of the trial.

Follow-up periods of clinical trials are short compared to the actual periods of treatment needed (one to two years versus life time exposure or follow-up) as a consequence of which long term toxicity can easily be missed. In the context of clinical trials, patients are monitored more closely and more regularly than in daily practice. Finally, the options for and effects of toxicity management are usually not the objective of research.

For these reasons, post-marketing research is warranted for all new medicines (120, 121). Manufacturers are instructed to conduct post-marketing research and to report all adverse effects to the authorities. However, in view of potential conflicts of interest, independent follow-up of new agents must be performed as well.

There are a number of research methods for post-marketing surveillance. In the Netherlands, physicians and pharmacists are encouraged to report adverse effects to the national pharmaco-vigilance foundation of the Netherlands (LAREB). Unusual and

severe underreporting of adverse effects, especially in highly specialized areas such as HIV/AIDS. Nevertheless, case reports arising from such a system can be informative and may trigger further studies. Epidemiological studies in well-defined populations, on the other hand, provide better insight into incidences of and risk factors for adverse effects under everyday circumstances and into potentially preventive and curative strategies (122, 123).

The ATHENA cohort

In November 1997, the National Health Insurance Council provided a grant for a project on the implications for the course of HIV-disease, public health and health care as well as the costs and benefits of the new antiretroviral treatment. This led to the set up of the ATHENA cohort, a nationwide observational cohort including patients treated with HIV-protease inhibitors or other new antiretroviral agents. The project was carried out in a collaborative action of all 22 hospitals in the Netherlands, which provide antiretroviral treatment to HIV-infected individuals. The resulting cohort, which covers a well-defined patient population, provided a unique opportunity for the conduct of epidemiological studies on antiretroviral agents in the post-marketing setting.

Outline of this thesis

The objectives of this thesis were to provide more insight into the risk and risk factors of adverse drug reactions associated with HIV-protease inhibitor treatment under non-experimental everyday circumstances. By recognition of risk factors, patients at risk can be identified beforehand and risk management can be targeted more efficiently, hence ultimately improving the safety of HIV-protease inhibitor treatment. In the studies presented in this thesis we applied multiple research strategies, in which the nationwide ATHENA cohort played a central role.

References

1. Coffin JM. HIV population dynamics in vivo: implications for genetic variation, pathogenesis, and therapy. Science 1995;267:483-9.
2. Rosenberg ZF, Fauci AS. Immunopathogenesis of HIV infection. Faseb J 1991;5::2382-90.
3. Deng H, Liu R, Ellmeier W, Choe S, Unutmaz D, Burkhart M, et al. Identification of a major co-receptor for primary isolates of HIV-1. Nature 1996;381:661-6.
4. Dragic T, Litwin V, Allaway GP, Martin SR, Huang Y, Nagashima KA, et al. HIV-1 entry into CD4+ cells is mediated by the chemokine receptor CC-CKR-5. Nature 1996;381:667-73.

5. Bleul CC, Farzan M, Choe H, Parolin C, Clark-Lewis I, Sodroski J, et al. The lymphocyte chemoattractant SDF-1 is a ligand for LESTR/fusin and blocks HIV-1 entry. Nature 1996;382:829-33.
6. Oberlin E, Amara A, Bachelerie F, Bessia C, Virelizier JL, Arenzana-Seisdedos F, et al. The CXC chemokine SDF-1 is the ligand for LESTR/fusin and prevents infection by T-cell-line-adapted HIV-1. Nature 1996;382:833-5.
7. Robins T, Plattner J. HIV protease inhibitors: their anti-HIV activity and potential role in treatment. J Acquir Immune Defic Syndr 1993;6:162-70.
8. Pearl LH, Taylor WR. A structural model for the retroviral proteases. Nature 1987;329:351-4.
9. Kohl NE, Emini EA, Schleif WA, Davis JC, Heimbach JC, Dixon R, et al. Active human immunodeficiency virus protease is required for viral infectivity. Proc Natl Acad Sci USA 1988;85:4686-90.
10. Miller M, Jaskolski M, Rao JK, Leis J, Wlodawer A. Crystal structure of a retroviral protease proves relationship to aspartic protease family. Nature 1989;337:576-9.
11. Wolthers KC, Bea G, Wisman A, Otto SA, de Roda Husman AM, Schaft N, et al. T cell telomere length in HIV-1 infection: no evidence for increased CD4+ T cell turnover. Science 1996;274:1543-7.
12. Fahey JL, Taylor JM, Detels R, Hofmann B, Melmed R, Nishanian P, et al. The prognostic value of cellular and serologic markers in infection with human immunodeficiency virus type 1. N Engl J Med 1990;322:166-72.
13. Phillips AN, Lee CA, Elford J, Janossy G, Timms A, Bofill M, et al. Serial CD4 lymphocyte counts and development of AIDS. Lancet 1991;337:389-92.
14. Mellors JW, Rinaldo C, Jr., Gupta P, White RM, Todd JA, Kingsley LA. Prognosis in HIV-1 infection predicted by the quantity of virus in plasma. Science 1996;272:1167-70.
15. Hirsch MS, Conway B, D'Aquila RT, Johnson VA, Brun-Vezinet F, Clotet B, et al. Antiretroviral drug resistance testing in adults with HIV infection: implications for clinical management. International AIDS Society--USA Panel. JAMA 1998;279:1984-91.
16. Saravolatz LD, Winslow DL, Collins G, Hodges JS, Pettinelli C, Stein DS, et al. Zidovudine alone or in combination with didanosine or zalcitabine in HIV-infected patients with the acquired immunodeficiency syndrome or fewer than 200 CD4 cells per cubic millimeter. Investigators for the Terry Beirn Community Programs for Clinical Research on AIDS. N Engl J Med 1996;335:1099-106.
17. Hammer SM, Katzenstein DA, Hughes MD, Gundacker H, Schooley RT, Haubrich RH, et al. A trial comparing nucleoside monotherapy with combination therapy in HIV-infected adults with CD4 cell counts from 200 to 500 per cubic millimeter. AIDS Clinical Trials Group Study 175 Study Team. N Engl J Med 1996;335:1081-90.
18. Graham NM, Jacobson LP, Kuo V, Chmiel JS, Morgenstern H, Zucconi SL. Access to therapy in the Multicenter AIDS Cohort Study, 1989-1992. J Clin Epidemiol 1994;47:1003-12.
19. Graham NM, Zeger SL, Park LP, Vermund SH, Detels R, Rinaldo CR, et al. The effects on survival of early treatment of human immunodeficiency virus infection. N Engl J Med 1992;326:1037-42.
20. Anonymous. New drugs for HIV infection. Med Lett Drugs Ther 1996;38:35-7.
21. Anonymous. Indinavir marketed under accelerated-approval program. Am J Health Syst Pharm 1996;53:982.
22. Ho DD. Time to hit HIV, early and hard [editorial; comment] . N Engl J Med 1995;333:450-1.

23. Moyle G, Gazzard B. Current knowledge and future prospects for the use of HIV protease inhibitors. Drugs 1996;51:701-12.
24. Winslow DLa, Otto MJ. HIV protease inhibitors. AIDS 1995;9 (suppl A):S183-S192.
25. Deeks SG, Smith M, Holodniy M, Kahn JO. HIV-1 protease inhibitors. A review for clinicians. [59 refs]. JAMA 1997;277:145-53.
26. Borleffs JJC, Danner SA, Boer K, de Groot R, Kauffmann R, Reiss P, et al. [Guidelines for HIV-suppressing therapy 1998]. National guidelines; 1998.
27. Carpenter CC, Cooper DA, Fischl MA, Gatell JM, Gazzard BG, Hammer SM, et al. Antiretroviral therapy in adults. Updated recommendations of the International AIDS Society -USA Panel. JAMA 2000;283:381-390.
28. Anonymous. British HIV Association guidelines for antiretroviral treatment of HIV seropositive individuals. BHIVA Guidelines Co-ordinating Committee . Lancet 1997;349:1086-92.
29. Kaplan AH, Zack JA, Knigge M, Paul DA, Kempf DJ, Norbeck DW, et al. Partial inhibition of the human immunodeficiency virus type 1 protease results in aberrant virus assembly and the formation of noninfectious particles. J Virol 1993;67:4050-5.
30. Ledergerber B, Egger M, M. O, Telenti A, Hirschel B, Battegay M, et al. Clinical progression and virological failure on highly active antiretroviral therapy in HIV-1 patients: a prospective cohort study. Lancet 1999;353:863-8.
31. Palella FJ, Delany KM, Moorman AC, Loveless MO, Fuhrer J, Satten GA, et al. Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. N Engl J Med 1998;338:853-860.
32. Mocroft A, Vella S, Benfield TL, Chiesi A, Miller V, Gargalianos P, et al. Changing patterns of mortality across Europe in patients infected with HIV-1. EuroSIDA Study Group. Lancet 1998;352:1725-30.
33. Markowitz M, Mo H, Kempf DJ, Norbeck DW, Bhat TN, Erickson JW, et al. Selection and analysis of human immunodeficiency virus type 1 variants with increased resistance to ABT-538, a novel protease inhibitor. J Virol 1995;69:701-6.
34. Jacobsen H, Hanggi M, Ott M, Duncan IB, Owen S, Andreoni M, et al. In vivo resistance to a human immunodeficiency virus type 1 proteinase inhibitor: mutations, kinetics, and frequencies. J Infect Dis 1996;173:1379-87.
35. Lorenzi P, Opravil M, Hirschel B, Chave JP, Furrer HJ, Sax H, et al. Impact of drug resistance mutations on virologic response to salvage therapy. Swiss HIV Cohort Study. AIDS 1999;13:F17-21.
36. Mellors JW, Munoz A, Giorgi JV, Margolick JB, Tassoni CJ, Gupta P, et al. Plasma viral load and CD4+ lymphocytes as prognostic markers of HIV-1 infection. Ann Intern Med 1997;126:946-54.
37. Condra JH, Schleif WA, Blahy OM, Gabryelski LJ, Graham DJ, Quintero JC, et al. In vivo emergence of HIV-1 variants resistant to multiple protease inhibitors. Nature 1995;374:569-71.
38. Schapiro JM, Winters MA, Lawrence J, Merigan TC. Clinical cross-resistance between the HIV-1 protease inhibitors saquinavir and indinavir and correlations with genotypic mutations. AIDS 1999;13:359-65.
39. Schmit JC, Ruiz L, Clotet B, Raventos A, Tor J, Leonard J, et al. Resistance-related mutations in the HIV-1 protease gene of patients treated for 1 year with the protease inhibitor ritonavir (ABT-538). AIDS 1996;10:995-9.

40. Kemper CA, Witt MD, Keiser PH, Dube MP, Forthal DN, Leibowitz M, et al. Sequencing of protease inhibitor therapy: insights from an analysis of HIV phenotypic resistance in patients failing protease inhibitors. AIDS 2001;15:609-15.
41. Karmochkine M, Si Mohamed A, Piketty C, Ginsburg C, Raguin G, Schneider-Fauveau V, et al. The cumulative occurrence of resistance mutations in the HIV-1 protease gene is associated with failure of salvage therapy with ritonavir and saquinavir in protease inhibitor-experienced patients. Antiviral Res 2000;47:179-88.
42. Hoetelmans RM, Reijers MH, Weverling GJ, ten Kate RW, Wit FW, Mulder JW, et al. The effect of plasma drug concentrations on HIV-1 clearance rate during quadruple drug therapy. AIDS 1998;12:F111-5.
43. Burger DM, Hoetelmans RM, Hugen PW, Mulder JW, Meenhorst PL, Koopmans PP, et al. Low plasma concentrations of indinavir are related to virological treatment failure in HIV-1-infected patients on indinavir-containing triple therapy. Antivir Ther 1998;3:215-20.
44. Reijers MH, Weigel HM, Hart AA, Ten Kate RW, Mulder JW, Reiss P, et al. Toxicity and drug exposure in a quadruple drug regimen in HIV-1 infected patients participating in the ADAM study. AIDS 2000;14:59-67.
45. Nieuwkerk PT, Sprangers MA, Burger DM, Hoetelmans RM, Hugen PW, Danner SA, et al. Limited patient adherence to highly active antiretroviral therapy for HIV-1 infection in an observational cohort study. Arch Intern Med 2001;161:1962-8.
46. Descamps D, Flandre P, Calvez V, Peytavin G, Meiffredy V, Collin G, et al. Mechanisms of virologic failure in previously untreated HIV-infected patients from a trial of induction-maintenance therapy. Trilege (Agence Nationale de Recherches sur le SIDA 072) Study Team). JAMA 2000;283:205-11.
47. Friedland GH, Williams A. Attaining higher goals in HIV treatment: the central importance of adherence. AIDS 1999;13 Suppl 1:S61-72.
48. Dieleman JP, Gyssens IC, van der Ende ME, de Marie S, Burger DM. Urological complaints in relation to indinavir plasma concentrations in HIV-infected patients. AIDS 1999;13:473-8.
49. Chun TW, Carruth L, Finzi D, Shen X, DiGiuseppe JA, Taylor H, et al. Quantification of latent tissue reservoirs and total body viral load in HIV-1 infection. Nature 1997;387:183-8.
50. Wong JK, Hezareh M, Gunthard HF, Havlir DV, Ignacio CC, Spina CA, et al. Recovery of replication-competent HIV despite prolonged suppression of plasma viremia. Science 1997;278:1291-5.
51. Embretson J, Zupancic M, Ribas JL, Burke A, Racz P, Tenner-Racz K, et al. Massive covert infection of helper T lymphocytes and macrophages by HIV during the incubation period of AIDS. Nature 1993;362:359-62.
52. Finzi D, Hermankova M, Pierson T, al. e. Indentification of a reservoir for HIV-1 in patients on highly active antiretroviral therapy. Science 1997;278:1295-1300.
53. Schrager LK, D'Souza MP. Cellular and anatomical reservoirs of HIV-1 in patients receiving potent antiretroviral combination therapy. JAMA 1998;280:67-71.
54. Cohen J. The daunting challenge of keeping HIV suppressed. Science 1997;277:32-3.
55. Collier AC, Coombs RW, Schoenfeld DA, Bassett RL, Timpone J, Baruch A, et al. Treatment of human immunodeficiency virus infection with saquinavir, zidovudine, and zalcitabine. AIDS Clinical Trials Group. N Engl J Med 1996;334:1011-7.

56. Danner SA, Carr A, Leonard JM, Lehman LM, Gudiol F, Gonzales J, et al. A short-term study of the safety, pharmacokinetics, and efficacy of ritonavir, an inhibitor of HIV-1 protease. European-Australian Collaborative Ritonavir Study Group. N Engl J Med 1995;333:1528-33.
57. Markowitz M, Saag M, Powderly WG, Hurley AM, Hsu A, Valdes JM, et al. A preliminary study of ritonavir, an inhibitor of HIV-1 protease, to treat HIV-1 infection. N Engl J Med 1995;333:1534-9.
58. Hammer SM, Squires KE, Hughes MD, Grimes JM, Demeter LM, Currier JS, et al. A controlled trial of two nucleoside analogues plus indinavir in persons with human immunodeficiency virus infection and CD4 cell counts of 200 per cubic millimeter or less. AIDS Clinical Trials Group 320 Study Team . N Engl J Med 1997;337:725-33.
59. Stein DS, Fish DG, Bilello JA, Preston SL, Martineau GL, Drusano GL. A 24-week open-label phase I/II evaluation of the HIV protease inhibitor MK-639 (indinavir). AIDS 1996;10:485-92.
60. Roca B, Gomez CJ, Arnedo A. A randomized, comparative study of lamivudine plus stavudine, with indinavir or nelfinavir, in treatment-experienced HIV-infected patients. AIDS 2000;14:157-61.
61. Erickson JW, Meidhart DJ, Van Drie J, Kempf DJ, Wank XC, Norbeck DW, et al. Design, activity and a 2.8 A crystal structure of a C2 symmetric inhibitor complexed to HIV-1 protease. Science 1990;249:527-533.
62. Wlodawer A. Rational drug design: the proteinase inhibitors. Pharmacotherapy 1994;14:9S-20S.
63. Wlodawer A, Miller M, Jaskolski M, Sathyanarayana BK, Baldwin E, Weber IT, et al. Conserved folding in retroviral proteases: crystal structure of a synthetic HIV-1 protease. Science 1989;245:616-21.
64. Dorsey BD, Levin RB, McDaniel SL, Vacca JP, Guare JP, Darke PL, et al. L-735,524: the design of a potent and orally bioavailable HIV protease inhibitor. J Med Chem 1994;37:3443-51.
65. Sham HL, Zhao C, Marsh KC, Betebenner DA, Lin S, McDonald E, et al. Potent inhibitors of the HIV-1 protease with good oral bioavailabilities. Biochem Biophys Res Commun 1995;211:159-65.
66. McQuade TJ, Tomasselli AG, Liu L, Karacostas V, Moss B, Sawyer TK, et al. A synthetic HIV-1 protease inhibitor with antiviral activity arrests HIV-like particle maturation. Science 1990;247:454-6.
67. Anonymous. Summary of product characteristics Invirase (saquinavir). Product characteristics: Roche Registration Ltd.
68. Anonymous. Norvir (ritonavir capsules, ritonavir oral solution). Product information: Abbott Laboratories (USA); 1996 feb. 1996. Report No.: 03-4657-R1.
69. Anonymous. Viracept (nelfinavir mesylate), brief summary. Package insert: Agouron Pharmaceuticals, Inc.; 1997 7/2/97. Report No.: 1.888.viracept.
70. Anonymous. Summary product characteristics Norvir (ritonavir). Product characteristics: Abbott Laboratories Ltd. (UK); 1996 25/6/1996.
71. Anonymous. Summary product characteristics Crixivan 400 mg. Product characteristics: Merck Sharp & Dohme ltd.; 1996 29/5/96.
72. Yeh KC, Deutsch PJ, Haddix H, Hesney M, Hoagland V, Ju WD, et al. Single-dose pharmacokinetics of indinavir and the effect of food. Antimicrob Agents Chemother 1998;42:332-8.
73. Carver PL, Fleisher D, Zhou SY, Kaul D, Kazanjian P, Li C. Meal composition effects on the oral bioavailability of indinavir in HIV-infected patients. Pharm Res 1999;16:718-24.
74. Lin JH, Chen IW, Vastag KJ, Ostovic D. pH-dependent oral absorption of l-735,524, a potent HIV protease inhibitor, in rats and dogs. Drug Metab Dispos 1995;23:730-735.

75. Chiba M, Hensleigh M, Lin JH. Hepatic and intestinal metabolism of indinavir, an HIV protease inhibitor, in rat and human microsomes. Major role of CYP3A. Biochem Pharmacol 1997;53:1187-95.
76. Fitzsimmons ME, Collins JM. Selective biotransformation of the human immunodeficiency virus protease inhibitor saquinavir by human small-intestinal cytochrome P4503A4: potential contribution to high first-pass metabolism. Drug Metab Dispos 1997;25:256-66.
77. Wood AJJ. Drug therapy: interactions among drugs for HIV and opportunistic infections. N Engl J Med 2001;344:984-96.
78. Eagling VA, Back DJ, Barry MG. Differential inhibition of cytochrome P450 isoforms by the protease inhibitors, ritonavir, saquinavir and indinavir. Br J Clin Pharmacol 1997;44:190-4.
79. Kumar GN, Rodrigues AD, Buko AM, Denissen JF. Cytochrome P450-mediated metabolism of the HIV-1 protease inhibitor ritonavir (ABT-538) in human liver microsomes. J Pharmacol Exp Ther 1996;277:423-31.
80. Balani SK, Woolf EJ, Hoagland VL, Sturgill MG, Deutsch PJ, Yeh KC, et al. Disposition of indinavir, a potent HIV-1 protease inhibitor, after an oral dose in humans. Drug Metab Dispos 1996;24:1389-94.
81. Baede-van Dijk PA, Hugen PW, Verweij-van Wissen CP, Koopmans PP, Burger DM, Hekster YA. Analysis of variation in plasma concentrations of nelfinavir and its active metabolite M8 in HIV-positive patients. AIDS 2001;15:991-8.
82. Zhang KE, Wu E, Patick AK, Kerr B, Zorbas M, Lankford A, et al. Circulating metabolites of the human immunodeficiency virus protease inhibitor nelfinavir in humans: structural identification, levels in plasma, and antiviral activities. Antimicrob Agents Chemother 2001;45:1086-93.
83. Lamotte C, Peytavin G, Farinotti R. Determination of nelfinavir, a potent HIV protease inhibitor, and its active metabolite M8 in human plasma by high-performance liquid chromatography with photodiode-array detection. J Chromatogr B Biomed Sci Appl 1999;735:159-70.
84. Heylen R, Miller R. Adverse effects and drug interactions of medications commonly used in the treatment of adult HIV positive patients: Part 2. Genitourin Med 1997;73:5-11.
85. Taburet AM, Singlas E. Drug interactions with antiviral drugs. Clin Pharmacokinet 1996;30:385-401.
86. Lillibridge JH, Liang BH, Kerr BM, Webber S, Quart B, Shetty BV, et al. Characterization of the selectivity and mechanism of human cytochrome P450 inhibition by the human immunodeficiency virus-protease inhibitor nelfinavir mesylate. Drug Metab Dispos 1998;26:609-16.
87. Hoetelmans RMW, Meenhorst PL, Mulder JW, Burger DM, Koks CHW, Beijnen JH. Clinical pharmacology of HIV protease inhibitors: focus on saquinavir, indinavir, and ritonavir. Pharmacy World & Science 1997;19:159-175.
88. Michalets EL. Update: Clinically significant cytochrome P-450 drug interactions. Pharmacotherapy 1998;18:84-112.
89. Bertz RJ, Granneman GR. Use of in vitro and in vivo data to estimate the likelihood of metabolic pharmacokinetic interactions. Clin Pharmacokinet 1997;32:210-58.
90. Burger DM, De Graaff M, Wuis EW, Kooopmans PP, Hekster YA. Determination of indinavir, an HIV-protease inhibitor, in human plasma by reversed-phase high-performance liquid chromatography. J Chromatogr B 1997;703:235-241.
91. Wu EY, Wilkinson JM, 2nd, Naret DG, Daniels VL, Williams LJ, Khalil DA, et al. High-performance liquid chromatographic method for the determination of nelfinavir, a novel HIV-1 protease inhibitor, in human plasma. J Chromatogr B Biomed Sci Appl 1997;695:373-80.

92. Ha HR, Follath F, Bloemhard Y, Krahenbuhl S. Determination of saquinavir in human plasma by high-performance liquid chromatography. J Chromatogr B Biomed Sci Appl 1997;694(2):427-33.
93. van Heeswijk RP, Hoetelmans RM, Harms R, Meenhorst PL, Mulder JW, Lange JM, et al. Simultaneous quantitative determination of the HIV protease inhibitors amprenavir, indinavir, nelfinavir, ritonavir and saquinavir in human plasma by ion-pair high-performance liquid chromatography with ultraviolet detection. J Chromatogr B Biomed Sci Appl 1998;719:159-68.
94. Frappier S, Breilh D, Diarte E, Ba B, Ducint D, Pellegrin JL, et al. Simultaneous determination of ritonavir and saquinavir, two human immunodeficiency virus protease inhibitors, in human serum by high-performance liquid chromatography. J Chromatogr B Biomed Sci Appl 1998;714:384-9.
95. Poirier JM, Robidou P, Jaillon P. Determination of indinavir in plasma by solid-phase extraction and column liquid chromatography. Ther Drug Monit 1999;21:404-10.
96. Hugen PW, Verweij-van Wissen CP, Burger DM, Wuis EW, Koopmans PP, Hekster YA. Simultaneous determination of the HIV-protease inhibitors indinavir, nelfinavir, saquinavir and ritonavir in human plasma by reversed-phase high-performance liquid chromatography. J Chromatogr B Biomed Sci Appl 1999;727:139-49.
97. Langmann P, Klinker H, Schirmer D, Zilly M, Bienert A, Richter E. High-performance liquid chromatographic method for the simultaneous determination of HIV-1 protease inhibitors indinavir, saquinavir and ritonavir in plasma of patients during highly active antiretroviral therapy. J Chromatogr B Biomed Sci Appl 1999;735:41-50.
98. Zhong L, Yeh KC. Determination of indinavir in human cerebrospinal fluid and plasma by solid-phase extraction and high-performance liquid chromatography with column switching. J Chromatogr B Biomed Sci Appl 1999;734:63-71.
99. Hsu A, Granneman GR, Bertz RJ. Ritonavir. Clinical pharmacokinetics and interactions with other anti-HIV agents. Clin Pharmacokinet 1998;35:275-91.
100. von Moltke LL, Greenblatt DJ, Grassi JM, Granda BW, Duan SX, Fogelman SM, et al. Protease inhibitors as inhibitors of human cytochromes P450: high risk associated with ritonavir. J Clin Pharmacol 1998;38:106-11.
101. van Heeswijk RP, Veldkamp AI, Hoetelmans RM, Mulder JW, Schreij G, Hsu A, et al. The steady-state plasma pharmacokinetics of indinavir alone and in combination with a low dose of ritonavir in twice daily dosing regimens in HIV-1-infected individuals. AIDS 1999;13:F95-9.
102. Rockstroh JK, Bergmann F, Wiesel W, Rieke A, Theisen A, Fatkenheuer G, et al. Efficacy and safety of twice daily first-line ritonavir/indinavir plus double nucleoside combination therapy in HIV-infected individuals. AIDS 2000;14:1181-1185.
103. Casado JL, Moreno A, Marti-Belda P, Sabido R, Garcia-Arata I, Perez-Elias MJ, et al. Increased Indinavir Levels Using Twice Daily Ritonavir/Indinavir at 100/800mg Improves Virological Response even after Multiple Failure (abstract no. 1170). In: 40th Interscience Conference on Antimicrobial Agents and Chemotherapy; September 2000; Toronto
104. Burger DM, Hugen PW, Aarnoutse RE, Dieleman JP, Prins JM, van Der Poll T, et al. A Retrospective, Cohort-Based Survey of Patients Using Twice-Daily Indinavir + Ritonavir Combinations: Pharmacokinetics, Safety, and Efficacy. J Acquir Immune Defic Syndr 2001;26:218-224.

105. Hsu A, Granneman GR, Cao G, Carothers L, Japour A, El-Shourbagy T, et al. Pharmacokinetic interaction between ritonavir and indinavir in healthy volunteers. Antimicrob Agents Chemother 1998;42:2784-91.

106. Kempf DJ, Marsh KC, Kumar G, Rodrigues AD, Denissen JF, McDonald E, et al. Pharmacokinetic enhancement of inhibitors of the human immunodeficiency virus protease by coadministration with ritonavir. Antimicrob Agents Chemother 1997;41:654-60.

107. Mitsuyasu RT, Skolnik PR, Cohen SR, Conway B, Gill MJ, Jensen PC, et al. Activity of the soft gelatin formulation of saquinavir in combination therapy in antiretroviral-naive patients. NV15355 Study Team. AIDS 1998;12:F103-9.

108. Gisolf EH, Jurriaans S, Pelgrom J, van Wanzeele F, van der Ende ME, Brinkman K, et al. The effect of treatment intensification in HIV-infection: a study comparing treatment with ritonavir/saquinavir and ritonavir/saquinavir/stavudine. Prometheus Study Group. AIDS 2000;14:405-13.

109. van Heeswijk RP, Veldkamp AI, Mulder JW, Meenhorst PL, Lange JM, Beijnen JH, et al. Once-daily dosing of saquinavir and low-dose ritonavir in HIV-1-infected individuals: a pharmacokinetic pilot study. AIDS 2000;14:F103-10.

110. Tanigawara Y. Role of P-glycoprotein in drug disposition. Ther Drug Monit 2000;22:137-40.

111. van Praag RME, Weverling GJ, Portegies P, Jurriaans S, Zhou X-J, Turner-Foisy ML, et al. Enhanced penetration of indinavir in cerebrospinal fluid and semen after the addition of low-dose indinavir. AIDS 2000;14:1187-1194.

112. Hugen PWH, Burger DM, ter Hofstede HJM, Koopmans PP, Stek M, Hekster YA, et al. Dose-finding study of a once-daily indinavir/ritonavir regimen. J Acquir Immun Def Syndr 2000;25:236-245.

113. Mallolas J, Blanco JL, Sarasa M, Giner V, Martinez E, Garcia-Viejo MA, et al. Dose-finding study of once-daily indinavir/ritonavir plus zidovudine and lamivudine in HIV-infected patients. J Acquir Immuno Def Syndromes 2000;25:229-235.

114. Anonymous. Crixivan® (indinavir sulfate) product monograph. Whitehouse Station: Merck & Co.; 1996 1996. Report No.: 9-97 CRX 96-W-6083-B.

115. Tebas P, Powderly WG. Nelfinavir mesylate. Expert Opin Pharmacother 2000;1:1429-40.

116. Jarvis B, Faulds D. Nelfinavir. A review of its therapeutic efficacy in HIV infection. Drugs 1998;56:147-67.

117. Cameron B, Heath-Chiozzi M, Kravcik S, Mills R, Potthoff A, Henry D, et al. Prolongation of life and prevention of AIDS in advanced HIV immunodeficiency with ritonavir (late breaker). In: 3rd Conference on Retroviruses and Oppotunistic Infections; 1996; Washington DC: Abstracts-On-Disk.

118. Markowitz M, Conant M, Hurley A, Schluger R, Duran M, Peterkin J, et al. A preliminary evaluation of nelfinavir mesylate, an inhibitor of human immunodeficiency virus (HIV)-1 protease, to treat HIV infection. J Infect Dis 1998;177:1533-40.

119. Moyle GJ, Youle M, Higgs C, Monaghan J, Prince W, Chapman S, et al. Safety, pharmacokinetics, and antiretroviral activity of the potent, specific human immunodeficiency virus protease inhibitor nelfinavir: results of a phase I/II trial and extended follow-up in patients infected with human immunodeficiency virus. J Clin Pharmacol 1998;38:736-43.

120. Leufkens HG, Heerdink ER. [Post-marketing surveillance]. Ned Tijdschr Geneeskd 1996;140:1201-2; discussion 1202.

121. Stricker BH, in 't Veld BA, Feenstra J. [The need for 'postmarketing surveillance']. Ned Tijdschr Geneeskd 1999;143:711-3.

122. Strom BL, Carson JL, Schinnar R, Snyder ES, Shaw M, Walter SL. No causal relationship between transdermal scopolamine and seizures: methodologic lessons for pharmacoepidemiology. Clin Pharmacol Ther 1991;50:107-13.

123. Strom BLe. Pharmacoepidemiology. 2nd ed. New York: John Wiley & Sons; 1994.

Chapter 2

Indinavir nephrotoxicity revisited

Chapter 2.1

Papillary necrosis associated with HIV-protease inhibitor indinavir

Summary

Background: HIV-protease inhibitor indinavir may cause nephrotlithiasis and interstitial nephritis. The renal consequences of indinavir associated nephrotoxicity are uncertain. We report a case of papillary necrosis in a patient treated with indinavir.

Patient and methods: An asymptomatic HIV-infected woman experienced right-sided renal colicky pain during treatment with indinavir. She passed a non-solid stone and continued indinavir treatment. Intravenous pyelogram twenty months later following an episode of left-sided colicky pain showed right-sided papillary necrosis. Indinavir associated nephrolithiasis and chronic interstitial nephritis were the only possible causes identified in this patient.

Conclusion: Physicians should be aware that indinavir nephrolithiasis may cause papillary necrosis

Introduction

The HIV-protease inhibitor indinavir causes symptoms of nephrolithiasis in 4-16% of patients and crystalluria in up to 20% of patients treated with indinavir 800 mg three times daily as part of Highly Active Antiretroviral Treatment (HAART) (1). Although crystalluria may remain asymptomatic, it is potentially harmful as illustrated by reports of interstitial nephritis with indinavir crystals in the interstitium (2). In addition, long term exposure to indinavir may be associated with renal atrophy (3). We report a case of papillary necrosis in a patient treated with indinavir.

Patient and methods

A non-symptomatic 34-year-old caucasian woman was diagnosed as having HIV-1 infection and hepatitis C virus infection, contracted through intravenous drug abuse. Since 1997, she received HAART, comprising of zidovudine (250 mg twice daily), lamivudine (150 mg twice daily) and indinavir (800 mg three times daily) because of a high viral load (7.51×10^4 HIV-1 RNA copies/mL, Roche Amplicor, detection limit 500 copies/mL) and a declining CD4 cell count (370 cells/μL). Her viral load has since been below the detection limit and her CD4 cell count has increased to 670 cells/μL. The patient also took 1 mg lorazepam daily and the occasional use of cocaine could not be excluded.

Results

Three months after starting HAART, patient complained of recurrent colicky pain in the right flank associated with gross haematuria. Symptoms improved after passing a non-solid stone, which unfortunately was lost. Twenty months later, two years after starting HAART, she reported left-sided colicky pain without gross haematuria or other urinary symptoms. She again passed a stone after which symptoms resolved. Again the stone was lost.

Throughout the course of HAART, urinalysis showed repeated pyuria of more than 75 cells/μL and mild microscopic haematuria with normal urine pH of 5 and negative nitrite tests for bacteria. Intermittent crystalluria of one to two crystals per 40 times magnified field was observed. Urine culture was repeatedly negative. There was mild albuminuria (albumin 3 to 6 g/mol creatinine, normal range <3.5 g/mol creatinine) and indinavir plasma concentrations were borderline increased (10.1 mg/L at 1 h after indinavir intake, 6.8 mg/L at 2.5 h and 0.3 mg/L at 8 h). Haematology and serum chemistry, including serum creatinine, were unremarkable. Abdominal X-ray, performed two years after starting HAART

Figure Intravenous pyelogram of the right kidney showing unilateral papillary necrosis of the lower pole calyx of the right kidney:smooth-margined saccular calyceal cavity formation.

during the last episode of renal colic did not show any stones, which is consistent with the radiolucent characteristic of indinavir stones (4). Subsequent intravenous pyelogram (IVP) did not reveal any residual stones either, but did show papillary necrosis in the right kidney (figure). There were no calcifications or other abnormalities.

Discussion

The assessment of a causal relationship between an adverse event and a drug requires knowledge of the temporal relationship and adequate exclusion of other potential causes. The onset of flank pain, passing a stone and pyuria after starting HAART and the temporal relationship are compatible with a causal relationship. Potential causal factors of papillary necrosis include the use of large amounts of non-steroidal anti-inflammatory drugs (NSAIDs) and phenacetin-containing analgesics. Other possible causes are diseases leading to renal ischemia such as diabetes mellitus, nephrolithiasis, chronic interstitial nephritis, urinary tract infection, tuberculosis, sickle cell haemoglobinopathy, anorexia nervosa and severe dehydration (5). Even though our patient had a history of drug abuse, the use of nephrotoxic medication or nephrotoxic drugs as the cause of papillary necrosis of our patient is unlikely because of its one-sided and localised presence. The patient admitted inadequate fluid intake prior to the first episode of renal colic, but was never severely dehydrated. Urine culture did not reveal any infection that could explain the continuous

pyuria. Because of the absence of other probable causes and the fact that the papillary necrosis was of a localised nature, HAART-attributed nephrolithiasis and interstitial nephritis were considered as the most probable cause of papillary necrosis in our patient. Given the concordance between the side of papillary necrosis and the side of first symptoms and the non-solid constitution of the first stone, we consider it likely that the papillary necrosis was caused by an indinavir stone. Indinavir-induced chronic interstitial nephritis might have contributed. Physicians should be aware that papillary necrosis is a potential consequence of nephrolithiasis caused by indinavir.

References

1. Kopp JB, Miller KD, Mican JA, Feuerstein IM, Vaughan E, Baker C, Pannell LK, Falloon J: Crystalluria and urinary tract abnormalities associated with indinavir. Ann Intern Med 1997;127:119-125.
2. Tashima KT, Horowitz JD, Rosen S: Indinavir nephropathy. N Engl J Med 1997;336:138-140.
3. Hanabusa H, Tagami H, Hataya H: Renal atrophy associated with long-term treatment with indinavir. N Engl J Med 1999;340:392-393.
4. Blake SP, McNicholas MM, Raptopoulos V: Nonopaque crystal deposition causing ureteric obstruction in patients with HIV undergoing indinavir therapy. AJR Am J Roentgenol 1998;171:717-720.
5. Bach PH, Nguyen TK: Renal papillary necrosis--40 years on. Toxicol Pathol 1998;26:73-91.

Chapter 2.2

Urologic complaints in relation to indinavir plasma concentrations in HIV-infected patients

Abstract

Objective: to assess the association between indinavir associated urologic complaints and indinavir plasma concentrations. Design: case-series, comparing indinavir plasma concentrations in cases with average concentrations in a control group.

Methods: patients taking 800 mg indinavir tid, who presented with overt urologic complaints (renal colic, flank pain and/or hematuria) were selected for the study. Plasma indinavir concentrations were measured by means of a standardized high performance liquid chromatography method. Plasma samples taken at 1.5-8 hours after the last indinavir ingestion were included for evaluation. Results were compared with the full pharmacokinetic curves of indinavir plasma concentrations from a control group of 14 patients taking 800 mg indinavir tid without urologic complaints, and expressed as concentration ratios. A ratio of 1 indicated a plasma concentration equalling the average concentration in the control population at the same point in time following indinavir intake.

Results: 17 patients (5 women) were enrolled and indinavir concentrations of 15 patients could be evaluated. 14 (93%) patients had a concentration above the mean of the controls and 12 (80%) patients had a concentration above the upper 95%-confidence limit (95%CL), and 1 (7%) had a concentration below the lower 95%CL. The mean indinavir concentration in patients with urologic complaints (ratio range 0.55–11.49) was significantly higher than the average concentration and the upper 95%CL of the control group ($p<0.05$). The results could not be explained by differences in weight, gender or drug interactions. Two patients had chronic active hepatitis B infection. In 6 patients with indinavir concentrations above the upper 95%CL, indinavir was reduced to 600 mg tid. Upon repeat measurement after the dose adjustment, their indinavir plasma concentrations fell within the 95%-confidence interval around the mean of the control population. All 6 patients remained asymptomatic and had viral loads <500 copies/ml after a follow-up of 5-16 months.

Conclusions: urologic complications occurring during indinavir treatment were associated with elevated indinavir plasma concentrations in 80% of patients in this study. Indinavir plasma concentrations should be monitored upon presentation of urologic complaints on the basis of which dose reductions may be applied if brief interruption and increased hydration are ineffective.

Introduction

Antiretroviral treatment with the HIV-protease inhibitor indinavir is associated with urologic complaints. Urologic complications occur in approximately 8% of patients on an indinavir dosage of 800 mg tid and vary from nephrolithiasis (3-4% of all patients) to symptomatic crystalluria with dysuria or back pain (7% of all patients) (1, 2). These adverse reactions can usually be resolved by hydration and short treatment interruption of 1 to 3 days, but may occasionally lead to acute renal failure which requires urologic intervention to relieve obstruction (3-5). In a majority of indinavir users with recurrent renal colic episodes, treatment with indinavir is abandoned (6).

Independently of the indinavir concentration, up to 20% of absorbed indinavir is excreted into the urine, more than half of which is excreted in the intact form (1, 7). Due to this route of elimination, high indinavir plasma concentrations result in high indinavir urine concentrations, depending on the urine volume, which may induce indinavir precipitates if urinary solubility is exceeded. Accordingly, the calculi voided by patients are of a radiolucent, gelatinous nature and consist of indinavir base monohydrate (intact indinavir) (2, 5, 6, 8). Apart from dilution of urine through higher fluid intake, to prevent and relieve urologic symptoms, avoidance of high indinavir plasma concentrations may be an important measure for reducing the rate of urologic complications. In this study, we investigated the relationship between indinavir plasma concentrations and urologic complaints occurring during indinavir treatment in HIV-infected patients.

Methods

Case series

The patient population of the Erasmus Medical Centre Rotterdam (EMCR) comprises approximately 500 adult HIV-infected patients, 104 (21 women, 20%) of whom currently receive indinavir treatment. Indinavir treated patients, who presented with overt urologic complaints between January and December 1997, were enrolled in the study. These patients received indinavir at a dosage of 800 mg three times a day (tid) as part of the Highly Active Antiretroviral Therapy (HAART) for HIV-infection. Urologic complaints were defined as episodes of renal colic, flank pain, dysuria and/or hematuria. Information on viral load, assessed by HIV-RNA polymerase chain reaction (Amplicor HIV-1 monitor, Roche, Basel), urinalysis, routinely performed by semi-quantitative dipstick test (Rapignost® total-screen L dipstick, Behringwerke AG, D-35001 Marburg, Germany), and concomitant medication was retrieved retrospectively from patient records. All assessments were done

during the regular outpatient clinic visit of each patient, which did not necessarily coincide with the manifestation of urologic symptoms.

Control patients

Indinavir concentrations in patients with urologic complaints were compared to a standard concentration curve as derived from the full pharmacokinetic curves of 14 HIV-infected patients (2 women) from the University Hospital Nijmegen, taking 800 mg of indinavir tid (Fig. 1). This control group had an average age of 41.4 ± 7.3 years (range 31-52) and an average weight of 72.1 ± 9 kg (range 61-89). After an average duration of indinavir therapy of 6.9 ±3.3 months (range 1-12), 10 patients had <200 copies/ml HIV-RNA. All control patients were free of overt signs of intoxication (including urologic complications). The pharmacokinetic parameters of this group (AUC, Cmax, Cmin) were consistent with the data as presented in the Crixivan® product monograph (1).

Sample collection

A blood sample was collected from each patient with overt urologic complaints at variable time points after the last ingestion of indinavir. The time of last ingestion was based on patient interview. The time lapse between the occurrence of urological symptoms and indinavir concentration assessment was not assessed. In order to attain comparable indinavir concentrations between cases and controls, samples were evaluable only if drawn at least 1.5 h after the last intake, to avoid variance due to absorption, and less than 8 h after the last intake, as there were no measurement points for control patients after 8 h. Seven ml of blood was drawn from an antecubital vein into a lithium heparin vacutainer, from which plasma was separated by centrifugation which was stored in the refrigerator until transfer to the Department of Clinical Pharmacy at the University Hospital Nijmegen.

Analytical methods

Indinavir concentrations in plasma were determined using a validated High Performance Liquid Chromatography (HPLC) assay, which has been described previously (9). In brief, plasma is mixed with acetonitrile before centrifugation. The subsequent supernatant is evaporated to dryness, the residue of which is dissolved in eluent and injected into the HPLC system. The system contains an Octadecyl column with a mobile phase of acetonitrile-water and uses ultraviolet detection at 210 nm.

Results of indinavir plasma concentration measurements were expressed as 'concentration ratios'. The ratio was calculated by dividing the indinavir concentration in each patient with urologic complaints by the average concentration of the control group at the same or nearest time interval following indinavir intake. For this purpose,

concentration means of the control group were extrapolated to 15 minute time intervals so that the maximum time difference between controls and cases was 7.5 minutes.

Statistical Analysis

Continuous variables such as age, weight and duration of therapy, were tested with a Mann-Whitney U test. The average concentration ratios of patients with urologic complaints was analysed for statistical significance against the expected concentration ratio of 1.0 by calculating the 95%-confidence intervals around the mean ratio of patients with urologic complaints, based on a student-distribution, after normalising the distribution by log-transformation. A ratio of 1.0 indicated an indinavir concentration equalling the mean concentration of the control population at the same time interval following indinavir ingestion. We also did a sensitivity analysis in which the upper 95%-confidence limit instead of the mean of the control group was chosen as the denominator in the ratio.

Results

Seventeen patients with overt urologic complaints, 12 men and 5 women, representing 16 % of the population treated with indinavir at the EMCR, were included in the study. Their mean age was 37.4 ± 8.6 (range 27-57), their mean weight was 65.6 ± 9.2 kg (range 46-85) and they had been on indinavir treatment for an average duration of 7.1 ± 4.8 (range 0.4-14.8) months (table 1). In 15 (88%) patients the plasma HIV-RNA had been reduced to < 500 copies/ml.

Table 1 Baseline characteristics

Characteristic#	Control	Cases	p-value
Male (%)	12 (86)	12 (71)	
Female (%)	2 (14)	5 (29)	
Age (years ± SD)	41.4 ± 7.3	37.4 ± 8.6	p= 0.1521
Weight (kg ± SD)	72.1 ± 9	65.6 ± 9	p= 0.1029
Duration of indinavir therapy (months ± SD)	6.9 ± 3.3	7.1 ± 4.8	p= 0.8580
Undetectable HIV-RNA (%)	10 (71)*	15 (88)¥	

\# SD= standard deviation
* detection limit 200 copies/mL
¥ detection limit 500 copies/mL

Table 2 provides an overview of the results. Twelve (71%) cases occurred during the spring and summer. Fifteen patients had an evaluable indinavir concentration measurement. Concentration ratios ranged from 0.55 to 11.49 (Fig. 1). Except for 1 patient, all (93%) had a concentration above the mean of the controls and 12 (80%) patients had a concentration

above the upper 95%-confidence limit of the control population (Fig.1). The log-corrected mean ratio was 2.64 (95%-confidence interval 1.68-4.14), which was significantly different from the expected ratio of 1.0. In the sensitivity analysis the log-corrected mean ratio was 1.96 (95%-confidence interval 1.23-3.03).

Table 2 Test results of patients with indinavir associated urologic complaints

Assessments			Results
Major Complaint*	Renal colic	n (%)	7 (41)
	Flank pain	n (%)	6 (35)
	Hematuria	n (%)	3 (18)
	Dysuria	n (%)	1 (6)
Urinalysis#	PH (n=11)	Mean (range)	5.9 (5 - 9)
	Leucocyturia (n=11)	n (%)	10 (91)
	Hematuria (n=11)	n (%)	8 (73)
	Proteinuria (n=10)	n (%)	9 (90)
Concentration ratio¥	800 mg tid (n=15)	Mean (range)	2.64 (0.55 – 11.49)
		95%-CI	1.68 – 4.14
	600 mg tid (n=6)	Mean (range)	0.88 (0.63 – 1.37)
		95%-CI	0.63 – 1.23

* Most severe complaints are listed, patients may have suffered a combination of symptoms
\# Semi-quantitative dipstick test (Rapignost□ total-screen L dipstick); leucocyturia: >15 cells/L; hematuria: >10 cells/L; proteinuria: >0.15 g/L
¥ Ratios are calculated by dividing the measured concentration by the concentration mean of controls at the same point in time following last indinavir intake; means are log-corrected; CI= confidence intervals.
¶ Six patients had a dose reduction and subsequent repeat indinavir concentration assessment

The average urine pH was 5.9 (n=11, range 5-9). Urinalysis (semi-quantitative dipstick test) further revealed the presence of leucocytes (ie. >15 leucocytes/µL) in 10 of 11 (91%) cases in absence of infection (ie. dipstick test negative for bacteria), and erythrocytes (ie. >10 erythrocytes/µL) in 8 of 11 (73%) cases. Nine of 10 (90%) patients showed slight proteinuria of 0.15-0.30 g/L. All symptoms cleared without urologic intervention. Concomitant medication included co-trimoxazole, diclofenac, haloperidol, cetirizine, dapson, megestrol, metoprolol, fluconazol, clindamycin, psylliumseed and butylscopolamin, none of which is known to significantly affect indinavir concentrations. In 1 patient occasional use of heroin could not be excluded.

In 6 patients with urologic symptoms and an indinavir concentration higher than the upper 95%-confidence limit (ratio range 2.25-11.49), the dose of indinavir was reduced to 600 mg tid. Repeat indinavir concentration ratios, measured at 58 ± 44 days after dose reduction, approached 1.0 (range 0.63-1.37) in all 6 patients (table2). Five out of 6 remained free of urologic symptoms and one patient remained free of urologic symptoms when having extra fluid intake. All 6 continued to have viral loads <500 copies/ml following indinavir dose reduction for follow-up periods of 5-16 months. As for the other patients, six discontinued indinavir treatment, five because of gastro-intestinal complaints, 3 of whom

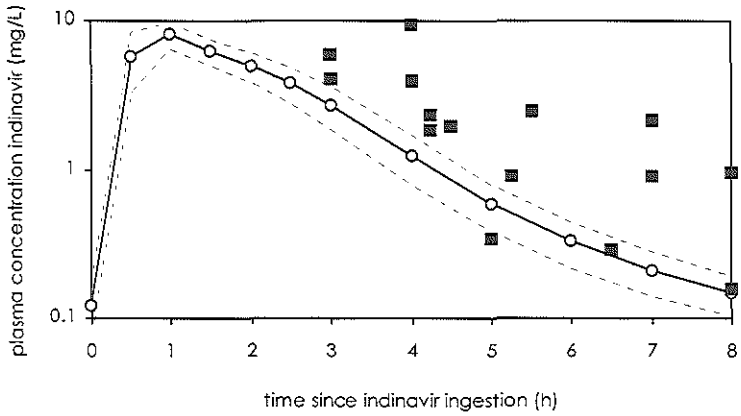

Figure 1 Indinavir plasma concentrations as a function of time following last ingestion The solid line represents the average pharmacokinetic curve of a control group (n=14). Broken lines represent the 95%-confidence bands of the concentration means in the control group. Bullet markers indicate measurement points in the control group. Square markers indicate indinavir plasma concentrations as measured in 15 individual patients with urologic complaints. All patients took indinavir at a dosage of 800 mg three times a day,measurements were done after ingestion of 800 mg indinavir.

had first tried dose reductions other than 600 tid, and one because of lipodystrophy. Two had modified the dose to a twice daily regimen and suffered residual urologic complaints. One patient continued indinavir at 800 mg tid without major problems, but HIV-RNA had raised to 2560 copies/mL after 8.5 months. There may be a problem with compliance in this patient. As for the two patients with non-evaluable indinavir concentration assessments, one reduced the dose to 400 mg tid, but later appeared to have a low indinavir plasma concentration and high viral load for which reason indinavir was stopped. The other patient had no dose reduction, but indinavir treatment was withdrawn due to gastro-intestinal intolerance.

Discussion

The relationship between indinavir exposure and antiretroviral effect has been reported, but an exposure-toxicity relationship has not been described, so far (1, 10, 11). The present results suggest an association between indinavir plasma concentrations and the occurrence of urologic complaints during indinavir containing antiretroviral therapy. Patients with urologic complaints in this study consistently presented with high indinavir plasma concentrations in comparison to a control group without urologic complaints.

Although this study did not analyse urinary crystals, it was shown that complaints often coincided with sterile leucocyturia, hematuria and proteinuria, consistent with a pattern of mucosal irritation due to (micro)lithiasis. As indinavir urinary concentrations and clearance were not measured in this study, it is not known as to how these relate to high indinavir plasma concentrations and the occurrence of indinavir associated urologic symptoms. The process of indinavir crystal formation has been reported to be enhanced by high urinary pH and low urinary volume (2, 12). All patients were advised to drink at least 1.5 L/day. Although most patients claimed that their intake was maximal, precise intake and urinary volumes are not known. In fact, many urologic complaints occurred during periods of hot weather when optimal hydration may have been difficult to achieve (13). Therefore, we cannot exclude urinary volume as the cause of urologic complaints in our patients.

High indinavir plasma concentrations may have a multifactorial origin. Oral absorption of indinavir is pH-dependent and subsequent elimination depends on the liver cytochrome P-450 system (iso-enzyme CYP3A4) and urinary clearance (12, 14). None of the patients used any drugs known to significantly affect the indinavir pharmacokinetics. Two patients had a chronic active hepatitis B infection, with possibly impaired liver clearance of indinavir. None of the patients was known with impaired renal function, but renal clearance may have been reduced by insufficient hydration. It is not known how this would affect the plasma concentration of indinavir as the kidneys play a minor part in indinavir clearance. The high indinavir plasma concentrations could not be attributed to low weight, as the patients with urologic complaints and patients from the control group had similar weights. A recent study observed an influence of gender on pharmacokinetics of indinavir, which would suggest higher propensity for women to develop urologic complications (15).

Importantly, patients remained free of urologic complaints following dose reduction to 600 mg tid. Subsequent indinavir plasma concentrations fell within the limits of the control group and viral suppression was maintained during follow-up. Thus, indinavir dose reduction may be a useful and safe control measure for urologic complaints associated with a high plasma indinavir concentration. Although indinavir concentration may not predictive of urologic complaints, it might be worthwhile to perform a cost-benefit analysis of monitoring indinavir concentrations in all patients in the prevention of urologic complaints.

In this pilot study indinavir concentrations were compared to an historical control group. More research is needed to confirm the findings and to learn the long-term effects of dose reduction in patients with high indinavir plasma concentrations. Further research should also be aimed at finding an explanation for the high incidence of urologic symptoms seen in our patient population. The cumulative incidence was 16%, which is much higher than reported so far (1, 2). This might reflect population characteristics or may be a

consequence of close patient monitoring (13, 15). In addition, the following points should be recognised. First, urologic complaints, occurring during indinavir treatment, may not always be attributable to indinavir. Second, possible causes of increased indinavir plasma concentrations, that can be removed, should be taken into account before applying any dose adjustment. Evidently, dose reductions should only be applied on the basis of indinavir plasma concentration measurements. Finally, it should be noted that this study compared single point measurements of indinavir plasma concentration in individual patients with the average concentration of an independent control group. The results of this control group were believed to be a proper reference, but it is not known how single point measurements of indinavir plasma concentrations relate to other pharmacokinetic parameters.

In conclusion, this study showed that urologic complaints, occurring during indinavir treatment, are associated with elevated indinavir plasma concentrations. If urologic complaints persist despite maximal fluid intake and indinavir plasma concentration is above the upper 95%-confidence limit, dose adjustment may be considered, so that patients can safely continue taking indinavir. In our patients a dose reduction to 600 mg tid was found to be effective. More research is needed to further elucidate the relationship between indinavir plasma concentrations and urologic complaints and to learn the long term effects of dose reductions. The influence of climate on indinavir pharmacokinetics and indinavir related urologic complaints should be clarified.

References

1. Crixivan® (indinavir sulfate) product monograph. 1996 Merck & Co., Whitehouse Station, N.J., USA.
2. Kopp JB, Miller KD, Mican JA, et al.: Crystalluria and urinary tract abnormalities associated with indinavir. Ann Intern Med 1997;127:119-125.
3. Berns JS, Cohen RM, Silverman M, Turner J: Acute renal failure due to indinavir crystalluria and nephrolithiasis: report of two cases. Am J Kidney Dis 1997;30:558-560.
4. Sutherland SE, Reigle MD, Seftel AD, Resnick MI: Protease inhibitors and urolithiasis. J Urol 1997;158:31-33.
5. Bruce RG, Munch LC, Hoven AD, et al.: Urolithiasis associated with the protease inhibitor indinavir. Urology 1997;50:513-518.
6. Gentile DL, Stoller ML, Jarrett TM, Ward JF, Geib KS, Wood AF: Protease inhibitor-induced urolithiasis. Urology 1997;50:508-511.
7. Balani SK, Woolf EJ, Hoagland VL, et al.: Disposition of indinavir, a potent HIV-1 protease inhibitor, after an oral dose in humans. Drug Metab Dispos 1996;24:1389-1394.
8. Daudon M, Estepa L, Viard JP, Joly D, Jungers P: Urinary stones in HIV-1-positive patients treated with indinavir. Lancet 1997;349:1294-1295.

9. Burger DM, De Graaff M, Wuis EW, Kooopmans PP, Hekster Y: Determination of indinavir, an HIV-protease inhibitor, in human plasma by reversed-phase high-performance liquid chromatography. J Chromatogr B 1997;703:235-241.
10. Stein DS, Fish DG, Bilello JA, Preston SL, Martineau GL, Drusano GL: A 24-week open-label phase I/II evaluation of the HIV protease inhibitor MK-639 (indinavir). AIDS 1996;10:485-492.
11. Burger DM, Koopmans PP, Brinkman K, etal.: Therapeutic drug monitoring of the HIV-protease inhibitor indinavir. 37th International Conference on Antimicrobial Agents and Chemotherapy. Toronto, September 1997 [abstract A-19].
12. Lin JH, Chen IW, Vastag KJ, Ostovic D: pH-dependent oral absorption of L-735,524, a potent HIV protease inhibitor, in rats and dogs. Drug Metab Dispos 1995;23:730-735.
13. Bach MC, Godofsky EW: Indinavir nephrolithiasis in warm climates. J Acquir Immune Defic Syndr Hum Retrovirol 1997;14:296-298.
14. Chiba M, Hensleigh M, Lin JH: Hepatic and intestinal metabolism of indinavir, an HIV protease inhibitor, in rat and human microsomes. Major role of CYP3A. Biochem Pharmacol 1997;53:1187-1195.
15. Burger DM, Koopmans PP, Brinkman K: Influence of gender on indinavir pharmacokinetics. Sixth European Conference on Clinical Aspects and Treatment of HIV-Infection. Hamburg, October1997 [abstract 252].

Chapter 2.3

Risk factors for urological symptoms in a cohort of users of HIV-protease inhibitor indinavir.

The ATHENA cohort.

Abstract

Background: Nephrolithiasis is a well-known complication of indinavir treatment and may result in a variety of urological symptoms (US) ranging from renal colic to renal insufficiency. In order to be able to adequately prevent or resolve US, further knowledge regarding the incidence and risk factors is needed.

Methods: The present study was performed in the ATHENA cohort, a cohort of HIV-infected patients on antiretroviral therapy in the Netherlands. The incidence rate of US was estimated in a sub-cohort of patients starting PI-treatment after 1996 (n=1219). US were defined as the first report of nephrolithiasis, renal colic, flank pain, hematuria, renal insufficiency or nephropathy. Risk factors for US during indinavir-treatment were subsequently studied among the subset of patients who started indinavir after 1996 (n=644), using multivariate Cox's regression analysis.

Results: The incidence of US was 8.3 per 100 treatment years of indinavir versus 0.7 per 100 treatment years with other PIs. Risk factors for US during indinavir were low weight (RR:2.1, 95%CI:1.1-3.9), low lean body mass (LBM) (RR:1.7, 95%CI:1.0-2.9), undetectable HIV-1 RNA when starting indinavir (RR:3.2, 95%CI:1.5-6.0), prior treatment change due to intolerance (RR:2.4, 95%CI:1.2-5.1), indinavir-regimens of ≥1000 mg bid (RR:3.1, 95%CI:1.3-8.2) and high environmental temperatures (RR:3.9, 95%CI:1.7-8.8). Risk estimates were highest among patients with a low LBM.

Conclusions: Increased alertness for US is warranted for patients with viral successful suppression when starting indinavir, patients with a low LBM, during indinavir-regimens of ≥1000 mg bid and during warm weather periods.

Introduction

Adverse effects of HIV-protease inhibitor-containing Highly Active Antiretroviral Treatment (HAART) constitute a threat to the quality of life, and treatment adherence of HIV-infected patients (1-3).

Nephrolithiasis is a well-known complication of treatment with indinavir, 20% of which is cleared by the renal system (4-6). Symptoms range from renal colic, flank pain, dysuria and gross hematuria to the passing of a stone. Other symptoms related to the renal effects of indinavir are crystalluria and renal insufficiency, which may go undetected without specific monitoring. Symptoms may occur early or late during treatment and occasionally occur even after treatment discontinuation (7-12). The incidence of urological symptoms among patients who used indinavir 800 mg three times daily (tid) was estimated at 4% in clinical trials and 8-16% in other studies (4, 9-13).

It is generally assumed that urological symptoms are secondary to sludging of indinavir crystals in the urinary tract, probably due to the low solubility of indinavir in aqueous conditions (14, 15). Therefore, the major risk factor for urological symptoms seems to be insufficient fluid intake, which leads to concentrated urine and a diminished urinary flow, thus facilitating indinavir precipitation and crystal aggregation (4, 16). Nevertheless, abundant fluid intake is not always sufficient for the prevention of urological symptoms, which suggests that other factors play a role as well (12).

High environmental temperatures, female gender, concurrent hepatitis C infection, and high indinavir plasma concentrations have also been suggested as risk factors for urological symptoms (8, 9, 13, 17-20). Indinavir plasma concentrations depend on the indinavir dosing regimen used, gastric pH, distribution volume, and both hepatic and renal clearance (5, 6, 21-23). Any factors affecting these aspects are potential determinants for the development of urological symptoms.

We performed a population-based cohort study to estimate the incidence of urological symptoms among patients using an HIV-protease inhibitor as part of HAART and to identify potential risk factors for urological symptoms during the use of indinavir. We studied the role of various indinavir dosing regimens, environmental temperatures and lean body mass (LBM) in particular.

Patients and methods

Setting

We conducted a cohort study within the ATHENA-cohort. This is a large national cohort of HIV-infected patients in the Netherlands, who have been or are being treated with at least one of the antiretroviral drugs which were introduced after July 1996 (all HIV-protease inhibitors, all non-nucleoside reverse transcriptase inhibitors and the newer nucleoside analogue reverse transcriptase inhibitors such as lamivudine and stavudine) and who gave written informed consent. Patient entry effectively started in May 1998 and continues to date. All 22 Dutch hospitals providing treatment to HIV-infected patients participate in the ATHENA-project. The project has been approved by the Ethics Committees of all participating centres.

According to the national guidelines for HIV-treatment in the Netherlands, patients are seen at approximately three-monthly intervals for regular follow-up (24). Data for the ATHENA-cohort are collected from the medical records on standardised forms by trained research nurses and treating physicians. This is done retrospectively for the period prior to consent and prospectively thereafter and continues to date. The resulting database contains information on gender, age, route of HIV-transmission, height and weight. Start and stop dates as well as dose frequency of any antiretroviral medication and prophylactic treatment against opportunistic infections, reasons for stopping such treatments, date of onset and resolution of HIV-related diseases, CD4-cell counts, plasma HIV-1 RNA load and abnormal laboratory values are all recorded in a standardised manner. Information on adverse events in the ATHENA database comprises all events that lead to a change in antiretroviral treatment and a number of pre-specified adverse events, among which nephrolithiasis. In addition, physicians are requested to report otherwise remarkable events and laboratory abnormalities. On site data monitoring of at least 10% of completed study forms by central data monitors takes place at regular time intervals. In addition, central data verification through automated database consistency checks are performed, the resulting queries of which are resolved by local research nurses. Data that had been entered, monitored and verified before March 2000 were available for the present analysis.

Cohort definition

On the basis of the treatment data in the ATHENA database we selected two cohorts. The first cohort of patients commencing treatment with any one of the HIV-protease inhibitors was used to estimate the incidence rate of urological symptoms during use of different HIV-protease inhibitors. Patients were eligible for entry if they started the first HIV-protease inhibitor no earlier than 1997, when the HIV-protease inhibitors became available as

prescription drugs through community pharmacies in the Netherlands. Patients entered the cohort upon the start of the first HIV-protease inhibitor and were followed until development of urological symptoms, death, loss to follow-up or the date of last data collection, whichever came first. Cohort participants were allowed to change between HIV-protease inhibitors and to have treatment interruptions during the follow-up period. The duration of use of each HIV-protease inhibitor was calculated. Consequently, each person could contribute to multiple specific protease inhibitor exposure categories.

Since urological symptoms occur primarily in indinavir users we formed a second cohort of indinavir users (the indinavir-cohort) to further specify incidence rates and to identify risk factors for urological symptoms during the use of indinavir. Patients entered the indinavir-cohort upon first start of indinavir in 1997 or thereafter, independent of previous HIV-protease inhibitor treatment and previous experience of urological symptoms. Follow-up lasted from the start of indinavir until the development of urological symptoms, discontinuation of indinavir, an interruption of indinavir for more than seven days, death, loss to follow-up or the date of last data collection whichever was earliest.

Patients for whom we had inadequate information regarding treatment, or who had urological symptoms at cohort entry were excluded from both the HIV-protease inhibitor cohort and the indinavir-cohort.

Outcome measure

A wide range of symptoms has been attributed to the effects of indinavir on the renal system, including both clinical and sub-clinical symptoms. Given the way data are collected, it can be expected that the ATHENA-data capture most overt clinical symptoms. There may be a relative underreporting of sub-clinical symptoms, such as laboratory abnormalities and renal insufficiency, depending on the intensity of patient monitoring. Assuming that any resulting misclassification is random within hospitals, the primary outcome measure of this study was defined as the occurrence of urological symptoms ranging from nephrolithiasis, renal colic, flank pain and gross hematuria to a diagnosis of renal insufficiency, nephritis or nephropathy as made by the treating physician. The date of onset of first symptoms was defined as the index date.

Co-variates

Potential determinants for urological symptoms generally available in the ATHENA database are gender, weight, HIV-1 RNA load (lower limit of quantification was set at 1000 copies/mL for the purpose of this analysis), which might be considered as a marker for compliance, and high plasma concentrations, primary reason for prior treatment change (possible reasons including intolerance, treatment failure, patient request, pharmacological reason,

other reason with specification, or unknown), concomitant use of ritonavir and co-trimoxazole, and dosing regimen of indinavir. Body mass index (BMI [kg/m²]) and lean body mass (LBM, [(height in cm-152)*0.9+50 for men, (height in cm-152)*0.9+45.5 for women]) were calculated from available data. Other factors investigated were the route of HIV-transmission, CDC-disease classification (25), CD4 cell counts, the occurrence of any type of hepatitis, calendar time and concomitant use of other antiretroviral medications. The monthly average environmental temperatures from 1997 until the end of 1999 were retrieved from the internet-site of the Royal Netherlands Institute of Meteorology (KNMI, www.knmi.nl).

Indinavir-regimens were divided into the standard regimen of 800 mg tid, 800 mg indinavir combined with 100 mg ritonavir bid (800/100 mg bid), 400 mg indinavir combined with 400 mg ritonavir bid (400/400 mg bid), 1000 mg or more bid (≥ 1000 mg bid), any other indinavir-regimen (including combinations with other HIV-protease inhibitors or non-nucleoside NRTIs). Continuous variables were dichotomised based on the lower quartile or the median.

Analysis

Crude incidence rates of urological symptoms with 95%-confidence intervals (95%CI) and relative risks (RR) were calculated based on a Poisson distribution. The one-year cumulative incidence was estimated using life table analysis. Potential risk factors for urological symptoms in the indinavir cohort were identified by means of univariate and multivariate Cox-regression analysis with the duration of indinavir use as follow-up time. We matched by treatment centre in order to control for potential bias from selective prescribing behaviour combined with increased diagnostic attention for urological symptoms or intensity of patient monitoring. Factors that may vary over time and potentially alter the risk of urological symptoms (weight, BMI, CD4 cell count, HIV-1 RNA load, indinavir-regimen, type of nucleoside reverse transcriptase inhibitors (NRTI) and environmental temperature) were included in the models as time-varying co-variates. Effect modification was explored by means of stratified multivariate Cox-regression analysis. All analyses were conducted with SPSS 8.0 for Windows.

Results

The ATHENA source population comprised a total of 2470 patients, 2362 (96%) of whom ever used an HIV-protease inhibitor. In total 1239 (52%) patients started the first HIV-protease inhibitor in 1997 or thereafter. We excluded 18 patients because of incomplete data on HIV-protease inhibitor treatment and 2 because of presence of urological symptoms at the start

of HIV-protease inhibitor treatment. The final HIV-protease inhibitor cohort therefore comprised 1219 patients, 445 of whom used indinavir at any time.

For the indinavir-cohort, we included all 445 patients from the HIV-protease inhibitor cohort who used indinavir. In addition, we included four patients who had incomplete data on initial HIV-protease inhibitor treatment but complete data on indinavir treatment and 195 patients who started HIV-protease inhibitors before 1997 but who had the first prescription for indinavir in 1997 or thereafter. The final indinavir-cohort therefore comprised 644 patients.

Incidence of urological symptoms

Within the HIV-protease inhibitor cohort, a first occurrence of urological symptoms was reported for 49 (4%) patients, 45 (92%) of whom developed these symptoms during the use of an HIV-protease inhibitor. Other reasons for end of follow-up (right censoring) were death (3%), loss to follow-up (0.4%) and last data collection (93%). The overall incidence rate of urological symptoms was 2.8 (95%CI: 2.0-3.6) per 100 person years of HIV-protease inhibitor treatment. Table 1 shows the HIV-protease inhibitor specific incidence rates. The majority of cases (73%) occurred during treatment with indinavir. The risk of urological symptoms during use of indinavir-containing regimens was 8.7 fold higher (95%CI: 7.4-10.2) than during use of regimens without indinavir.

The indinavir-cohort was larger than the indinavir component in the HIV-protease inhibitor cohort because patients were allowed to have used another HIV-protease inhibitor before 1997. Conversely, the indinavir-cohort had relatively shorter follow-up because follow-up was ended upon discontinuation of indinavir. The indinavir-cohort was used for further specification of the incidence rates during the use of indinavir and the identification of risk factors for indinavir-associated urological symptoms.

A first occurrence of urological symptoms during the use of indinavir was reported for 58 (9%) patients. Symptoms ranged from nephrolithiasis (n= 38), renal colic or flank pain (n= 6), gross hematuria (n= 6), renal insufficiency (n= 6), nephritis (n= 1) to nephropathy (n= 1). Other reasons for end of follow-up (right censoring) were discontinuation of indinavir (33%), interruption of indinavir for more than 7 days (6%), death (2%), loss to follow-up (1%) and last data collection (50%).

The overall incidence rate of urological symptoms was 8.3 (95%CI: 6.3-10.8) per 100 person years of indinavir treatment. The highest incidence rate was observed during indinavir-regimens of 1000 mg or more twice daily (bid) and during the first six months of indinavir-treatment (Table 2). Following the first six months, urological symptoms continued to occur but at a steady lower rate as is illustrated in figure 1.

Table 1 Incidence rates of urological symptoms (events) during the use of specific HIV-protease inhibitors

Type of treatment	Patients[a]		Person years	Events		Events per 100 person years	
	N	(%)	N	N	(%)	IR[b]	(95%CI)
Using any PI	1219	(100)	1619.4	45	(92)	2.8	(2.0-3.7)
Any IDV-containing	445	(37)	511.4	36	(73)	7.0[c]	(4.9-9.8)
IDV	382	(31)	462.3	34	(69)	7.4	(5.1-10.3)
IDV/RTV	82	(7)	44.6	2	(4)	4.5	(5.0-16.0)
IDV/other PI(s)	9	(1)	4.5	0	(0)	0	(0-73.3)
Non-IDV-containing	950	(78)	1108.0	9	(18)	0.8[c]	(0.4-1.5)
SQV/NFV	44	(4)	44.9	1	(2)	2.2	(0.03-12.4)
NFV	289	(24)	186.1	2	(4)	1.1	(0.1-3.9)[e]
RTV/SQV	374	(31)	402.2	4	(8)	1.0	(0.3-2.5)[e]
RTV	237	(19)	279.4	2	(4)	0.7	(0.1-2.6)[e]
SQV	170	(14)	193.6	0	(0)	0	(0-1.9)[e]
Other PI(s)	6	(1)	1.8	0	(0)	0	(0-183)
Not using a PI[d]	602	(49)	305.2	4	(8)	1.3	(0.4-3.4)[e]
Overall PI-cohort including periods with and without PIs	1219	(100)	1924.6	49	(100)	2.5	(1.9-3.4)

IDV= indinavir; NFV= nelfinavir; PI= HIV-protease inhibitor; RTV= ritonavir; SQV= saquinavir

a Total does not add up since patients may have used more than one HIV-protease inhibitor at different points in time

b IR= incidence rate per 100 person years, 95%-confidence intervals based on a Poisson distribution

c Relative risk during the use of IDV versus the use of other PI = 8.7 (95%CI: 7.4-10.2)

d HIV-protease inhibitor treatment interruption or after discontinuation

e Statistically significantly lower than IDV regimens with and without other PI(s)

Table 2 Incidence rates of urological symptoms (events) during the use of different indinavir-regimens and during sequential follow-up periods

Type of IDV treatment	Patients[a]		Person years	Events		Events per 100 person years	
	N	(%)	N	N	(%)	IR[b]	(95%CI)
IDV-regimen							
800 mg tid	486	(75)	539.2	37	(64)	6.9	(4.8-9.5)
≥ 1000 mg bid	63	(10)	28.9	7	(12)	24.2	(9.7-50)
800/100 mg bid	48	(7)	20.9	2	(3)	9.6	(1.1-34.4)
400/400 mg bid	61	(9)	31.2	0	(0)	0	(0-11.8)
Other IDV-regimen	154	(24)	75.8	9	(16)	11.9	(5.4-22.5)
Treatment period							
0 to 6 months	644	(100)	273.9	31	(53)	11.3	(7.7-16.1)
6 to 12 months	451	(70)	190.4	12	(21)	6.3	(3.3-11.0)
12 to 18 months	312	(48)	127.6	8	(14)	6.3	(2.7-12.3)
18 to 24 months	206	(32)	72.6	6	(10)	8.3	(3.0-17.9)
24 to 30 months	83	(13)	26.4	1	(2)	3.8	(0.1-21.4)
30 to 36 months	31	(5)	8.2	0	(0)	0	(0-46)
Overall	644	(100)	696.0	58	(100)	8.3	(6.3-10.8)

IDV= indinavir; ≥ 1000 mg= IDV dosages of 1000 mg or more; 800/100 mg= 800 mg IDV combined with 100 mg ritonavir; 400/400 mg= 400 mg IDV combined with 400 mg ritonavir

a Total does not add up since patients may have used more than one IDV-regimen

b IR= incidence rate per 100 person years based on a Poisson distribution

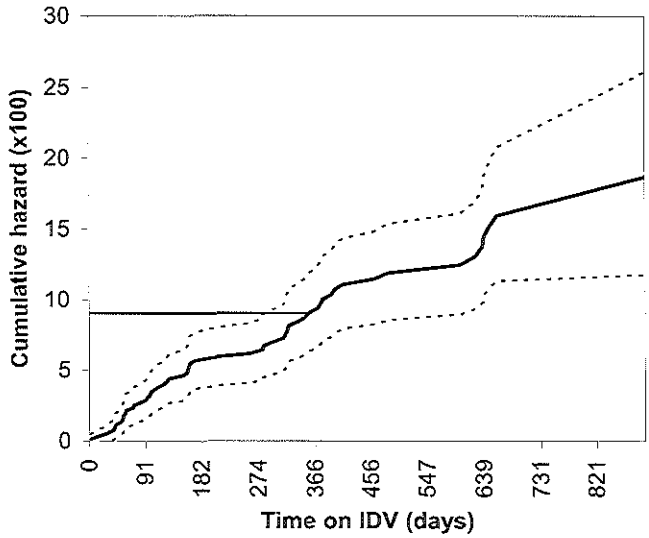

Figure 1 Hazard of urological symptoms during the use of indinavir
Solid bold line represents the hazard of urological symptoms over time. Dotted lines represent the standard error. The straight horizontal line indicates the cumulative hazard times 100 over one year of indinavir treatment, which was 9.0 (95%-confidence interval: 6.3-11.8).

Table 3 summarises the baseline characteristics of the indinavir-cohort. Overall, patients had a median age of 39 years, were predominantly male homosexuals with CDC-disease classification A or B. The median weight, BMI and LBM were 70 kg, 22 kg/m² and 73 kg respectively. The majority had more than 200 CD4-cells/microL and more than 1000 HIV-1 RNA copies/mL. Most patients had had prior antiretroviral treatment, started indinavir in 1997 and the most common first indinavir dosing regimen was 800 mg indinavir tid combined with AZT and 3TC, usually without concomitant co-trimoxazole.

Risk factors for urological symptoms

Baseline characteristics associated with urological symptoms were: LBM below the lowest quartile (68 kg), undetectable HIV-1 RNA (≤1000 copies/mL), absence of co-trimoxazole use and prior change of an antiretroviral treatment component due to any type of intolerance. Prior change due to intolerance and the absence of use of co-trimoxazole were both closely related to low HIV-1 RNA (Pearson's Chi-square test, p=0.000 and p=0.002) whereas low LBM was not (p=0.647).

Table 3 Baseline characteristics of the indinavir-cohort at start of indinavir

Characteristic at start of IDV		Outcome						P-Value[a]
		No US (n=586)		US (n=58)		Total (n=644)		
		N	(%)	N	(%)	N	(%)	
Follow-up (wks)	Median (IQR)	54	(24-91)	23	(10-53)	50	(23-89)	-
Age (years)	Median (IQR)	39	(33-46)	39	(33-42)	39	(33-45)	Ns
Gender	Female	87	(15)	9	(15)	96	(15)	Ns
Weight (kg)	Median (IQR)	70	(63-90)	64	(61-75)	70	(63-79)	Ns
	> 70	196	(33)	14	(24)	210	(33)	Ns
	≤ 70	200	(34)	23	(48)	228	(35)	
	Missing	190	(32)	16	(28)	206	(32)	
BMI (kg/m²)	Median (IQR)	22	(20-25)	22	(20-24)	22	(20-25)	Ns
	> 22	195	(33)	22	(38)	217	(34)	Ns
	≤ 22	175	(30)	20	(35)	195	(30)	
	Missing	216	(37)	16	(28)	232	(36)	
LBM (kg)	Median (IQR)	73	(68-78)	71.6	(66-76)	73	(68-78)	Ns
	> 68	398	(68)	35	(60)	433	(67)	0.0638
	≤ 68	145	(25)	23	(40)	168	(26)	
	Missing	43	(7)	0	(0)	43	(7)	
Route of HIV-Transmission	MSM	372	(68)	37	(67)	409	(68)	Ns
	IVdrugs	27	(5)	5	(9)	32	(5)	
	Other	152	(28)	13	(24)	165	(27)	
Stage of HIV-infection	Non-aids	523	(89)	52	(90)	575	(89)	Ns
	Aids	63	(11)	6	(10)	69	(11)	
CD4-cells (10^6/L)	Median (IQR)	205	(72-359)	209	(75-423)	206	(75-360)	Ns
	Missing	82	(14)	9	(16)	91	(14)	
HIV-1 RNA (copies/mL)	> 1000	425	(73)	37	(64)	462	(72)	0.0026
	≤ 1000	70	(12)	14	(24)	84	(13)	
	Missing	91	(16)	7	(12)	98	(15)	
Ever any type of hepatitis		9	(2)	1	(2)	10	(2)	Ns
Prior urological symptoms		2	(0.3)	1	(2)	3	(0.5)	Ns
Co-trimoxazole		244	(42)	17	(29)	261	(40)	0.0425
Prior ART		350	(60)	35	(60)	385	(60)	Ns
Reason prior change ART	None	318	(54)	26	(45)	344	(53)	0.0181
	Other	200	(34)	19	(33)	219	(34)	
	Intolerance	68	(12)	13	(22)	81	(13)	
Year of start IDV	1997	357	(61)	44	(76)	401	(62)	Ns
	1998	163	(28)	12	(21)	175	(27)	
	1999	66	(11)	2	(3)	68	(11)	
First IDV-regimen	800 mg tid	424	(72)	48	(73)	472	(73)	Ns
	≥ 1000 mg bid	27	(5)	3	(5)	21	(3)	
	800/100 mg bid	21	(4)	0	(0)	46	(7)	
	400/400 mg bid	45	(8)	1	(2)	30	(5)	
	Other	69	(12)	6	(9)	75	(12)	
NRTI combination	AZT/3TC	256	(44)	32	(55)	288	(45)	Ns
	3TC/d4T	176	(30)	13	(22)	189	(29)	
	d4T/ddI	68	(12)	8	(14)	76	(12)	
	Other	79	(14)	5	(9)	84	(13)	
	None	7	(1)	0	(0)	7	(1)	
Environmental temperature (°C)	Median (IQR)	9	(7-16)	10	(7-15)	9	(7-16)	Ns
	< 15	420	(72)	44	(76)	464	(72)	
	15-18	142	(24)	14	(24)	156	(24)	
	>18	24	(4)	0	(0)	24	(4)	

ART= antiretroviral treatment; BMI= body mass index; IDV= indinavir; IVdrug= intravenous drug use; LBM= lean body mass; MSM= man having sex with man; reason prior change ART= primary reason for changing prior ART (other includes failure, patient request, pharmacological, other with specification or unknown); US= urological symptoms; ≥ 1000 mg= IDV dosages of 1000 mg or more; 800/100 mg= 800 mg IDV combined with 100 mg ritonavir; 400/400 mg= 400 mg IDV combined with 400 mg ritonavir

a Cox-regression with days on IDV as follow-up time; Ns= p>0.1

Table 4 Risk factors for urological symptoms within the indinavir-cohort (n= 644)

Characteristic		Crude[a]		Adjusted[b]		LBM (kg) ≤ 68		LBM (kg) > 68	
		RR	(95%CI)	RR	(95%CI)	RR	(95%CI)	RR	(95%CI)
Baseline characteristics									
LBM (kg)	> 68	1		1					
	≤ 68	1.7	(1.0-2.9)	1.6	(0.9-2.9)				
	Missing	-	-	-	-				
HIV-1 RNA	>1000	1		1		1		1	
(copies/mL)	≤1000	3.2	(1.5-6.0)	3.1	(1.5-6.3)	33.6	(3.5-324.0)	2.1	(0.8-5.3)
	Missing	1.0	(0.4-2.3)	0.9	(0.4-2.3)	3.4	(0.7-15.6)	0.6	(0.1-2.8)
Co-trimoxazole	No	1							
	Yes	0.6	(0.3-1.0)						
Reason prior	None	1							
change ART	Other	1.3	(0.7-2.4)						
	Intolerance	2.4	(1.2-5.1)						
Time dependent characteristics									
Weight (kg)	> 70	1							
	≤ 70	2.1	(1.1-3.9)						
	Missing	1.8	(0.8-4.0)						
IDV-regimen	800 mg tid	1		1		1		1	
	≥1000 mg bid	3.1	(1.3-8.2)	3.2	(1.2-8.7)	8.7	(1.2-65.7)	2.0	(0.4-8.9)
	800/100 mg bid	1.0	(0.2-4.9)	1.1	(0.2-5.5)	7.0	(0.6-75.0)	-	-
	400/400 mg bid	-	-	-	-	-	-	-	-
	Other	1.7	(0.8-3.6)	1.9	(0.9-4.1)	3.8	(0.8-19.4)	1.3	(0.4-3.7)
Environmental	< 15	1		1		1		1	
temperature (°C)[c]	15-18	0.5	(0.2-1.2)	0.5	(0.2-1.2)	0.6	(0.2-2.4)	0.3	(0.1-1.1)
	> 18	3.9	(1.7-8.8)	4.1	(1.7-9.5)	6.4	(1.2-34.5)	2.7	(0.8-8.7)

LBM= lean body mass; Reason prior change ART= primary reason for changing any prior antiretroviral treatment (other includes failure, patient request, pharmacological, other with specification or unknown); ≥ 1000 mg= IDV dosages of 1000 mg or more; 800/100 mg= 800 mg IDV combined with 100 mg ritonavir; 400/400 mg= 400 mg IDV combined with 400 mg ritonavir

a Cox-regression analysis stratified for hospital with days on IDV as follow-up time

b Adjusted for HIV-1 RNA at baseline, LBM, IDV-regimen and environmental temperature

c environmental temperature as average temperature in the month of the index date (history of weather reports on internet www.knmi.nl)

In addition to the baseline variables, we assessed whether time-varying co-variates such as weight, BMI, CD4-cell count, HIV-1 RNA load, indinavir dosing regimen, type of NRTIs and environmental temperatures altered the risk of urological symptoms. Of these, low weight but not BMI, indinavir-regimens of ≥ 1000 mg bid, and high environmental temperatures were associated with urological symptoms.

Table 4 shows the crude and adjusted relative risks for all factors that were univariately associated with the development of urological symptoms at a p-value of 0.1. Adjustment for age and gender alone (data not shown) and adjustment for univariately associated variables did not change any of the associations. Weight was excluded from the multivariate analysis because of its close relation to LBM.

Restricting the analysis to clinically overt symptoms such as nephrolithiasis, renal colic, flank pain or gross hematuria did not lead to different conclusions (data not shown). Unfortunately, the data did not allow further breakdown of urological symptoms due to small patient numbers.

Stratification for LBM showed that the effects of undetectable HIV-1 RNA load (≤ 1000 copies/mL), indinavir-regimens of ≥ 1000 mg bid and 800/100 mg bid and environmental temperature above 18 °C were more pronounced in the low LBM stratum as reflected in higher RRs.

A further description of the course of events was made to provide insight into the reversibility of symptoms. Twenty (34%) patients with urological symptoms discontinued indinavir, 16 (28%) of whom had subsequent resolution of their symptoms within a maximum of 86 days. On ther other hand, we observed resolution of urological symptoms for 37 (64%) patients without or before discontinuation of indinavir. For 5 (9%) patients resolution of urological symptoms had not been recorded, 4 of whom had discontinued indinavir.

Discussion

In this large population based cohort of HIV-infected patients in the Netherlands the risk of urological symptoms during the use of indinavir was eight-fold higher as compared to other HIV-protease inhibitors. The large difference in incidence between indinavir and other HIV-protease inhibitors is an expected finding, although the incidence among indinavir users was lower than reported elsewhere (4, 9, 12, 13, 20). The majority of urological symptoms occurred within the first six months of indinavir-treatment, but the incidence remained elevated thereafter albeit at a reduced steady rate.

Risk factors for urological symptoms were low LBM, undetectable HIV-1 RNA (≤ 1000 copies/mL) at the start of indinavir and inherent to this prior stopping of an antiretroviral drug

due to any type of intolerance and the absence of concomitant co-trimoxazole use. In addition, indinavir-regimens of ≥ 1000 mg bid, low weight and monthly environmental temperatures above 18 °C were identified as risk factors. Neither gender nor concomitant use of co-trimoxazole, which have been suggested as risk factors before, were associated with urological symptoms in our study (8, 18).

The finding that LBM rather than BMI was associated with urological symptoms can be explained by the fact that a low LBM reflects a low distribution volume which may lead to higher indinavir plasma concentrations. Moreover, indinavir has low lipid solubility resulting in low fatty tissue penetration and therefore little protective effect of the amount of fatty tissue (6).

Patients with undetectable HIV-1 RNA at the start of indinavir represent a selection of patients who previously had successful treatment with ART. These patients are more likely to have therapeutic or even high plasma drug concentrations than patients without viral suppression. As a consequence they may be more susceptible to plasma concentration dependent adverse effects such as indinavir associated urological symptoms [13]. We can only speculate on this association since we did not measure indinavir plasma concentrations. However, a large proportion of patients with viral suppression had prior intolerance leading to a treatment change, which confirms the fact that these patients may indeed be more prone to development of adverse effects (26). Another possible cause of urological symptoms in this group of patients might be for example nausea leading to less fluid intake and increased urine concentration.

The finding that indinavir dosing regimens of ≥ 1000 mg bid, which achieve higher indinavir plasma peak concentrations (Cmax) and larger areas under the curve (AUC), were associated with urological symptoms is consistent with earlier reports of the relation between urological symptoms and high plasma indinavir concentrations (13, 27, 28). It is not known which pharmacokinetic parameter, high Cmax or increased AUC, is the more important determinant of the risk for urological symptoms. Unfortunately the number of patients using the indinavir/ritonavir combination of 800/100 mg bid, a regimen with a Cmax comparable to that of indinavir 800 mg tid and a large AUC (28, 29), was too small to differentiate between the effects of high Cmax and large AUC.

It has been reported before that a high environmental temperature increases the risk of urological symptoms (17, 20). As suggested before, reduced urine production as a consequence of increased perspiration is the most likely explanation for the association between environmental temperatures and urological symptoms. The current advice for patients to drink at least 1.5 L per day may not be sufficient under warm weather circumstances (30).

Potential limitations of this study mainly concern information bias and bias due to diagnostic suspicion for urological symptoms among indinavir users rather than users of other HIV-protease inhibitors. We dealt with these biases as much as possible. To avoid information bias, we included only patients who started in 1997 or thereafter, thus eliminating a period of accustomisation to the new HIV-treatment entities and preventing major underreporting due to relative unawareness of the symptoms. Still, sub-clinical urological symptoms of indinavir such as mild renal insufficiency that goes undetected if renal function is not regularly assessed may have been underreported. Furthermore, certain urological symptoms may have been misdiagnosed as urinary tract infection. Underreporting or misclassification may have led to an underestimation of the incidence rate. It is however unlikely that it has affected the identification of risk factors for urological symptoms since we matched on treatment centre within which the degree of reporting and preference for indinavir-regimens can be expected to be equal between patients. Diagnostic suspicion bias is unlikely to have played a role in the identification of risk factors either as we restricted this analysis to indinavir users only. Potential effect modifying factors in the observed associations such as dietary changes and fluid intake were not available for analysis.

Given the fact that indinavir plasma concentrations play an important role in the development of urological symptoms, inhibitors of the cytochrome P450 CYP3A4 as well as hepatic dysfunction might aggravate the effects observed in this study as effect modifiers (9, 16, 31-33). As the use of interacting drugs probably is rare, because of an automated warning system for incompatible drug combinations as operated by the community pharmacists in the Netherlands (34), and the prevalence of clinically overt hepatitis is low in our cohort (2%), these factors are unlikely to have caused a major impact on our results.

In conclusion, we found an incidence rate of urological symptoms of up to 8.3 per 100 person years of indinavir treatment in this large population based cohort study. Low LBM, low HIV-1 RNA at the start of indinavir, the use of indinavir-dosing regimens higher than the standard dosing of 800 mg tid and warm weather days were all identified as independent risk factors for the development of urological symptoms. As high indinavir plasma concentrations appear to play a key role, it may be useful to include indinavir plasma concentration monitoring as a preventive strategy for urological symptoms. Alternative indinavir/ritonavir dosing regimens with lower Cmax and/or smaller AUCs may have to be considered. Our study focussed on the identification of risk factors for urological factors. Any of the proposed control measures will have to be tested for their ability to reverse and prevent urological symptoms. Finally, the results endorse the need for increasing fluid intake during warm weather days or comparable circumstances during which urine output may be decreased.

References

1. Deeks SG, Smith M, Holodniy M, Kahn JO. HIV-1 protease inhibitors. A review for clinicians. JAMA 1997;277:145-53.
2. Vanhove GF, Schapiro JM, Winters MA, Merigan TC, Blaschke TF. Patient compliance and drug failure in protease inhibitor monotherapy. JAMA 1996;276:1955-6.
3. Flexner C. HIV-protease inhibitors. N Engl J Med 1998;338:1281-1292.
4. Anonymous. Product Information Crixivan (indinavir sulfate). Haarlem, The Netherlands: Merck Sharp & Dohme B.V.; 1997.
5. Balani SK, Woolf EJ, Hoagland VL et al. Disposition of indinavir, a potent HIV-1 protease inhibitor, after an oral dose in humans. Drug Metab Dispos 1996;24:1389-94.
6. Lin JH, Chen IW, Vastag KJ, Ostovic D. pH-dependent oral absorption of I-735,524, a potent HIV protease inhibitor, in rats and dogs. Drug Metab Dispos 1995;23:730-735.
7. Antony SJ. Rapid development of indinavir-induced asymptomatic crystalluria in a human immunodeficiency virus-negative patient. Clin Infect Dis 1998;27:911-2.
8. Boubaker K, Sudre P, Bally F et al. Changes in renal function associated with indinavir. AIDS 1998;12:F249-54.
9. Brodie SB, Keller MJ, Ewenstein BM, Sax PE. Variation in incidence of indinavir-associated nephrolithiasis among HIV-positive patients. AIDS 1998;12:2433-7.
10. Kopp JB, Miller KD, Mican JA et al. Crystalluria and urinary tract abnormalities associated with indinavir. Ann Intern Med 1997;127:119-25.
11. Padberg J, Fritsche L, Bergmann F, Schurmann D, Suttorp N. Nephropathy and renal colic in patients treated with indinavir, ritonavir plus indinavir or ritonavir plus saquinavir. AIDS 1999;13:2173-4.
12. Reiter WJ, Schon-Pernerstorfer H, Dorfinger K, Hofbauer J, Marberger M. Frequency of urolithiasis in individuals seropositive for human immunodeficiency virus treated with indinavir is higher than previously assumed. J Urol 1999;161:1082-4.
13. Dieleman JP, Gyssens IC, van der Ende ME, de Marie S, Burger DM. Urological complaints in relation to indinavir plasma concentrations in HIV-infected patients. AIDS 1999;13:473-8.
14. Gentile DL, Stoller ML, Jarrett TW, Ward JF, Geib KS, Wood AF. Protease inhibitor-induced urolithiasis. Urology 1997;50:508-11.
15. Gagnon RF, Tsoukas CM, Watters AK. Light microscopy of indinavir urinary crystals. Ann Intern Med 1998;128:321.
16. Perazella MA. Crystal-induced acute renal failure. Am J Med 1999;106:459-65.
17. Bach MC, Godofsky EW. Indinavir nephrolithiasis in warm climates. J Acquir Immune Defic Syndr Hum Retrovirol 1997;14:296-7.
18. Burger DM, Koopmans PP, Brinkman K. Influence of gender on indinavir pharmacokinetics. Sixth European Conference on Clinical Aspects and Treatment of HIV-Infection. Hamburg; 1997:abstract 32.
19. Lin JH, Chiba M, Chen IW, Nishime JA, Vastag KJ. Sex-dependent pharmacokinetics of indinavir: in vivo and in vitro evidence. Drug Metab Dispos 1996;24:1298-306.

20. Martinez E, Leguizamon M, Mallolas J, Miro JM, Gatell JM. Influence of environmental temperature on incidence of indinavir-related nephrolithiasis. Clin Infect Dis 1999;29:422-5.
21. Chiba M, Hensleigh M, Lin JH. Hepatic and intestinal metabolism of indinavir, an HIV protease inhibitor, in rat and human microsomes. Major role of CYP3A. Biochem Pharmacol 1997;53:1187-95.
22. Guardiola JM, Mangues MA, Domingo P, Martinez E, Barrio JL. Indinavir pharmacokinetics in haemodialysis-dependent end-stage renal failure. AIDS 1998;12:1395.
23. Yeh KC, Deutsch PJ, Haddix H et al. Single-dose pharmacokinetics of indinavir and the effect of food. Antimicrob Agents Chemother 1998;42:332-8.
24. Borleffs JJC, Danner SA, Boer K et al. [Guidelines for HIV-suppressing therapy 1998]; 1998.
25. Revision of HIV classification codes. MMWR Morb Mortal Wkly Rep 1988;36:821.
26. Bini T, Testa L, Chiesa E et al. Outcome of a second-line protease inhibitor-containing regimen in patients failing or intolerant of a first highly active antiretroviral therapy. J Acquir Immune Defic Syndr 2000;24:115-122.
27. Gatti G, Vigano A, Sala N et al. Indinavir pharmacokinetics and parmacodynamics in children with human immunodeficiency virus infection. Antimicrob Agents Chemother 2000;44:752-5.
28. van Heeswijk RP, Veldkamp AI, Hoetelmans RM et al. The steady-state plasma pharmacokinetics of indinavir alone and in combination with a low dose of ritonavir in twice daily dosing regimens in HIV-1-infected individuals. AIDS 1999;13:F95-9.
29. Hsu A, Granneman GR, Cao G et al. Pharmacokinetic interaction between ritonavir and indinavir in healthy volunteers. Antimicrob Agents Chemother 1998;42:2784-91.
30. Polhemus ME, Aronson NE. Persistent nephrolithiasis after discontinuation of indinavir therapy. Clin Infect Dis 1998;27:1536.
31. Malavaud B, Dinh B, Bonnet E, Izopet J, Payen JL, Marchou B. Increased incidence of indinavir nephrolithiasis in patients with hepatitis B or C virus infection. Antivir Ther 2000;5:3-5.
32. Melvin DC, Lee JK, Belsey E, Arnold J, Murphy RL. The impact of co-infection with hepatitis C virus and HIV on the tolerability of antiretroviral therapy. AIDS 2000;14:463-5.
33. Schwarz A, Perez-Canto A. Nephrotoxicity of antiinfective drugs. Int J Clin Pharm Ther 1998;36:164-7.
34. de Gier JJ. Clinical pharmacy in primary care and community pharmacy. Pharmacotherapy 2000;20:278S-81S.

Chapter 2.4

Indinavir crystallisation under loop of Henle conditions: experimental evidence

Abstract

Objective: To determine the probable site of the nephron and the plasma indinavir (IDV) concentration at which intra-renal IDV crystallisation occurs.

Design: We performed in-vitro crystallisation experiments in IDV solutions simulating the conditions encountered in the nephron.

Method: To determine intra-renal IDV concentrations at which the conditions in the nephron allow crystallisation, several concentrations of IDV base solutions (0-800 mM) were titrated from pH 4.0 to higher pH values until crystals occurred within one minute. Based on the combination of pH and ionic strength at which crystals occurred, the site of the nephron to which these apply was determined. Based on the concentrating capacity up to that site, the corresponding plasma IDV concentration was calculated.

Results: Under conditions applying to the proximal tubule, pH 6.7 and ionic strength of 200 mM, IDV crystallised at 200 mg/L. Under conditions applying to the loop of Henle, pH 7.4 and ionic strength of 200 mM, IDV crystallised at 125 mg/L, which would correspond to a plasma IDV concentration of 8 mg/L.

Conclusion: IDV crystallisation is most likely to occur in the loop of Henle and may already start at plasma IDV concentrations of 8 mg/L. Increasing hydration will not reduce the risk of IDV crystallisation in the loop of Henle, but rather prevent IDV crystallisation and aggregation in the lower urinary tract. It remains to be confirmed whether prevention of high IDV plasma concentrations will reduce the risk of IDV crystallisation in the loop of Henle.

Introduction

Treatment with the widely prescribed HIV-protease inhibitor indinavir (IDV) is associated with renal intolerance, the spectrum of which ranges from a-symptomatic crystalluria (20-32%) and interstitial nephritis to symptomatic nephrolithiasis (4-12%) (1-4). Renal failure and renal atrophy have been described (5-10).

Mass-spectrometry has shown that urinary crystals and calculi from IDV treated patients primarily consist of the IDV base monohydrate (3,11). Little is known about the exact site of IDV crystal formation. IDV crystals have been found in the cortical and medullar collecting ducts and in interstitial cells of the kidney suggesting that IDV crystallisation may already start in the renal tubuli (3,6,8,12-14). For the development of preventive strategies for renal complications from IDV it is important to understand where and under which conditions intra-tubular IDV-crystal formation may occur.

Oral IDV is rapidly absorbed and 24% of the ingested dose is excreted and secreted into urine within 24 hours about half of which as the parent molecule, IDV monohydrate (12,15,16). IDV monohydrate is a weak base with pKa values of 5.9 and 3.7. It is poorly soluble in aqueous solutions of high pH (17). Given the physico-chemical properties of IDV, intra-tubular crystallisation of IDV most likely is supersaturation driven.

During passage of the glomerular filtrate (GF) through the tubuli the fluid passes zones of varying pH-values and ionic strength (IS) while the GF is increasingly concentrated. These conditions have been described in a model taking into account normal glomerular filtration rate, tubular function, tubular reabsorption and secretion processes, tubular dimensions and tubular flow rates (table 1) (18-20). In brief, the pH reaches a maximum of 7.4 in the longest loops of Henle (LH), coinciding with an high IS and high GF concentration. Subsequently the pH gradually decreases to urine pH and the GF is further concentrated depending on the hydration status of the body. The model has been used previously for example to explain how calcium salt crystalluria can be a frequent finding while de novo calcium salt crystal formation in urine is difficult. The reason is that the conditions in urine do not reflect the intra-tubular conditions (18-20).

The lowest solubility for IDV in the renal tubuli is probably reached in the LH due to the high pH values. Since the conditions in the respective parts of the nephron are relatively stable under normal physiological conditions, variations in the local IDV concentration depend primarily on variations in the unbound fraction of plasma IDV that is filtered by the glomeruli. Not taking into account the IS in the tubuli and assuming a two-fold secretion component, it can be calculated that the fluid in the LH becomes saturated at plasma IDV concentrations of 1.5 mg/L (16,17). However, it is not known at which level of saturation

(supersaturation) crystallisation occurs, if IS affects the solubility of IDV and if crystals can grow to a large enough size during the available time in the nephron.

We performed crystallisation experiments taking into account the existing conditions in the nephron in order to determine the part of the nephron in which IDV crystallisation is most likely to occur and to determine the plasma IDV concentration needed for this.

Methods

In the crystallisation experiments the IS and the pH were varied to simulate the normal physiological conditions in the various segments of the nephron as described in the model presented in table 1. Based on the plasma IDV concentrations of patients with urological complaints and the known conditions in the tubuli, IDV concentrations of 105 mg/L to 260 mg/L may be expected in the LH of these patients (Figure 1).

Table 1 Model describing the changing physiological conditions and fluid compositions during passage through the various parts of the nephron (19,20)

Part of the nephron	PH	IS (mM)	Passage time (s)	Degree of concentration
GF	7.25	136-145	0	0
PT	6.7	140-200	24	3
LH	7.4	200-870	40	20
Ascending LH	6.7	870-73	100	5-20
DT[a]	Regulation to urine pH	73 to 30-50	31	5-20
CD[a]	Regulation to urine pH	30-50 to 45-582	29-49	20-200

GF= glomerular ultra filtrate, PT= proximal tubule, LH= loop of Henle, DT= distal tubule, CD= collecting duct, IS= ionic strength

a Conditions largely depend on the hydration status of the body

Crystallisation experiments

Varying amounts of pure IDV base (IDV monohydrate) provided by Merck & Co., were dissolved to concentrations of 100, 125, 150, 200, 250, 500 and 750 mg/L in purified water solutions with IS of 0, 200, 400 and 800 mM NaCl at a low pH of 4.0. In a test run the pH required for supersaturation and particle formation was established. Particle formation was monitored by means of optical density of the solution (21). Subsequently, three runs of the same experiment were done in which the IDV solutions were titrated to higher pH-values with 0.1 mM NaOH to rapidly approach the state of supersaturation and titrated further with 0.01 nM NaOH until particle formation (crystallisation) occurred within one minute. One minute was considered as the time that a given test solution must remain particle free in order to allow a safe passage through the specific part of the nephron (18,19). The pH-value

at which particle formation occurred was recorded. Solutions were filtered through a 0.1 µm nucleopore polycarbonate filter after one minute and the precipitate was examined for presence of IDV crystals by infrared spectrometry, light microscopy and scanning electron microscopy. All experiments were performed in triplicate at room temperature. Results were expressed as the average pH-value at which particle formation (crystallisation) occurred at a certain IS and IDV concentration.

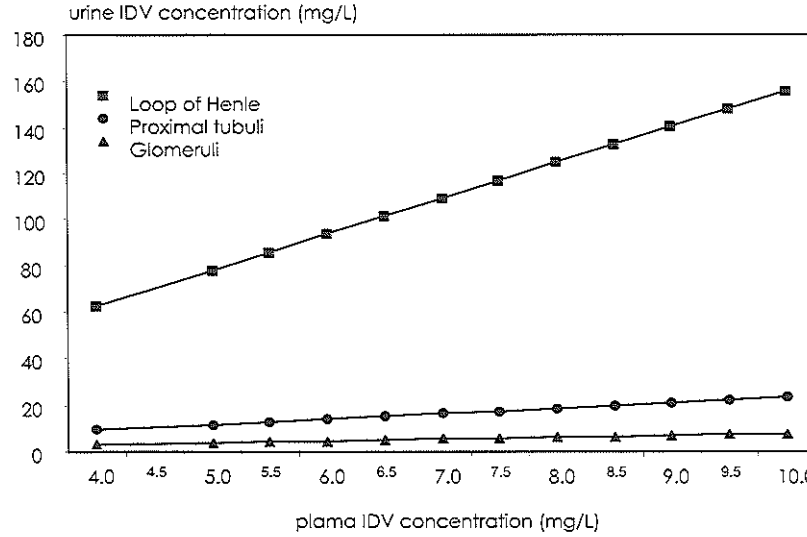

Figure 1 indinavir (IDV) concentrations expected in the respective parts of the nephron given certain plasma IDV concentrations
Figure shows the relationship between relevant IDV concentrations encountered in plasma (x-axis) and the nephron (y-axis) as predicted by the model described in table 1. The glomeruli harbour a pH of 7.4, along the proximal tubuli the pH decreases to 6.7 and in the longest loops of Henle the pH reaches 7.4. Solubility data suggest that the fluid in the loop of Henle is saturated with IDV at all indicated plasma IDV concentrations (17).

The part of the nephron to which this pH and IS applied was determined according to table 1. The degree of fluid concentration present in this part of the nephron was then used to calculate the IDV plasma concentration at which crystallisation would first occur, assuming 100% filtration of plasma IDV, a two-fold secretion component in the PT and 61% plasma protein binding of IDV (Figure 2).

$$[IDV]_{plasma}{}^{a} = \frac{[IDV]_{nephron}{}^{b}}{\text{IDV secretionc} * \text{concentrating capacityd} * \text{fraction of filterable plasma IDVe}}$$

Figure 2 Interpolation to plasma indinavir (IDV) concentrations

a Plasma IDV concentration
b IDV concentration in the part of the nephron of interest
c IDV secretion in the proximal tubulus estimated at 2 (16)
d Estimated concentrating capacity in the part of the nephron of interest (20 for the LH)
e Estimated free fraction of filterable plasma IDV= 0.39 (39%)

Results

Table 1 shows the fluid compositions (IS and pH) during passage of GF through the various parts of the nephron. Up to the descending LH, the pH first decreases and then increases to arrive at a maximum of 7.4. The IS of the filtrate also increases to a maximum in the descending LH where the concentrating capacity is highest. From the ascending LH to the distal tubule the pH decreases to urine pH and the IS first decreases and then increases in the collecting ducts to its final urine value.

Crystallisation occurred very rapidly after exceeding supersaturation. Table 2 shows the pH and IS at which certain IDV concentrations formed crystals within one minute. According to table 1 the only sites of the nephron to which these conditions of pH and IS apply are the PT and the LH. Crystallisation occurred at a local IDV concentration of 200 mg/L and 125 mg/L in the PT and LH respectively. Light and scanning electron microscopy analysis demonstrated the typical starburst appearance of IDV crystals, which are also seen in IDV crystalluria (3,9,22,23).

Assuming 39% unbound plasma IDV, a normal glomerular and tubular function and a two-fold secretion component, an IDV concentration of 200 mg/L at the end of the PT corresponds to a plasma IDV concentration of 85 mg/L, which is not attainable with current IDV dosing regimens. An IDV concentration of 125 mg/L at the end of the descending limb of the LH would correspond to a total plasma IDV concentration of 8 mg/L, which is attainable with current dosing regimens. These results imply that intra-tubular IDV crystallisation probably is unlikely to occur at plasma IDV concentrations below 8 mg/L, but might occur at plasma concentrations above this level. Crystallisation first occurs at the bend of the LH and probably does not occur in the PT or the descending and ascending limbs of the LH.

Table 2 pH (SD) at which indinavir (IDV) crystals are formed within one minute for a given IDV concentration and ionic strength

$[IDV]_{GF}$ (mg/L)	Ionic Strength (mM)			
	0 mM	200 mM	400 mM	800 mM
125	7.36 (0.04)	***7.41 (0.01)***[a]	7.53 (0.04)	7.50 (0.02)
150	7.08 (0.02)	***7.23 (0.23)***[a]	***7.34 (0.09)***[a]	***7.44 (0.05)***[a]
200	6.01 (0.11)	***6.65 (0.13)***[b]	6.86 (0.04)	6.80 (0.12)
250	5.79 (0.08)	6.10 (0.04)	6.31 (0.01)	6.29 (0.04)
500	5.17 (0.03)	5.41 (0.06)	5.65 (0.02)	5.61 (0.03)
750	4.95 (0.03)	5.31 (0.02)	5.47 (0.05)	5.42 (0.06)

$[IDV]_{GF}$ = IDV concentration in the glomerular filtrate. Values are pH-values with one standard deviation between brackets (n=3); bold italic print indicates physiological conditions present in the renal tubuli

a Physiological conditions present in the loop of Henle

b Physiological conditions present in the proximal tubuli

Discussion

The results of the crystallisation experiments support the hypothesis that the risk of IDV crystal formation in the nephron is highest under conditions existing at the bend of the LH. It was also shown that crystal formation occurs upon exceeding saturation and that crystals can grow rapidly within the available passage time once supersaturation is reached. Our data do not provide an exact upper limit for tolerable plasma IDV concentrations, but suggest that IDV crystallisation in the LH might already occur at IDV plasma concentrations of 8 mg/L, which should be interpreted as an indication rather than a statement.

Since concentrations of 8 mg/L are common with IDV regimens of 800 mg tid (24), intra-renal IDV crystallisation might be a frequent event not necessarily leading to clinically apparent nephrolithiasis. A previous study showed that clinical symptoms were associated with plasma IDV concentrations of more than twice the population mean (25). Intra-renal crystallisation might even be more frequent with ritonavir boosted IDV regimens, such as twice daily 800 mg IDV combined with small doses ritonavir, since plasma IDV peak concentrations are increased (16,26-29). In addition, such boosted regimens yield an increased risk of IDV supersaturation in the lower urinary tract because of the longer periods of high plasma IDV concentrations, resulting in larger amounts of IDV excreted into urine (16,28).

Increasing hydration, which is the current advice for controlling urological complications from IDV, will only increase the urine flow beyond the LH in the collecting duct and therefore be ineffective against the potential formation and effects of IDV crystals in the LH (1,7). The increased urine flow and dilute urine caused by increasing hydration rather is effective against crystal aggregation and new IDV crystallisation in the lower parts of the urogenital tract. Adequate hydration therefore is essential in the prevention and alleviation of urological complications, but may not always be sufficient. Given a direct

association between the free fraction of plasma IDV and the degree of supersaturation in the LH, avoidance of high plasma IDV concentrations by means of dose adjustments might be an effective control measure against IDV crystal formation in the LH. In doing so it must be ensured that adequate plasma IDV trough concentrations are maintained for optimal viral suppression (24), for which the combination of indinavir with ritonavir offers possibilities.

The results of this study are based on a model that has been used successfully to explain calcium salt crystallisation processes (18). Obviously these results need substantiation in a clinical setting. In addition, the following points should be taken into consideration. First, the IDV concentration of 8 mg/L as the suggested upper tolerable concentration may need to be corrected for variations in plasma protein binding and secretion of IDV. We assumed a free fraction of IDV of 39% and a two-fold secretory component (1,12,16,28). The degree of plasma protein binding appears to vary from 54 to 70% and is lower at higher plasma IDV concentrations, suggesting an additive risk for high plasma peak concentrations (30).

Second, we did not take into consideration the possibility of re-absorption of IDV. Weak bases like IDV may have the capacity to migrate along the epithelium into the interstitium. Re-absorption in the PT would lead to lower intra-tubular IDV concentrations and less risk of crystallisation.

Third, the experiments were conducted at room temperature whereas physiologic temperature is 37°C. Since IDV solubility might be slightly better at higher temperatures the actual risk of IDV crystallisation might be slightly lower. Furthermore, we assumed that IDV crystallisation in the LH is a sole consequence of supersaturation independent of any promoters or inhibitors. Other factors, such as proteins, might either promote or delay the IDV crystallisation process. Calcium phosphate crystals, which are usually present in the LH, might for example promote IDV crystallisation, whereas citrate may inhibit this process (31-34).

Finally, we assumed normal glomerular and tubular function. Reduced glomerular filtration would lead to lower intra-tubular IDV concentrations and hence a lower risk of intra-tubular crystallisation. Tubular dysfunction might lead to higher intra-tubular pH values and hence a higher risk of crystallisation. However, given the concentrating capacities of the various parts of the nephron, the pH in the PT needs to be extremely high to induce IDV crystallisation. In the distal tubuli a pH of 7.4 is needed to equal the risk of IDV crystallisation in the LH. In the collecting duct the risk of crystallisation may easily be increased at higher pH because of the high IDV concentrations reached. An association between urine pH and IDV crystals has indeed been described elsewhere (2,34,35).

In conclusion, the results suggest that plasma IDV concentrations of 8 mg/L may already cause IDV crystallisation in the LH, the conditions of which cannot be influenced by modifying hydration. Therefore, dose adjustments to minimise the maximum plasma IDV

concentrations while maintaining adequate trough concentrations might be indicated for patients with urological adverse effects from IDV. The conclusions are based on the assumptions that the model employed is an accurate approximation of the changing conditions in the nephron, that there is no tubular re-absorption of IDV, normal renal function and no influence of promoters and inhibitors. It remains to be confirmed whether avoidance of plasma IDV concentrations of 8 mg/L reduces the risk of IDV associated urological complications in a clinical setting.

References

1. Anonymous. Product Information Crixivan (indinavir sulfate). Haarlem, The Netherlands: Merck Sharp & Dohme B.V., 1997.
2. Hortin GL, King C, Miller KD, Kopp JB. Detection of indinavir crystals in urine: dependence on method of analysis. Arch Pathol Lab Med 2000;124:246-50.
3. Kopp JB, Miller KD, Mican JA, Feuerstein IM, Vaughan E, Baker C, Pannell LK, Falloon J. Crystalluria and urinary tract abnormalities associated with indinavir. Ann Intern Med 1997;127:119-25.
4. Reiter WJ, Schon-Pernerstorfer H, Dorfinger K, Hofbauer J, Marberger M. Frequency of urolithiasis in individuals seropositive for human immunodeficiency virus treated with indinavir is higher than previously assumed. J Urol 1999;161:1082-4.
5. Berns JS, Cohen RM, Silverman M, Turner J. Acute renal failure due to indinavir crystalluria and nephrolithiasis: report of two cases. Am J Kidney Dis 1997;30:558-60.
6. Hanabusa H, Tagami H, Hataya H. Renal atrophy associated with long-term treatment with indinavir. N Engl J Med 1999;340:392-3.
7. Perazella MA. Crystal-induced acute renal failure. Am J Med 1999;106:459-65.
8. Tashima KT, Horowitz JD, Rosen S. Indinavir nephropathy. N Engl J Med 1997;336:138-40.
9. Tsao JW, Kogan SC. Images in clinical medicine. Indinavir crystalluria. N Engl J Med 1999;340:1329.
10. Witte M, Tobon A, Gruenenfelder J, Goldfarb R, Coburn M. Anuria and acute renal failure resulting from indinavir sulfate induced nephrolithiasis. J Urol 1998;159:498-9.
11. Daudon M, Estepa L, Viard JP, Joly D, Jungers P. Urinary stones in HIV-1-positive patients treated with indinavir. Lancet 1997;349:1294-5.
12. Anonymous. Crixivan® (indinavir sulfate) product monograph. Whitehouse Station: Merck & Co., 1996.
13. Marroni M, Gaburri M, Mecozzi F, Baldelli F. Acute interstitial nephritis secondary to the administration of indinavir. Ann Pharmacother 1998;32:843-4.
14. Martinez F, Mommeja-Marin H, Estepa-Maurice L, Beaufils H, Bochet M, Daudon M, Deray G, Katlama C. Indinavir crystal deposits associated with tubulointerstitial nephropathy. Nephrol Dial Transplant 1998;13:750-3.
15. Balani SK, Woolf EJ, Hoagland VL, Sturgill MG, Deutsch PJ, Yeh KC, Lin JH. Disposition of indinavir, a potent HIV-1 protease inhibitor, after an oral dose in humans. Drug Metab Dispos 1996;24:1389-94.

16. Hsu A, Granneman GR, Cao G, Carothers L, Japour A, El-Shourbagy T, Dennis S, Berg J, Erdman K, Leonard JM, Sun E. Pharmacokinetic interaction between ritonavir and indinavir in healthy volunteers. Antimicrob Agents Chemother 1998;42:2784-91.
17. Lin JH, Chen IW, Vastag KJ, Ostovic D. pH-dependent oral absorption of I-735,524, a potent HIV protease inhibitor, in rats and dogs. Drug Metab Dispos 1995;23:730-5.
18. Kok DJ, Khan SR. Calcium oxalate nephrolithiasis, a free or fixed particle disease. Kidney Int 1994;46:847-54.
19. Kok DJ. Crystallization and stone formation inside the nephron. Scann Microsc 1996;10:471-86.
20. Kok DJ. Intratubular crystallization events. World J Urol 1997;15:219-28.
21. Antinozzi PA, Brown CM, Purich DL. Calcium oxalate monohydrate crystallization: Citrate inhibition of nucleation and growth steps. J Crystal Growth 1992;125:215-22.
22. Gagnon RF, Tsoukas CM, Watters AK. Light microscopy of indinavir urinary crystals. Ann Intern Med 1998;128:321.
23. Schwartz BF, Schenkman N, Armenakas NA, Stoller ML. Imaging characteristics of indinavir calculi. J Urol 1999;161:1085-7.
24. Burger DM, De Graaff M, Wuis EW, Kooopmans PP, Hekster YA. Determination of indinavir, an HIV-protease inhibitor, in human plasma by reversed-phase high-performance liquid chromatography. J Chromatogr B 1997;703:235-41.
25. Dieleman JP, Gyssens IC, van der Ende ME, de Marie S, Burger DM. Urological complaints in relation to indinavir plasma concentrations in HIV-infected patients. AIDS 1999;13:473-8.
26. Casado JL, Moreno A, Martí-Belda P, Sabido R, Garcia-Arata I, Perez-Elias MJ, Muñoz V, Moreno S. Increased Indinavir Levels Using Twice Daily Ritonavir/Indinavir at 100/800mg Improves Virological Response even after Multiple Failure (abstract no. 1170) 40th Interscience Conference on Antimicrobial Agents and Chemotherapy. Toronto, 2000:301.
27. van Heeswijk RP, Veldkamp AI, Hoetelmans RM, Mulder JW, Schreij G, Hsu A, Lange JM, Beijnen JH, Meenhorst PL. The steady-state plasma pharmacokinetics of indinavir alone and in combination with a low dose of ritonavir in twice daily dosing regimens in HIV-1-infected individuals. AIDS 1999;13:F95-9.
28. Hugen PWH, Burger DM, ter Hofstede HJM, Koopmans PP, Stek M, Hekster YA, Reiss P, Lange JMA. Dose-finding study of a once-daily indinavir/ritonavir regimen. J Acquir Immune Defic Syndr 2000;25:236-45.
29. Mallolas J, Blanco JL, Saerasa M, Giner V, Martinez E, Garcia-Viejo MA, Arnaiz JA, Cruceta A, Soy D, Tuset M, Soriano A, Codina C, Pumarola T, Carne X, Gatell JM. Dose-finding study of once-daily indinavir/ritonavir plus zidovudine and lamivudine in HIV-infected patients. J Acquir Immune Defic Syndr 2000;25:229-35.
30. Anderson PL, Brundage RC, Bushman L, Kakuda TN, Remmel RP, Fletcher CV. Indinavir plasma protein binding in HIV-1-infected adults. AIDS 2000;14:2293-7.
31. Dussol B, Berland Y. Urinary kidney stone inhibitors. What is the news? Urol Int 1998;60:69-73.
32. Gentle DL, Stoller ML, Jarrett TW, Ward JF, Geib KS, Wood AF. Protease inhibitor-induced urolithiasis. Urology 1997;50:508-11.

33. Hess B, Jordi S, Zipperle L, Ettinger E, Giovanoli R. Citrate determines calcium oxalate crystallization kinetics and crystal morphology - studies in the presence of Tamm-Horsfall protein of a healthy subject and a severely recurrent calcium stone former. Nephrol Dial Transplant 2000;15:366-74.
34. Kohan AD, Armenakas NA, Fracchia JA. Indinavir urolithiasis: an emerging cause of renal colic in patients with human immunodeficiency virus. J Urol 1999;161:1765-8.
35. Daudon M, Estepa L, Kebede M, Viard JP, Montagnac R, Deray G, Bricaire F. [Urinary calculi and crystalluria in HIV+ patients treated with indinavir sulfate]. Presse Med 1997;26:1612-5..

Chapter 2.5

Persistent leukocyturia and loss of renal function in a prospectively monitored cohort of HIV-infected patients treated with indinavir

Abstract

Background: Symptomatic nephrotoxicity is a well-known complication of indinavir. However, little is known about the clinical relevance of leukocyturia during use of indinavir.

Objectives: To estimate the prevalence of leukocyturia and to determine risk factors for persistent leukocyturia and changes in renal function.

Methods: Consecutive patients on indinavir visiting three adult outpatient clinics, underwent urinalysis at each regular visit (once every three months) between August 1998 and September 2000. Urinalysis included dipstick pH, erythrocytes and leukocytes, microscopy for indinavir crystals and urine albumin/creatinine ratio. Serum creatinine and indinavir plasma concentrations were measured and urological symptoms were retrieved from medical records. Presence of urinary tract infection was excluded.

Results: 184 patients underwent at least one urinalysis, 35% of whom had leukocyturia (ie. >75 cells/μL) at any time during the study period. Leukocyturia coincided with mild albumin loss, erythrocyturia and crystalluria. Thirty-two of 134 (24%) patients with two or more assessments had persistent leukocyturia (ie. ≥2 occasions). Risk factors were indinavir plasma concentration above 9 mg/L, urine pH above 5.7 and crystalluria. Persistent leukocyturia was associated with a gradual loss of renal function, but was not associated with urological symptoms.

Conclusion: Leukocyturia is a frequent finding which is associated with a loss of renal function independently of urological symptoms. Patients should be monitored for renal function during indinavir treatment. Indinavir plasma concentrations, urine pH and indinavir crystalluria are associated with persistent leukocyturia.

Introduction

The HIV-protease inhibitor indinavir is widely used in the treatment of HIV-infection. As prolonged viral suppression can now be achieved by chronic treatment with highly active antiretroviral treatment (HAART), long-term adverse effects become of increasing concern.

Indinavir may cause symptoms of nephrolithiasis through the formation of indinavir crystals, particularly in urine with a high pH (1-5). However, damage to the tubular epithelium may also occur without symptoms. This sub-clinical phase can be followed by symptomatic renal injury (6). There have been several reports of renal failure (7-10), renal atrophy (11, 12), interstitial nephritis (6, 13-15) and papillary necrosis (16) following the use of indinavir.

We performed a prospective cohort study among patients on indinavir to systematically screen for sub-clinical signs of nephrotoxicity with the objective to estimate the prevalence of mild and non-symptomatic urine abnormalities as indicator of mild renal injury (17-19). Furthermore, we determined risk factors for persistent leukocyturia and changes in renal function.

Patients and methods

Setting

Between August 1, 1998 and September 1, 2000 the outpatient clinics of the Erasmus Medical Centre Rotterdam (Erasmus MC), the University Medical Centre Nijmegen and the Walcheren Hospital in Vlissingen in the Netherlands carried out an indinavir nephrotoxicity monitoring program, which was approved of by the Institutional Review Board. Patients treated with an indinavir containing antiretroviral regimen were monitored on their three-monthly visits to the outpatient clinic.

For the present analysis, our cohort comprised all patients 18 years and older in the monitoring program (both prevalent and incident users of indinavir). Cohort members were followed from the moment of inclusion in the monitoring program until the end of the study period, one visit after discontinuation of indinavir, departure from the clinic or death, whichever event occurred first.

Laboratory assessments

We assessed midstream urinary pH, presence of leukocytes, erythrocytes, crystals and bacteria in urine, proteinuria as a marker of tubular damage (urine albumin and urine creatinine concentration ratio) as well as serum creatinine levels and indinavir plasma

concentrations. Assessment of urinary pH, presence of leukocytes, erythrocytes and bacteria was done by means of a dipstick analysis (Combur-Test M, Roche DiagnosticsGmbH, Mannheim, Germany). Presence of leukocytes, erythrocytes and bacteria was confirmed by light microscopy. Urine light microscopy using a polarisation filter was performed to identify the presence of indinavir crystals. Urine samples were analysed with a maximum delay of two hours after voiding.

Urine samples from five consecutive patients with qualitative signs of leukocyturia were used for leukocyte differentiation on cytospins. For this purpose fresh urine samples were centrifuged at 1400 rounds per minute (rpm) at 6°C for 10 minutes. The resulting pellet was washed twice in 500 µL phosphate-buffer solution (PBS) with centrifugation steps in between. The final cell suspension was diluted to reach a cell density of $2*10^6$ cells/mL. Cytospin filtration paper on a slide was humidified by adding 50 µL of 0.9% sodium chloride solution, which was rotated at 500 rpm for one minute. Subsequently, 50 µL cell suspension was added and the slide was rotated again at 50 rpm for ten minutes. Finally, slides were fixated and stained with the May-Grünwald-Giemsa staining method after which an experienced laboratory technician performed cell differentiation.

Creatinine clearance was estimated from serum creatinine using the equation of Cockcroft and Gault (20). At one hour after the last ingestion of indinavir, seven mL of blood was sampled from an antecubital vein into a lithium heparin vacutainer, for estimation of the maximum indinavir plasma concentrations. Indinavir plasma concentrations were determined at the University Hospital in Nijmegen, the Netherlands according to a previously validated high performance liquid chromatography assay (21). The maximal plasma indinavir concentration for each patient was used for data analysis.

Additional data collection

Baseline data regarding serum creatinine levels and weight prior to the start of indinavir and details on height, indinavir treatment, concomitant medication, HIV-1 RNA, CD4 cell counts and urological symptoms during treatment were collected from the medical records. Urological symptoms included notes of renal colic, flank pain, the passing of a stone, dysuria and gross hematuria and were validated independently by three authors blinded to the presence of leukocyturia, indinavir dose frequency and indinavir plasma concentration. Symptoms were classified as either indicators of nephrolithiasis, such as the passing of a stone and flank pain with or without hematuria, or other associated urological symptoms not attributable to urinary tract infection.

Case definition

Since we considered sterile leukocyturia as an indicator for mucosal damage in the urinary tract, this was the primary outcome of our study. We considered sterile leukocyturia to be present if the dipstick test showed more than 75 cells/μL and urinary tract infection was absent. We further determined the presence of persistent sterile leukocyturia (persistent leukocyturia), defined as the presence of sterile leukocyturia on two or more occasions during the use of indinavir, since persistent rather than incidental mild renal injury is likely to have more serious implications.

For diagnosis of urinary tract infection we applied the CDC-definitions for nosocomial infections based on clinical symptoms, dipstick analysis and urine culture (22). Urine culture was performed if signs of bacteria were detected by dipstick or urine light microscopy or if there was suspicion of urinary tract infection. The results of urine cultures were judged by an experienced infectious diseases specialist.

Secondary outcomes were erythrocyturia (ie. more than 60 erythrocytes/μL by dipstick analysis), crystalluria (more than two indinavir crystals per 40x magnified field), mild albumin loss (ie. albumin/creatinine > 3.5 mg/μmol), urological symptoms and a decrease in renal function (ie. more than 45 μmol/L increase in serum creatinine since the start of indinavir).

Statistical analyses

To describe the prevalence of leukocyturia and other urological abnormalities over time we first categorized all measurements into 12-week assessments thereby ignoring extra intermediate assessments (which may have been triggered by abnormal findings at routine monitoring). Proportions and 95%-confidence intervals (95%CI) were calculated based on a binomial distribution. The six-month hazard of leukocyturia among incident users of indinavir was calculated by means of Kaplan-Meier survival analysis. Associations between the presence of leukocyturia and other urine abnormalities were tested with a Chi-square test or Fisher's exact test for categorical variables and the Mann-Whitney U test for continuous non-normally distributed variables in a cross-sectional analysis of the first assessment of prevalent indinavir users.

In a second step, we calculated the prevalence of persistent leukocyturia among patients who had two or more urine assessments during the use of indinavir. Potential risk factors for persistent leukocyturia were identified through univariate and multi-variate risk ratio analysis. Results were expressed as relative risks (RR) with 95%CI. In the subpopulation of patients with two or more urine assessments we also compared renal function (serum creatinine and creatinine clearance) during follow-up between patients with and without persistent leukocyturia by means of General Linear Model for repeated measurements

adjusted for baseline values. For all analyses statistical significance was accepted at a two-sided p-value lower than 0.05.

Results

During the monitoring period, we included 184 adult patients with a median age of 39 years of whom 80% were male. Patients were predominantly prevalent users of indinavir (n=161, 88%), who had been using indinavir for a median of 67 (inter quartile range [IQR]: 12-109) weeks prior to entry in the monitoring program (Table 1). The overall median follow-up while being monitored was 48 (IQR: 24-72) weeks. Patients who were monitored from the start of indinavir use (incident users) had a median follow-up of 39 (14-64) weeks. During follow-up, we obtained a total of 622 urine samples with a median of three analyses (IQR: 1-4) per patient.

Table 1 Characteristics of patients included in the indinavir nephrotoxicity monitoring program (n=184)

Characteristic at the first assessment		
Age (years)	39	(IQR: 34-48)
Male	148	(80%)
Lean body mass (kg) [n=168]	72	(IQR: 65-77)
CDC-C classification [n=164]	66	(40%)
Hepatitis C infection [n=76]	11	(14%)
Concomitant use of Co-trimoxazole	43	(23%)
Prevalent use of indinavir	161	(88%)
Weeks on indinavir (prevalent users)	67	(IQR: 12-109)
CD4 cell count [n=140]	330	(IQR: 170-508)
HIV-1 RNA < 500 copies/mL [n=84]	46	(55%)

IQR= inter quartile range

During follow-up 64 (35%, 95%CI: 28-42) patients had sterile leukocyturia in at least one urine specimen. Eight of 23 (35%, 95%CI: 16-54) incident indinavir users presented with sterile leukocyturia with a six-month cumulative hazard of 0.31 (95%CI: 0.20-0.36). The prevalence of crystalluria was highest at the start of the monitoring program (Figure 1). Erythrocyturia and urological symptoms were constant at a prevalence of approximately 10% of patients every three months. We did not observe any seasonal variation in the prevalence of symptoms.

Cross-sectional analysis of the first assessment of prevalent indinavir users showed that sterile leukocyturia (n=19, 20%) was associated with erythrocyturia (p=0.043) and mild albumin loss (p=0.008) but not with crystalluria (p=0.393) or urological symptoms (p=0.905) (Figure 2). Presence of leukocyturia coincided with greater increases in serum creatinine

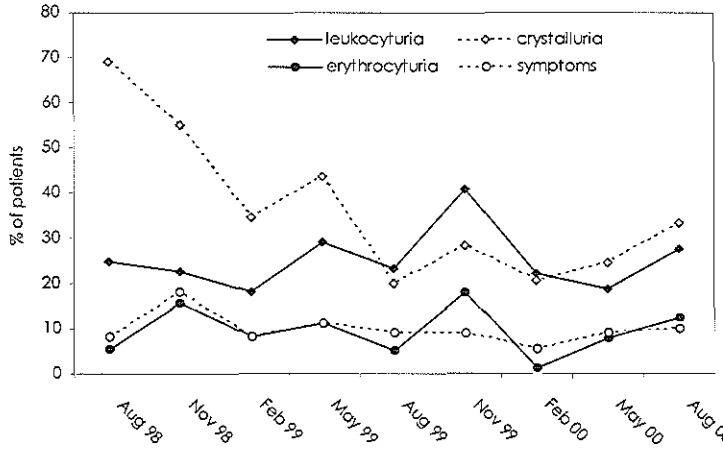

Figure 1 Urine abnormalities and urological symptoms during follow-up over calendar time

from baseline (p=0.008). Leukocyturia was the least prevalent (4%) among patients using indinavir 400 mg with ritonavir 400 mg bid (n=25), whereas the prevalence during other indinavir regimens (800 mg tid, n=89; 800 mg with 100 mg ritonavir bid, n=30; other, n=16) ranged from 20% to 30%. Urinary pH did not differ between samples with and without leukocyturia (p=0.740).

Persistent sterile leukocyturia

A total of 134 patients provided two or more urine samples during use of indinavir and thereby defined the subpopulation for the assessment of abnormalities associated with sterile leukocyturia and with persistent sterile leukocyturia (persistent leukocyturia). Reasons for not having two urine samples were omitting sampling during outpatient clinic visits (n=20), discontinuation of indinavir (n=16), late study entrance (n=8) and lost to follow-up (n=6). Apart from an under-representation of incident indinavir users (9% vs 22%) and patients with CDC-C stage of HIV-infection (35% vs 58%) in those with two or more visits versus those with one visit, both groups did not differ.

During follow-up 56 (42%, 95%CI:34-50) patients with two or more visits ever presented with sterile leukocyturia. Persistent sterile leukocyturia was observed in 32 (24%, 95%CI: 17-31) patients, 10 (31%) of whom were newly identified (ie. leukocyturia was absent at cohort entry) during follow-up. Leukocyte differentiation (n=5) showed that leukocyturia consisted predominantly of neutrophils (76%) and a small proportion of eosinophils (3%).

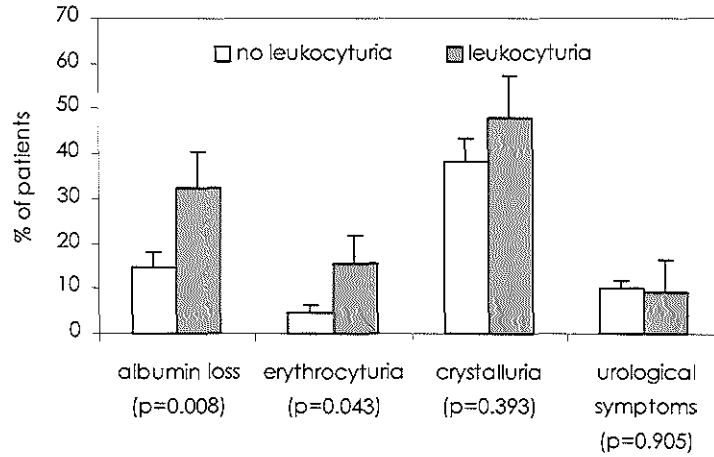

Figure 2 Urine abnormalities associated with leukocyturia (n=19) in a cross-sectional analysis of the first urine assessment among prevalent users of indinavir (n=161). Small bars indicate standard errors.

Thirteen (41%) of 32 patients with persistent leukocyturia also presented with symptoms of nephrolithiasis (n=8) or other urological symptoms (n=5), which was not significantly different from patients without persistent leukocyturia (RR=1.5, 95%CI: 0.7-3.1). Three patients discontinued indinavir because of urological symptoms.

Risk factors for persistent leukocyturia

As potential risk factors for persistent leukocyturia we identified high plasma indinavir concentrations as well as urine pH and crystalluria (Table 2). The median plasma indinavir concentration one hour after indinavir ingestion was 7.3 (IQR: 4.2-9.6, n=90). Patients with concentrations above 9 mg/L had a 2.6-fold increased risk for persistent leukocyturia compared to patients with concentrations lower than or equal to 9 mg/L. Patients with an average urine pH value above the cohort median of 5.7 (IQR: 5.3-6.1) had a 3.1-fold increased risk for persistent leukocyturia and patients with crystalluria (n=39, 29%) had a 2.4-fold increased risk (Table 2). These factors remained independent predictors upon multivariate analysis modelling.

Age, gender, lean body mass, CDC-C stage of HIV-infection, hepatitis C infection, the use of concomitant co-trimoxazole, prevalent indinavir use at cohort entry, time on indinavir at cohort entry, CD4 cell count and HIV-1 RNA and were not associated with persistent leukocyturia.

Table 2 Potential risk factors for persistent sterile leukocyturia among patients who had at least two assessments in the indinavir nephrotoxicity monitoring program (n=134)

Parameter	N (%) / Median (IQR)		RR (95% CI)* Univariate		Multivariate#	
Median plasma indinavir concentration (mg/L)Ψ	7.3	(IQR: 4.2-9.6)	1.1	(0.98-1.2)		
> 6 mg/L vs ≤ 6 mg/L	55	(60)	1.4	(0.6-3.4)		
> 7 mg/L vs ≤ 7 mg/L	50	(55)	1.8	(0.7-4.3)		
> 8 mg/L vs ≤ 8 mg/L	41	(45)	2.1	(0.9-5.1)		
> 9 mg/L vs ≤ 9 mg/L	29	(32)	2.6	(1.1-5.9)	2.3	(1.01-5.5)
Urine pH > 5.7 vs ≤ 5.7 mg/L♣	66	(49)	3.1	(1.4-6.9)	3.4	(1.3-8.7)
Indinavir crystalluria vs no crystalluria	39	(29)	2.4	(1.2-4.9)	2.3	(0.99-5.5)

IQR= inter quartile range; RR= relative risk; CI= confidence interval;

* calculated by means of Cox-regression analysis with a fixed follow-up time

\# plasma indinavir concentration above or below 9 mg/L, urine pH and crystalluria included into the model

Ψ maximum value measured at one hour after ingestion (n=90)

♣ mean dipstick pH value per patient over all assessments during follow-up (median= 5.7)

The one patient with persistent leukocyturia who switched to 400 mg indinavir with 400 mg ritonavir twice daily and five of six patients who stopped indinavir experienced resolution of leukocyturia within three months. Persistent leukocyturia did not resolve in the four patients who switched to 800 mg indinavir with 100 mg indinavir.

Renal function in patients with persistent leukocyturia

Patients with persistent leukocyturia showed a statistically significant increase in serum creatinine during follow-up compared to patients without persistent leukocyturia (Figure 3, p=0.027). The creatinine clearance rate reduced accordingly. A quarter (26%) of patients with persistent leukocyturia had an increase of more than 45 µmol/L in serum creatinine since the start of indinavir (range: 47 to 76 µmol/L). Creatinine concentrations did not differ between patients with or without urological symptoms and men or women. Serum creatinine concentrations did not normalise within three months after discontinuation of indinavir.

Discussion

In this prospective cohort study of patients on indinavir we observed a high rate of leukocyturia associated with mild albuminuria, indinavir crystalluria and micro-hematuria. Persistent leukocyturia as represented by persistent presence of leukocyturia, was observed in almost 25% of patients, most of whom were prevalent users of indinavir at the start of our monitoring program. Even though persistent leukocyturia remained sub-clinical, it was

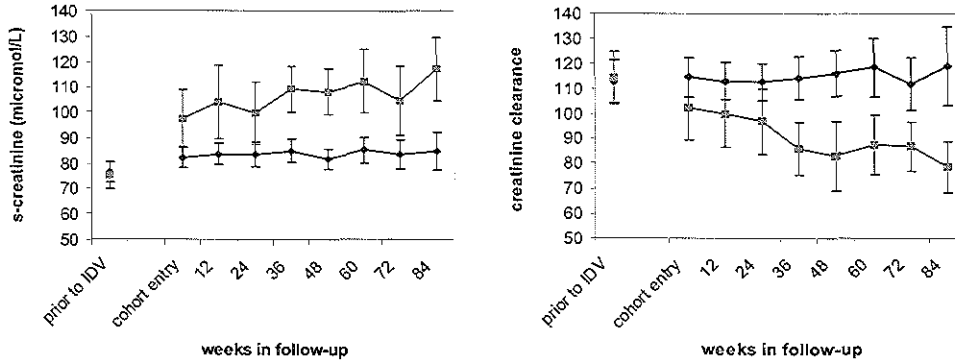

Figure 3 Course of mean serum creatinine (s-creatinine) and mean creatinine clearance during indinavir (IDV) treatment (n=134) for patients with (n=32, square marks) and patients without (triangle marks) persistent sterile leukocyturia. Bars represent 95%-confidence intervals. IDV= indinavir

accompanied by a gradual loss of renal function, an observation, which has been described before (17).

As with urological symptoms, persistent leukocyturia was associated with high indinavir plasma concentrations (23). The risk of persistent leukocyturia was increased at plasma concentrations of 9 mg/L or higher at one hour after indinavir ingestion. It was our aim to estimate the maximum plasma concentration (Cmax) for each patient by drawing blood samples one hour after ingestion. It is, however, possible that we hence underestimated the Cmax among patients who took ritonavir concomitantly (24-26), especially among those taking 800 mg bid indinavir with 100 mg bid ritonavir.

The risk of persistent leukocyturia was also increased at a urine pH above 5.7, which may be related to the poor solubility of indinavir at high pH values (5). A high urine pH might be caused by a high pH of the glomerular filtrate in the proximal renal tubuli as can be seen with tubular dysfunction, but it might be caused by dietary influences in the distal tubuli as well.

We observed an association between the detection of indinavir crystalluria and persistent leukocyturia, which is in line with the assumption that indinavir crystals are part of the patho-physiological mechanism underlying leukocyturia (1, 2, 17, 18, 27). The assessment of indinavir crystals in urine samples should, however, be regarded with caution since crystals might be formed in standing urine in the bladder or canister (28). Moreover, the high prevalence of crystalluria at the start of the monitoring program suggests the presence of bias regarding assessment of crystalluria. Overestimation of crystalluria at the beginning might have occurred due to initial diagnostic suspicion by laboratory technicians or by

longer lag periods between voiding and urine analysis in the beginning. On the other hand, underestimation of crystalluria at the end might have occurred due to decreased alertness.

Persistent leukocyturia occurred independently of factors such as gender, hepatitis C and concomitant use of co-trimoxazole, which have previously been reported to be associated with urological symptoms (3, 19, 29-34).

It is not known if continued renal injury as indicated by persistent leukocyturia will eventually lead to severe renal damage. So far, none of the patients with persistent leukocyturia in our population developed severe renal failure during follow-up. However, on the basis of our data we cannot exclude the risk of irreversible renal damage after prolonged exposure.

Since our study was based on a monitoring program that did not apply exclusion criteria there are several limitations. The most important limitation is the low number of incident indinavir users who were followed from the start of therapy. This implied that we could not estimate the incidence of and lag time for persistent leukocyturia. It also implied the absence of baseline measurements before the start of indinavir. However, in absence of the baseline rate of leukocyturia, the positive de-challenge after discontinuation of indinavir at least pointed towards a causal relation between indinavir and leukocyturia. Since we had many prevalent users of indinavir, there was a potential selection of patients who are able to tolerate indinavir. Such a bias might have caused an underestimation of the actual prevalence and effects on renal function by depletion of susceptibles.

In conclusion, we observed a high prevalence of indinavir associated (persistent) leukocyturia. Leukocyturia was not always accompanied by urological symptoms. This subclinical nephrotoxicity is of clinical relevance given the reduction in renal function, which we observed. It emphasizes the need for systematic monitoring of renal function during the use of indinavir. The long-term consequences are not known at present, but should be monitored by regular assessment of renal function and imaging procedures. Alternatively, there should be studies on the efficacy and effectiveness of regimens with lower indinavir plasma concentrations since increasing hydration alone may not protect against leukocyturia.

References

1. Kopp JB, Miller KD, Mican JA, et al. Crystalluria and urinary tract abnormalities associated with indinavir. Ann Intern Med 1997;127:119-125.
2. Clayman RV. Crystalluria and urinary tract abnormalities associated with indinavir. J Urol 1998;160:633.

3. Trainor LD, Steinberg JP, Austin GW, Solomon HM. Indinavir crystalluria: identification of patients at increased risk of developing nephrotoxicity. Arch Pathol Lab Med 1998,122:256-259.
4. Antony SJ. Rapid development of indinavir-induced asymptomatic crystalluria in a human immunodeficiency virus-negative patient. Clin Infect Dis 1998,27:911-912.
5. Yeh KC, Deutsch PJ, Haddix H, et al. Single-dose pharmacokinetics of indinavir and the effect of food. Antimicrob Agents Chemother 1998,42:332-338.
6. Kopp JB, Miller KD, Falloon J. Indinavir and interstitial nephritis. Ann Intern Med 1998,128:320-321.
7. Berns JS, Cohen RM, Silverman M, Turner J. Acute renal failure due to indinavir crystalluria and nephrolithiasis: report of two cases. Am J Kidney Dis 1997,30:558-560.
8. Chen SC, Nankivell BJ, Dwyer DE. Indinavir-induced renal failure. AIDS 1998,12:440-441.
9. Witte M, Tobon A, Gruenenfelder J, Goldfarb R, Coburn M. Anuria and acute renal failure resulting from indinavir sulfate induced nephrolithiasis. J Urol 1998,159:498-499.
10. Perazella MA. Crystal-induced acute renal failure. Am J Med 1999,106:459-465.
11. Hanabusa H, Tagami H, Hataya H. Renal atrophy associated with long-term treatment with indinavir. N Engl J Med 1999,340:392-393.
12. Cattelan AM, Trevenzoli M, Naso A, Meneghetti F, Cadrobbi P. Severe hypertension and renal atrophy associated with indinavir. Clin Infect Dis 2000,30:619-621.
13. Sarcletti M, Petter A, Zangerle R. Indinavir and interstitial nephritis. Ann Intern Med 1998,128:320.
14. Marroni M, Gaburri M, Mecozzi F, Baldelli F. Acute interstitial nephritis secondary to the administration of indinavir. Ann Pharmacother 1998,32:843-844.
15. Jaradat M, Phillips C, Yum MN, Cushing H, Moe S. Acute tubulointerstitial nephritis attributable to indinavir therapy. Am J Kidney Dis 2000,35:E16.
16. Dieleman JP, van der Feltz M, Bangma CH, Stricker BH, van der Ende ME. Papillary necrosis associated with the HIV protease inhibitor indinavir. Infection 2001,29:232-233.
17. Gagnon RF, Tecimer SN, Watters AK, Tsoukas CM. Prospective study of urinalysis abnormalities in HIV-positive individuals treated with indinavir. Am J Kidney Dis 2000,36:507-515.
18. Gagnon RF, Tecimer SN, Watters AK, Hatzakis GE, Tsoukas CM. The natural history of leukocyturia associated with indinavir treatment in HIV+ individuals. Am J Nephrol 2000,20:448-454.
19. Sarcletti M, Petter A, Romani N, et al. Pyuria in patients treated with indinavir is associated with renal dysfunction. Clin Nephrol 2000,54:261-270.
20. Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. Nephron 1976,16:31-41.
21. Hugen PW, Verweij-van Wissen CP, Burger DM, Wuis EW, Koopmans PP, Hekster YA. Simultaneous determination of the HIV-protease inhibitors indinavir, nelfinavir, saquinavir and ritonavir in human plasma by reversed-phase high-performance liquid chromatography. J Chromatogr B Biomed Sci Appl 1999,727:139-149.
22. Garner JS, Jarvis WR, Emori TG, Horan TC, Hughes JM. CDC definitions for nosocomial infections, 1988. Am J Infect Control 1988,16:128-140.
23. Dieleman JP, Gyssens IC, van der Ende ME, de Marie S, Burger DM. Urological complaints in relation to indinavir plasma concentrations in HIV-infected patients. AIDS 1999,13:473-478.

24. Hsu A, Granneman GR, Cao G, et al. Pharmacokinetic interaction between ritonavir and indinavir in healthy volunteers. Antimicrob Agents Chemother 1998,42:2784-2791.
25. Hugen PWH, Burger DM, ter Hofstede HJM, et al. Dose-finding study of a once-daily indinavir/ritonavir regimen. J Acquir Immun Def Syndr 2000,25:236-245.
26. Burger DM, Hugen PW, van der Ende ME, et al. Once-daily indinavir plus ritonavir: preliminary results of the PIPO study. AIDS 2000,14:2621-2623.
27. Blake SP, McNicholas MM, Raptopoulos V. Nonopaque crystal deposition causing ureteric obstruction in patients with HIV undergoing indinavir therapy. AJR Am J Roentgenol 1998,171:717-720.
28. Salahuddin S, Hsu YS, Buchholz NP, Dieleman JP, Gyssens IC, Kok DJ. Is indinavir crystalluria an indicator for indinavir stone formation? AIDS 2001,15:1079-1080.
29. Anonymous. Product information Crixivan (indinavir sulfate). In. Haarlem, The Netherlands: Merck Sharp & Dohme B.V.; 1998.
30. Martinez E, Leguizamon M, Mallolas J, Miro JM, Gatell JM. Influence of environmental temperature on incidence of indinavir-related nephrolithiasis. Clin Infect Dis 1999,29:422-425.
31. Brodie SB, Keller MJ, Ewenstein BM, Sax PE. Variation in incidence of indinavir-associated nephrolithiasis among HIV-positive patients. AIDS 1998,12:2433-2437.
32. Rich JD, Ramratnam B, Chiang M, Tashima KT. Management of indinavir associated nephrolithiasis. J Urology 1997,158:2228.
33. Boubaker K, Sudre P, Bally F, et al. Changes in renal function associated with indinavir. AIDS 1998,12:F249-254.
34. Malavaud B, Dinh B, Bonnet E, Izopet J, Payen JL, Marchou B. Increased incidence of indinavir nephrolithiasis in patients with hepatitis B or C virus infection. Antivir Ther 2000,5:3-5.

Chapter 2.6

Persistent sterile leukocyturia is associated with impaired renal function in HIV-1 infected children treated with indinavir

Abstract

Background: Prolonged administration of indinavir is associated with the occurrence of a variety of renal complications in adults. These well documented side effects have restricted the use of this potent protease inhibitor in children.

Design: A prospective study to monitor indinavir-related nephrotoxicity in a cohort of 30 HIV-1 infected children treated with indinavir.

Methods: Urinary pH, albumin, creatinine, the presence of erythrocytes, leukocytes, bacteria, and crystals, and culture were analyzed every 3 months for 96 weeks. Serum creatinine levels were routinely determined at the same time points. Steady state pharmacokinetics of indinavir were done at week 4 after the initiation of indinavir.

Results: The cumulative incidence of persistent sterile leukocyturia (≥75 cells/μL in at least 2 consecutive visits) after 96 weeks was 53%. Persistent sterile leukocyturia was frequently associated with a mild increase in the urine albumin/creatinine ratio and by microscopic hematuria. The cumulative incidence of serum creatinine levels more than 50% above normal was 33% after 96 weeks. Children with persistent sterile leukocyturia more frequently had serum creatinine levels of 50% above normal (p=0.02) than those children without persistent sterile leukocyturia. In children younger than 5.6 years, persistent sterile leukocyturia was significantly more frequent(p= 0.05) than in older children. A higher cumulative incidence of persistent leukocyturia was found in children with an AUC >19 (mg/L*h) or a peak serum level of indinavir >12 (mg/L) (p=0.05 and p=0.02, respectively). In 4 children indinavir was discontinued because of nephrotoxicity. Subsequently, the serum creatinine levels decreased, the urine albumin/creatinine ratios returned to zero and the leukocyturia disappeared within 3 months.

Conclusion: Children treated with indinavir have a high cumulative incidence of persistent sterile leukocyturia. Children with persistent sterile leukocyturia more frequently had an increase in serum creatinine levels of more than 50% above normal. Younger children have an additional risk for renal complications. The impairment of the renal function in these children occurred in the absence of clinical symptoms of nephrolithiasis. Indinavir-associated nephrotoxicity must be monitored closely, especially in children with risk factors such as persistent sterile leukocyturia, age <5.6 years, an AUC of indinavir >19 (mg/L*h), and a Cmax >12(mg/L).

Introduction

Indinavir is a potent HIV-protease inhibitor that has been used successfully in adults in combination with nucleoside reverse transcriptase inhibitors to suppress infections by HIV-1. Discontinuation of the antiretroviral therapy rapidly results in virologic rebound, decreased immune function, and the redevelopment of AIDS-defining illness. Thus, antiretroviral drugs such as indinavir need to be continued for many years. This necessitates a careful surveillance for long-term toxicity of the medication.

The experience with the administration of indinavir to HIV-1-infected children has been limited because of the absence of a pediatric formulation and the well-documented side-effects of the drug on the upper and lower urinary tract in adults. Indinavir is metabolized by the liver, but approximately 20% of a single oral dose is excreted unchanged in the urine. (1) PH dependent crystallization of indinavir in renal tubuli may cause renal symptoms such as kidney stones, flank pain (even without evident stone formation), interstitial nephritis, elevation of the serum creatinine, dysuria and asymptomatic urine abnormalities such as hematuria, leukocyturia and crystalluria. indinavir crystals may illicit an inflammatory response in the *tubules*, leading to sterile leukocyturia and renal insufficiency. (2-7) To *minimize* renal side effects of indinavir, an increased fluid intake is advised. (8)

The incidence of indinavir associated nephrolithiasis in adults varies from 4 to 43%. (2, 9) Renal complications (including nephrolithiasis) from indinavir are found in 0 to 80% (10-15) and nephrolithiasis in 0 to 20% (10, 12-15) of children who have been treated with this drug.

Renal and urologic symptoms of indinavir crystals have been correlated with the serum levels of indinavir. (16) In children, sufficiently high area under the plasma-concentration curves (AUCs) for indinavir are required to achieve trough levels (13, 17) associated with an optimal virologic response. (18-20) The risk of nephrotoxicity in children might therefore be higher than in adults.

In contrast to symptomatic nephrolithiasis, other renal complications such as leukocyturia, microscopic hematuria, and crystalluria usually do not lead to a decision to discontinue indinavir, although an association between recurrent severe leukocyturia and renal damage by indinavir-induced crystalluria has been reported in adults. (6, 21)

Currently it is unknown whether and when these asymptomatic signs of renal damage lead to renal complications and long-term renal damage. This prospective study was performed to monitor renal and urinary complications in a cohort of 30 HIV-1-infected children treated with indinavir. We hypothesized that indinavir-related nephrotoxicity might

occur more frequently in children than in adults due to the higher risk for cellular damage to the still developing renal system.

Methods

In 1997 a prospective, open, uncontrolled, multicenter study was initiated to evaluate the clinical, immunologic and virologic response to combination therapy consisting of indinavir, zidovudine, and lamivudine in HIV-1-infected children (15). Children >3 months of age and one of the following 2 items: a decreased CD4+ T-cell count (<1 year: <1750/mm^3, 1-2 years: <1000/mm^3, 3-6 years: <750/mm^3, >6 years: <500/mm^3) or a HIV-1 RNA load >5000 copies/mL were included. The follow-up period was 96 weeks after the initiation of indinavir. Two years after the initiation of this multicenter study a separate study was started in one of the participating centers to analyze additional urinalysis parameters with a follow-up period of 96 weeks.

The Ethics Committee of the University Hospital Rotterdam approved the study. Patients and their caretakers provided written informed consent.

Laboratory parameters

The routinely analyzed laboratory parameters of the children included dipstick analysis *(Rapignost® total screen L, Behring Diagnostics Inc. Westwood, USA)* for urinary pH (at urine pH values below 5 solubility of indinavir increases (22)), erythrocytes, leukocytes and bacteria at baseline (before the use of indinavir) and every three months thereafter. Routine biochemistry tests included serum creatinine. Steady state pharmacokinetics of indinavir (400 mg/m^2 every 8 hours) were determined at week 4 after the initiation of indinavir. This procedure was repeated when a dosage adjustment of indinavir was necessary to normalize the area under the curve-concentration (AUC) curve to adult values (20 mg/L*h, range 10-30 mg/L*h). (17)

Demographic parameters, indinavir start date and stop date, indinavir dosing regimens, urinary tract symptoms, concomitant treatment, HIV-1 RNA, and CD4+ T-cell counts were recorded on structured data collection forms. Nephrolithiasis related symptoms included renal colic, flank pain, the passing of a stone, and gross hematuria.

Additional laboratory tests were performed in children included in one center, from March 1999 onwards. Urinary pH was measured by means of calibrated electrode technique. Urine albumin and creatinine were measured to calculate the albumin to creatinine ratio as an indicator of renal damage. Urine light microscopy for presence of

indinavir crystals under a polarized filter was perfomed and urine cultures were performed in patients with a positive dipstick test or microscopy for bacteria.

Sterile leukocyturia is considered to be caused by damage of renal tubuli. Therefore we considered this the principal endpoint this study. Leukocyturia was defined by the presence of a dipstick test with more than 75 cells/μL. Persistent sterile leukocyturia was present when leukocyturia with negative urine cultures was found at at least two consecutive visits, after the start of indinavir. In children with more than 150 leukocytes/μLat at least two visits and a negative urine culture a renal ultrasound was performed.

Analysis

Cumulative incidences of persistent leukocyturia and serum creatinine more than 50% above age and sex specific normal values (23) were calculated with Kaplan-Meier analysis. The influences of pharmacokinetic factors were determined with the logrank test. The relation between the occurrence of persistent leukocyturia and an increased creatinine and between persistent leukocyturia and nephrolithiasis related symptoms were analyzed using the Fisher's exact test. In order to describe urine abnormailties associated with leukocyturia, we performed a cross-sectional analysis 12 weeks after the start of the measurement of the additional urinalysis.

Results

Thirty HIV-1-infected children were enrolled between April 1997 and April 2000. Fifteen children were available for additional analyses between March 1999 and March 2001. The other 15 children were not available for additional analyses for various reasons: enrollment in a center different from that where the additional analyses were performed (n=8), discontinuation of indinavir (n=6: 5 children because of virological failure and one because of nephrotoxicity) and age > 18 years (n=1). Baseline characteristics of the children are presented in Table 1.

A good clinical, immunologic and virologic response was observed in all children who were treated with indinavir. Most of the children needed 600 mg/m^2 of indinavir every 8 hours to obtain an AUC of indinavir between 10 and 30 mg/L*h. The median (IQR) AUC was 19 (14-28) mg/L*h with a median (IQR) peak level of 9 (6-12) mg/L. Baseline and follow-up serum creatinine and urinalysis data were available from 30 children in whom indinavir was initiated.

Table 1 Patient Characteristics

Characteristic				
Age (y)		Median (range)	5.6	(2.4-9.9)
Male		N (%)	15	(50)
Body mass index (kg/m²)		Median (IQR)	16	(14-17)
HIV-1 RNA (copies/mL)		Median (IQR)	127,500	(18,400-661,000)
CD4 cells (cells/μL)		Median (IQR)	610	(230-880)
Indinavir regimen	400 mg/m² tid	N (%)	8	(27)
	500 mg/m² tid	N (%)	11	(37)
	600 mg/m² tid	N (%)	7	(23)
	≥700 mg/m² tid	N (%)	4	(13)
	500/100 mg/m² bid*	N (%)		
Serum creatinine (μmol/L)		Median (IQR)	24	(19-38)
Urine	leukocytes > 75 cells/μL			
	erythrocytes > 60 cells/μL	N (%)	0	(0)

* indinavir/ritonavir; IQR= interquartile range

The cumulative incidence of persistent sterile leukocyturia (≥2 times ≥75 cells/microliter)

Eleven out of thirty (37%) children developed persistent sterile leukocyturia (two times or more ≥75 leukocytes/μL). The cumulative incidence after 96 weeks was 53% with a mean time to leukocyturia of 74 weeks (95% confidence interval: 61-87 weeks) of combination therapy containing indinavir (Figure 1A).

The influence of age and sex on the cumulative incidence of persistent sterile leukocyturia was determined. Children were divided into two groups: younger and older than the median age of 5.6 years. Figure 1B shows that children younger than 5.6 years had a significantly higher cumulative incidence of persistent sterile leukocyturia than children older than 5.6 years (p=0.05). Sex did not influence the incidence of leukocyturia.

The cumulative incidence of a change of serum creatinine more than 50% above age and sex specific normal values

Six (20%) of 30 children had a change of serum creatinine more than 50% above age and sex specific normal values. The cumulative incidence after 96 weeks was 33% with a mean time to creatinine increase of 90 weeks (95% confidence interval: 82-98 weeks) of combination therapy containing indinavir. (Figure 1C)

Relation between persistent sterile leukocyturia and an increase of serum creatinine

One (5%) of the 19 children without persistent sterile leukocyturia had a change in serum creatinine more than 50% above age and sex specific normal values, whereas 5 (45%) of 11 children with persistent sterile leukocyturia had a change in serum creatinine more than 50% above age and sex specific normal values (p=0.02). The median (IQR) time to a creatinine increase among patients with leukocyturia was 24 (0-48) weeks.

Figure 1A

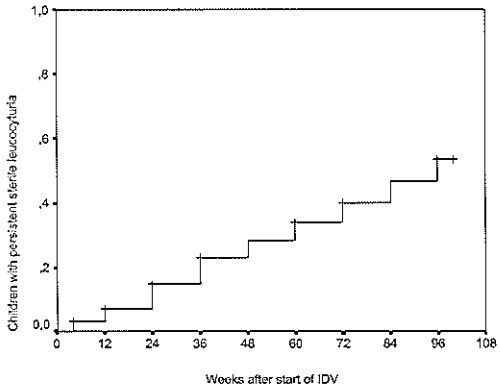

Figure 1B

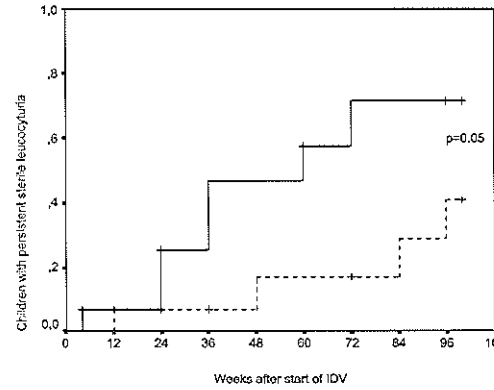

Figure 1C

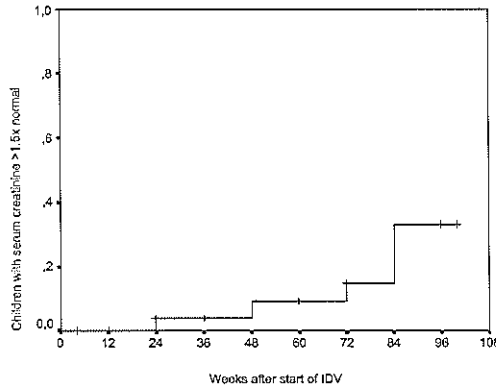

Figure 1D

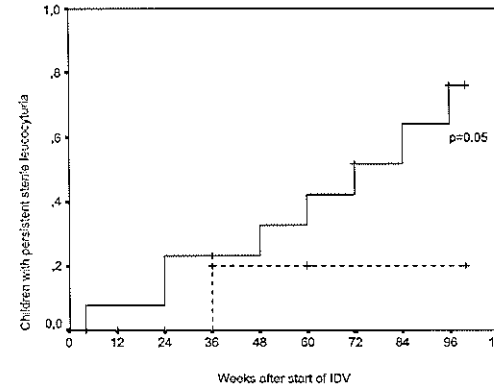

Figure 1E

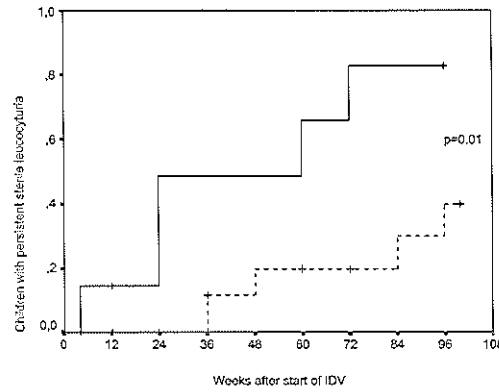

Figure 1A: Cumulative incidence of persistent sterile leukocyturia

Figure 1B: Cumulative incidence of persistent sterile leukocyturia in children < 5.6 years (dotted line) and in children >5.6 years

Figure 1C: Cumulative incidence of children with creatinine increase ≥ 50% above age and sex specific normal values

Figure 1D: Cumulative incidence of persistent sterile leukocyturia in children with AUC of indinavir ≤19 (dotted line) or >19 (mg/L*h)

Figure 1E: Cumulative incidence of persistent sterile leukocyturia in children with Cmax ≤12 (dotted line) or >12 (mg/L)

Relation between persistent sterile leukocyturia and pharmacokinetic parameters
Children with an AUC_{0-8} of indinavir higher than the median AUC of 19 (mg/L*h) had a significantly higher cumulative incidence of persistent sterile leukocyturia compared with those with an AUC ≤19 (mg/L*h) (p=0.05). After 96 weeks 8 (67%) of the 12 children with an AUC>19 (mg/L*h) had persistent sterile leukocyturia, in contrast with 2 (13%) of 16 of the children with an AUC<19 (mg/L*h). The cumulative incidences were 79% and 19%, respectively after 96 weeks. (Figure 1D) Having a maximum concentration (Cmax) of indinavir of 12 (mg/L) was significantly (p=0.02) associated with the presence of persistent sterile leukocyturia. (Figure 1E). Five of the 21 (24%) children with a Cmax <12 (mg/L) had persistent sterile leukocyturia, whereas in 5 of 7 (71%) children with a Cmax ≥12 (mg/L) this abnormality was observed. No relation was found between Cmin and the presence of persistent sterile leukocyturia, between age and AUC of indinavir and between age and Cmax.

Relation between persistent sterile leukocyturia and nephrolithiasis related symptoms
Four (19%) of the 21 children without persistent sterile leukocyturia presented with urological symptoms, whereas 7 (78%) of 9 children with persistent sterile leukocyturia had symptoms during the follow-up time (p=0.003).

Hematuria
Persistent hematuria (≥2x ≥60 cells/μL) was not detected in any child.

In addition to the standard analyses performed in the 30 children, urine creatinine, urine albumin, quantitative pH measurements and crystalluria were analysed in 15 children. At the time of the start of the additional analyses, these children were using indinavir for a median of 75 weeks (interquartile range (IQR): 8-77 weeks).

A cross-sectional analysis at week 12 after the start of the initiation of additional analysis showed that in these children (median time on indinavir (IQR): 87 (20-89) weeks) 33% of the patients had a change of serum creatinine more than 50% above age and sex specific normal values. In four of these 5 patients an albumin/creatinine ratio ≥ 3.5 g/mmol was observed. In two of these patients indinavir was discontinued because of nephrolithiasis on renal ultrasound.

Fourty-three% of the patients had leukocyturia, 21% had microscopic hematuria, 54% had crystalluria and 29% had an albumin/creatinine ratio ≥ 3.5 g/mmol. Urine cultures were all negative for bacteria.

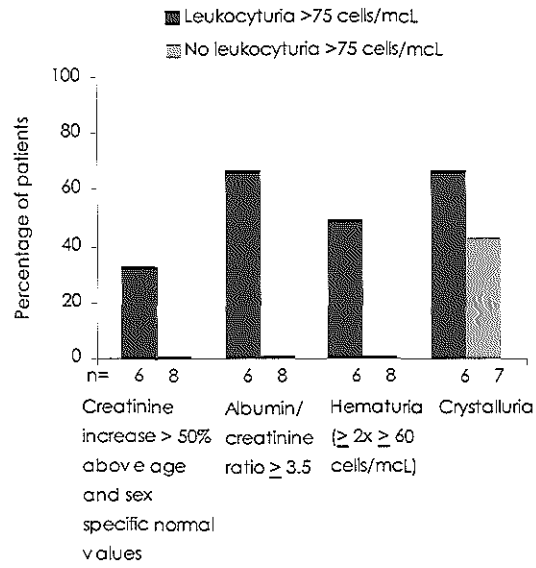

Figure 2 Urinary abnormalities associated with leukocyturia (cross-sectional week 12) in 15 children included in cohort B

In Figure 2 urine abnormalities associated with leukocyturia at week 12 are presented. An increase of serum creatinine levels of more than 50% above age and sex specific normal values, an albumin/creatinine ratio ≥ 3.5 g/mmol and hematuria were observed more frequently in children with leukocyturia. The median (IQR) albumin/creatinine ratio of children with and without persistent leukocyturia was 0.7 (0.6-2.5) and 2.8 (1.2-10.6) respectively. In contrast indinavir crystalluria was not detected more frequently in children with leukocyturia. The presence of symptoms, urinary pH>5, and the presence of crystalluria were not associated with persistent sterile leukocyturia.

Symptoms of nephrotoxicity after discontinuation of indinavir because of nephrotoxicity

In 4of the 15 children indinavir was discontinued because of nephrotoxic symptoms (n=2) or nephrolithiasis on renal ultrasound (n=2). In these children serum creatinine (µmol/l) levels decreased from a median (IQR) of 54 (49-75) at the last observation during the use of indinavir to 39 (28-42) 12 weeks after discontinuation of indinavir (p=0.07). The albumin/creatinine ratio decreased from 16 (8-44) to 0.7 (0.4-2.1) g/mmol (p=0.07). Leukocyturia disappeared within 3 months after the discontinuation of indinavir. Figure 3 shows the serum creatinine levels and the albumin/creatinine ratios of the four children that discontinued indinavir because of nephrotoxicity. Urine albumin/creatinine ratio increases

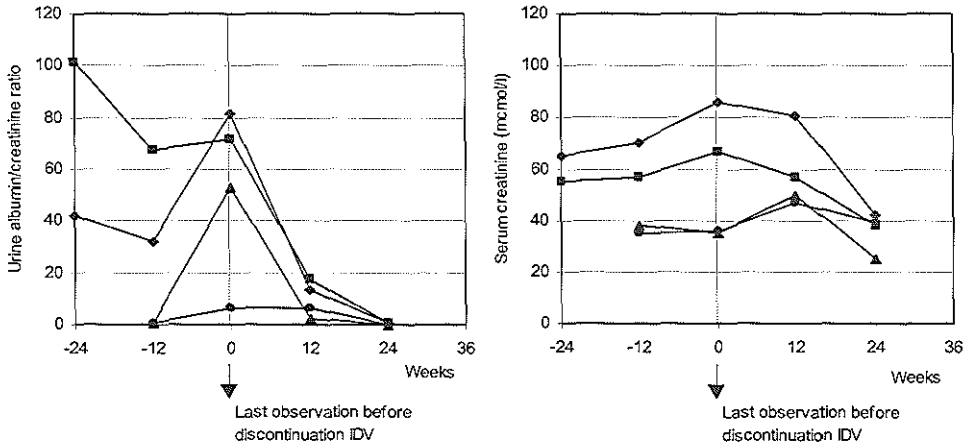

Figure 3 Urine albumin/creatinine ratio (g/mmol) and creatinine levels (µmol/l) during the use of indinavir and afer discontinuation of indinavir in 4 children.

preceded serum creatinine increases and may therefore be an early marker of renal impairment.

In three other children that discontinued indinavir for other reasons (virologic failure, n=2, and because of the poor taste of indinavir, n=1) serum creatinine levels did not decrease, whereas the albumin/creatinine ratio showed a decrease from 3.94 (0.51-11.2) to 0 g/mmol (p=0.10).

Discussion

We here present the first study to monitor nephrotoxicity in HIV-1 infected children with a prolonged treatment with indinavir. We hypothesized that indinavir related nephrotoxicity might occur more frequently in children than in adults due to the higher risk for cellular damage to the still developing renal system.

In our study a cumulative incidence of persistent sterile leukocyturia (≥2x ≥ 75 cells/µL) of 53% was observed after 96 weeks. This persistent sterile leukocyturia was frequently accompanied by a mild increase of the urine albumin/creatinine ratio and microscopic hematuria. The cumulative incidence of an increase in serum creatinine levels more than 50% above normal was 35% after 96 weeks. Children with persistent sterile leukocyturia more frequently had an increase of serum creatinine levels of more than 50%

above normal (p=0.02). This suggests that persistent sterile leukocyturia is an early indication for the development of renal damage.

Recently Gagnon et al. found a significant reduction in the renal function of 3 adults with recurrent severe leukocyturia. Our data confirm the observations in adults of Gagnon et al., that reduction of renal function is associated with recurrent severe leukocyturia, but not with isolated hematuria or crystalluria. (21) Renal damage resulting in leukocyturia and an increased creatinine may be a result of irritation of the tubular epithelium. This is supported by the pathologic finding of tubulointerstitial nephritis in patients on therapy with indinavir. (4, 24, 25)

The prevalence of persistent leukocyturia in adults screened on the same nephrotoxicity monitoring program was 22% (personal communication), which is substantially lower than we observed in children. One might hypothesize that indinavir crystals more easily congest in the small tubuli of young children which may lead to a higher incidence of nephrolithiasis. The more frequent presence of persistent sterile leukocyturia in younger children confirms this observation. However, nephrolithiasis was only diagnosed by renal ultrasound in two asymptomatic children with persistent sterile leukocyturia (26). Renal ultrasounds of the other children with persistent leukocyturia showed no nephrolithiasis. Since it is well documented that the occurrence of indinavir nephrolithiasis increases with a poor hydration status and high environmental temperatures (27), the Dutch climate with relatively moderate temperatures may contribute to a lower incidence of nephrolithiasis in our patients. Persistent leukocyturia might have been prevented by an increased fluid intake. Since it is more difficult to achieve a large fluid intake in young children, a relatively small fluid intake in younger children may be the cause of the more frequent occurrence of persistent sterile leukocyturia in children younger than 5.6 years (cumulative incidence after 96 weeks: 78%).

We did not observe an association between indinavir crystalluria and leukocyturia. Since indinavir crystals can develop in the urine canister (28), it is possible that crystalluria reflects the time lapse between urine collection and urinalysis.

We observed a higher cumulative incidence of persistent leukocyturia in children with an $AUC_{0\text{-}8}$ of indinavir of >19 (mg/L*h) and in children with a peak level of indinavir higher than 12 (mg/L). This is in accordance with previous publications on the relation between levels of indinavir and urological complications in adults. An AUC of indinavir less than 20 (mg/L*h) is associated with virological failure (17). This observation complicates the treatment of HIV-1-infected children with indinavir: to achieve optimal virologic suppression an AUC higher than 20 (mg/L*h) is required, but to avoid persistent leukocyturia an AUC less than 19 (mg/L*h) is needed.

These observations suggest that indinavir may be less useful in the treatment of HIV-1-infected children. However, indinavir is a very potent protease inhibitor which in combination with nucleoside analogues gives an excellent long-term clinical, virologic and immunologic response in adults and in children. (15, 29, 30) We therefore propose to monitor nephrotoxicity very closely in children treated with indinavir and change therapy only in the case of overt signs of renal impairment. In this respect it is reassuring that the signs of renal impairment are reversible after discontinuation of indinavir. Serum creatinine levels decreased in the 4 children with signs of nephrotoxicity who discontinued indinavir. The urine albumin/creatinine ratio returned to zero in all patients. It still remains unclear whether renal impairment is reversible in all stages of damage or that a chronic renal insufficiency will develop above a critical level of cellular damage.

Conclusion

Prolonged therapy with indinavir is associated with a high risk for persistent sterile leukocyturia in children especially in those younger than 5.6 years. The presence of sterile leukocyturia is associated with a significant increase in serum creatinine levels. A high AUC (>19 (mg/L*h)) and high peak levels (>12 (mg/L) of indinavir are associated with the occurrence of leukocyturia. Therapeutic drug monitoring of indinavir serum levels is therefore essential to estimate the risk of nephrotoxicity. Children with risk factors for the development of nephrotoxicity such as an age <5.6 years, AUC of indinavir > 19 (mg/L*h), Cmax >12 (mg/L)) should be monitored routinely by means of urinalysis and analysis of serum creatinine levels.

References

1. Balani SK, Arison BH, Mathai L, et al. Metabolites of L-735,524, a potent HIV-1 protease inhibitor, in human urine. Drug Metab Dispos. 1995;23:266-270.
2. Kopp JB, Miller KD, Mican JA, Feuerstein IM, et al. Crystalluria and urinary tract abnormalities associated with indinavir [see comments]. Ann Intern Med. 1997;127:119-125.
3. Tashima KT, Horowitz JD, Rosen S. Indinavir nephropathy. N Engl J Med. 1997;336:138-140.
4. Marroni M, Gaburri M, Mecozzi F, Baldelli F. Acute interstitial nephritis secondary to the administration of indinavir. Ann Pharmacother. 1998;32:843-844.
5. Martinez F, Mommeja-Marin H, Estepa-Maurice L, et al. Indinavir crystal deposits associated with tubulointerstitial nephropathy. Nephrol Dial Transplant. 1998;13:750-753.
6. Perazella MA, Kashgarian M, Cooney E. Indinavir nephropathy in an AIDS patient with renal insufficiency and pyuria. Clin Nephrol. 1998;50:194-196.
7. Sarcletti M, Zangerle R. Persistent flank pain, low-grade fever, and malaise in a woman treated with indinavir. AIDS Patient Care STDS. 1999;13:81-87.

8. Merck and Co. Indinavir Sulfate. West Point, Pennsylvania March 1996.
9. Saltel E, Angel JB, Futter NG, et al. Increased prevalence and analysis of risk factors for indinavir nephrolithiasis. J Urol. 2000;164:1895-1897.
10. Wintergerst U, Hoffmann F, Solder B, et al. Comparison of two antiretroviral triple combinations including the protease inhibitor indinavir in children infected with human immunodeficiency virus. Pediatr Infect Dis J. 1998;17:495-499.
11. Monpoux F, Sirvent N, Cottalorda J, Mariani R, Lefbvre JC. Stavudine, lamivudine and indinavir in children with advanced HIV-1 infection: preliminary experience [letter]. AIDS 1997;11(12):1523-5.
12. Mueller B, Sleasman J, Nelson R, et al. A phase I/II study of the protease inhibitor indinavir in children with HIV infection. Pediatrics. 1998;102:100-109.
13. Gatti G, Vigano A, Sala N, et al. Indinavir pharmacokinetics and parmacodynamics in children with human immunodeficiency virus infection. Antimicrob Agents Chemother. 2000;44:752-755.
14. Melvin AJ, Mohan KM, Arcuino LA, et al. Clinical, virologic and immunologic responses of children with advanced human immunodeficiency virus type 1 disease treated with protease inhibitors. Pediatr Infect Dis J. 1997;16:968-974.
15. Van Rossum AM, Niesters HG, Geelen SP, et al. Clinical and virologic response to combination treatment with indinavir, zidovudine, and lamivudine in children with human immunodeficiency virus-1 infection: A multicenter study in The Netherlands. J Pediatr. 2000;136:780-788.
16. Dieleman JP, Gyssens IC, van der Ende ME, de Marie S, Burger DM. Urological complaints in relation to indinavir plasma concentrations in HIV-infected patients. AIDS. 1999;13:473-478.
17. Burger DM, van Rossum AM, Hugen PW, et al. Pharmacokinetics of the protease inhibitor indinavir in human immunodeficiency virus type 1-infected children. Antimicrob Agents Chemother. 2001;45:701-705.
18. Burger DM, Hoetelmans RMW, Hugen PWH, et al. Low plasma concentrations of indinavir are related to virological treatment failure in HIV-1-infected patients on indinavir-containing triple therapy. Antivir Ther. 1998;3:215-220.
19. Harris M, Durakovic C, Rae S, et al. A pilot study of nevirapine, indinavir, and lamivudine among patients with advanced human immunodeficiency virus disease who have had failure of combination nucleoside therapy. J Infect Dis. 1998;177:1514-520.
20. Stein DS, Fish DG, Bilello JA, Preston SL, Martineau GL, Drusano GL. A 24-week open-label phase I/II evaluation of the HIV protease inhibitor MK-639 (indinavir). AIDS 1996;10:485-492.
21. Gagnon RF, Tecimer SN, Watters AK, Hatzakis GE, Tsoukas CM. The natural history of leukocyturia associated with indinavir treatment in HIV+ individuals. Am J Nephrol 2000;20(6):448-54.
22. John H, Muller NJ, Opravil M, Hauri D. Indinavir urinary stones as origin of upper urinary tract obstruction. Urol Int 1997;59(4):257-9.
23. Schwartz GJ, Haycock GB, Spitzer A. Plasma creatinine and urea concentration in children: normal values for age and sex. J Pediatr 1976;88(5):828-30.
24. Sarcletti M, Petter A, Zangerle R. Indinavir and interstitial nephritis. Ann Intern Med 1998;128(4):320-1.
25. Jaradat M, Phillips C, Yum MN, Cushing H, Moe S. Acute tubulointerstitial nephritis attributable to indinavir therapy. Am J Kidney Dis 2000;35(4):E16.

26. van Rossum AMC, Dieleman JP, Fraaij PLA, et al. Indinavir-associated asymptomatic nephrolithiasis and renal cortex atrophy in two HIV-1 infected children. AIDS. 2001;15:1745-1747
27. Martinez E, Leguizamon M, Mallolas J, Miro JM, Gatell JM. Influence of environmental temperature on incidence of indinavir-related nephrolithiasis. Clin Infect Dis 1999;29(2):422-5.
28. Salahuddin S, Hsu YS, Buchholz NP, Dieleman JP, Gyssens IC, Kok DJ. Is indinavir crystalluria an indicator for indinavir stone formation? AIDS 2001;15:1079-1080.
29. Gulick RM, Mellors JW, Havlir D, et al. 3-year suppression of HIV viremia with indinavir, zidovudine, and lamivudine. Ann Intern Med. 2000;133:35-39.
30. Vigano A, Dally L, Bricalli D, et al. Clinical and immuno-virologic characterization of the efficacy of stavudine, lamivudine, and indinavir in human immunodeficiency virus infection. J Pediatr. 1999;135:675-682.

Chapter 3

Lipodystrophy and HIV-protease inhibitor treatment

Chapter 3.1

Lipodystrophy and buffalo hump during treatment with HIV-protease inhibitors

Abstract

Many patients who are using HIV-protease inhibitors, gradually develop generalized lipodystrophy. The observed syndrome can be defined by peripheral fat wasting, central fat accumulation, hyperlipidaemia and insulin resistance. Typically the subcutaneous fatty tissue disappears resulting in prominent zygomata, veins and muscles and thinning of extremities and buttocks. In contrast, patients may accumulate visceral fat, resulting in abdominal complaints and abdominal distension. In addition to abdominal fat accumulation, there have been reports on the occurrence of a dorso-cervical fat pad, the so called buffalo hump. To date it is not clear whether this effect is part of the central fat accumulation seen with lipodystrophy an whether it is associated with exposure to a HIV-protease inhibitor. The pathogenesis and prognosis of lipodystrophy and buffalo hump are still unknown. Recognition is essential for adequate follow-up and possible treatment. In this paper we present three patients with typical signs of lipodystrophy with and without buffalo hump.

Introduction

The introduction of the HIV-protease inhibitors, saquinavir, indinavir en ritonavir, preluded a new era for the treatment of HIV-infection. In combination with two reverse transcriptase inhibitors HIV-protease inhibitors can induce a strong reduction in plasma HIV-RNA and an increase in the number of CD4 cells (1-5). The new developments caused a reduction in AIDS-related morbidity and mortality (6-9). Knowledge regarding the long-term effectiveness and the adverse effects was, however, scanty. Since the registration of HIV-protease inhibitors in 1996 a number of new adverse effects have been observed, including HIV-protease inhibitor associated diabetes mellitus and increased risk of haemorrhages for haemophiliacs (10, 11).

Recently it was noted that many patients treated with HIV-protease inhibitors develop an abnormal fat distribution, referred to as lipodystrophy (12-14). The peripheral (subcutaneous) fat disappears leading to thinning of the extremities and buttocks and prominence of zygoma and vascular drawing without muscle weakness or significant weight loss. As a result the patient acquires a pseudo-cachexic appearance. On the other hand, central (visceral) fat accumulates as shown by Dual Energy X-ray Absorptiometry (DEXA) and Computer Tomography-scanning (CT) (13). The overall body fat mass hardly changes. In addition, a dorso-cervical fat pad, or "buffalo hump" and hypertrophy of the breasts may occur (15, 16). The "buffalo hump" might, however, be a separate phenomenon as it is observed among HIV-patients who are not using HIV-protease inhibitors (17).

The dramatic body shape changes may have significant psychosocial consequences for patients and negatively affect adherence. With the below case histories and images we would like to draw attention to the clinical picture of this suspected adverse effect of HIV-protease inhibitors.

Patient 1

The first case concerned a 36-year old homosexual man, known with HIV-infection since 1992 and known with AIDS based on *Pneumocystis carini* pneumonia. At the end of 1996, triple therapy with stavudine (D4T, 40 mg bid), didanosine (ddI, 400 mg qd) and ritonavir (600 mg bid) was instigated. During treatment, the patient complained of diarrhoea especially after food consumption, abdominal pain and occasionally abdominal distension, which was attributed to ddI intake. During a regular clinic visit approximately one year and a half after starting triple therapy, a recently developed change in appearance was noted,

comprising prominent zygoma, apparent muscle and vascularity on the extremities and abdomen due to generalised subcutaneous fat loss, in absence of overtly increased abdominal girth (figure 1). Based on these signs a diagnosis of lipodystrophy was made.

Weight had decreased from 74 kg prior to starting triple therapy to 70 kg, of which the largest part happened during the past three months. During treatment serum triglycerides had been elevated (1.93 to 5.84 mmol/L, normal 0.5-1.7 mmol/L) with a maximum of 9.9 mmol/L one month prior to diagnosing lipodystrophy. Serum cholesterol was slightly elevated (6.7 mmol/L, normal < 6.5 mmol/L). During treatment serum glucose dropped regularly (3.4 mmol/L), whereas plasma C-peptide was elevated (2.10 nmol/L, normal 0.22-0.83 nmol/L) at the last assessment, consistent with insulin resistance. There were no abnormalities of plasma cortisol, steroid hormone binding globulin, luteinizing hormone, follicle-stimulating hormone, estradiol, testosterone and free testosterone at the moment of establishing the diagnosis lipodystrophy. The CD4 cell count increased from 220/mm^3 prior to therapy to 710/mm^3 one year and a half later. The viral load at these moments was 66,000 copies/mL and less than 80 copies/mL (NASBA-Nuclisens Organon) respectively. The patient did not experience any HIV-related events during treatment and treatment was not modified. Upon patient request, treatment with nandrolone (200 mg intramuscularly/2 weeks) was started in April 1998. By September 1998 weight had increased to 74.5 kg, but, despite a slight improvement of the facial symptoms, prominent nasolabial folds persisted.

Patient 2

The second case concerned a 35-year old woman, known to be HIV-infected since 1987, who contracted HIV through heterosexual contact. HIV-infection progressed asymptomatically and treatment with a combination of D4T (40 mg bid), 3TC (150 mg bid) and indinavir (800 mg tid) was started in January 1997. In April 1998, 15 months after the start of this treatment presence of a "buffalo hump" was noted during a routine clinic visit (Figure 2).

Thinned extremities, increased abdominal girth and prominent zygoma were observed as well on the basis of which the diagnosis lipodystrophy was made. In retrospect, signs started to develop approximately nine months after treatment instigation. Weight increased from 64.6 kg to 67.4 kg during treatment. The CD4 cell count increased from 220/mm^3 to 370/mm^3 and plasma HIV-RNA declined from 111,000 copies/mL to below 500 copies/mL (Amplicor HIV-1 monitor, Roche, Basel) within seven months. Plasma triglycerides appeared to be elevated (7.08 mmol/L, normal < 2.0 mmol/L) and serum HDL-cholesterol were reduced (0.74 mmol/L, normal > 1.1 mmol/L). Secondary diabetes mellitus was

diagnosed (glucose 15.1 mmol/L). Cortisol levels had not been determined. During treatment HIV-related events were not encountered. One month after diagnosing lipodystrophy, indinavir was replaced by nevirapine, a non-nucleoside reverse transcriptase inhibitor, four months after which facial symptoms had improved but not disappeared. High serum triglycerides (3.87 mmol/L) and low HDL persisted (1.35 mmol/L) and high serum cholesterol was measured (6.7 mmol/L, normal ≤ 5.0 mmol/L), whereas glucose concentrations had normalised.

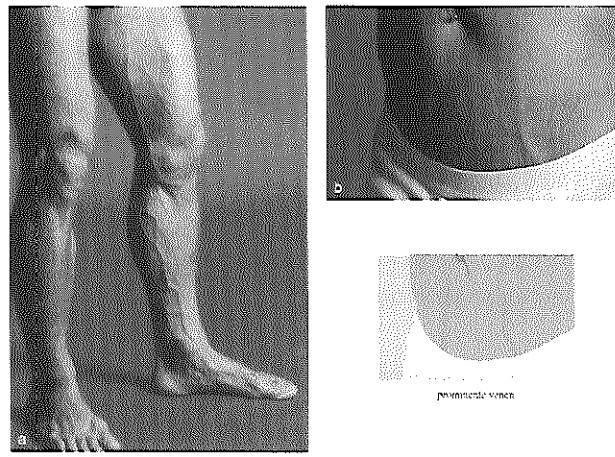

Figure 1 Prominent vascular drawing, typical for lipodystrophy during HIV-protease inhibitor treatment

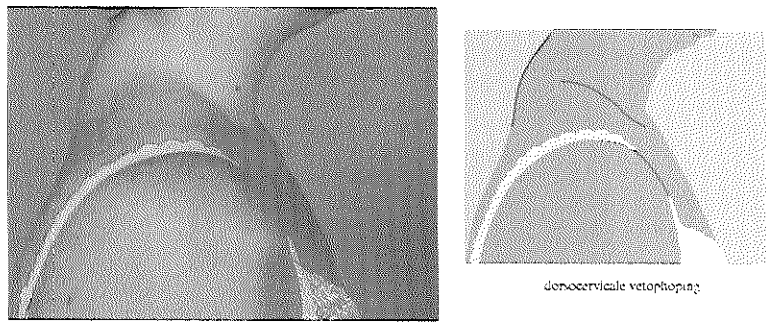

Figure 2 "Buffalo hump" during long-term use of an HIV-protease inhibitor

Patient 3

The third case concerned a 59-year old woman with a known heterosexually transmitted HIV-infection since 1989 which had progressed to AIDS because of wasting (CD4 cell count of less than 10 cells/mm^3). She was treated with saquinavir (600 mg tid), AZT (100 mg tid) and ddI (125 mg bid) from May 1996 until April 1997 and subsequently with D4T (40 mg bid), 3TC (150 mg bid) and indinavir (800 mg tid). During the latter regimen plasma HIV-RNA dropped below 500 copies/mL (Amplicor HIV-1 monitor, Roche, Basel) and CD4 cells rose to a maximum of 90/mm^3. Weight initially increased from 64 kg to 66 kg, but subsequently dropped to 62 kg. Because of abdominal pain, diarrhoea, nausea and vomiting, urological symptoms coinciding with elevated plasma indinavir concentrations, indinavir dosing was reduced to 600 mg tid. In April 1998, "buffalo hump" with concomitant thinned extremities and a strongly increased abdominal girth were noted upon which the diagnosis lipodystrophy was made (figure 3). According to the patients' account these body shape changes already existed for some time. During triple therapy no abnormalities in serum glucose were observed. Indinavir was replaced by nevirapine but improvement of the lipodystrophy syndrome has not been recorded to date.

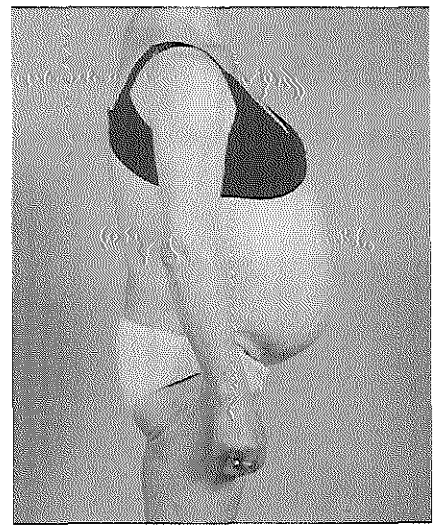

Figure 3 Central adipositas during the use of an HIV-protease inhibitor.

Discussion

Notwithstanding the uncertainties regarding the pathogenesis of lipodystrophy, there is little doubt about a causal relation with the current antiretroviral treatment, which typically contains an HIV-protease inhibitor. This is supported by the high prevalence of this otherwise rare condition among users of HIV-protease inhibitors, indinavir, saquinavir and ritonavir (14). Little is known about the risk of lipodystrophy during use of the newest HIV-protease inhibitor, nelfinavir. It is unclear as to whether lipodystrophy is caused by HIV-protease inhibitors directly or by an interaction with other factors.

An association between lipodystrophy and CD4 cell count, plasma HIV-RNA load, family history of diabetes mellitus, liver function disturbances, serum cortisol, testosteron, sex-hormone binding globuline, prolactine, C3 and TNF-a was denied (12, 14, 15). Similarly to the above case histories, there was, however, an association with triglycerides (elevated), insulin (elevated), insulin resistance, C-peptide (elevated) and with the duration of HIV-protease inhibitor containing treatment (14). So far, it is not known if HIV-protease inhibitor discontinuation or replacement by a non-nucleoside analogue reverse transcriptase inhibitor has a favourable effect on the course of lipodystrophy. Some patients attempt to improve their appearance by using anabolic steroids, but the effect of such interventions is uncertain. Adequate treatment strategies for lipodystrophy remain to be determined.

As hyperlipidemia, abdominal adipositas and diabetes mellitus predispose to premature development of cardiovascular diseases, patients with lipodystrophy probably are at an increased risk for these conditions (18, 19). Treated HIV-infected patients with cardiovascular diseases have already been described (20-24). Assessment of serum lipids and glucose, therefore, is useful and may facilitate early intervention. It is possible and recommended to treat hyperlipidemia as is customary with other patients (25). Other complications, which might occur in association with lipodystrophy are osteoporosis and liver steatosis (26, 27).

A possible explanation for lipodystrophy might be a recently recognised homology between HIV-protease and cytoplasmatic retinoic-acid binding protein type 1 (CRABP-1) en lipoprotein-receptor-related protein (LRP) (28). HIV-protease inhibitors could potentially bind to these proteins and hence stimulate apoptosis of peripheral adipocytes and disturb triglyceride uptake leading to an excess of circulating triglycerides. Triglycerides are subsequently stored in central adipocytes (intra-abdominal, dorso-cervical and mammae) leading to insulin resistance with secondary diabetes mellitus type 2. Another *in vitro* study, on the other hand, suggested that HIV-protease inhibitors do in fact stimulate adipogenesis (29).

The number of patients developing abnormalities of the lipid metabolism is unknown, but a small cross-sectional study observed a prevalence of 60% (13). In order to determine the incidence, prospective research is needed. Hopefully, research on the pathogenesis will soon provide more insight into prognosis and possible treatment strategies. Research on the natural course of lipodystrophy, with and without treatment modifications, and the risks of cardiovascular diseases, liver steatosis and osteoporosis is equally important. As children are now being treated with HIV-protease inhibitor combination therapy as well, potential disturbances in fat metabolism, which may manifest in a different way, should be traced. In order to study lipodystrophy and its complications more adequately objective assessment tools such as serum lipid spectrum, glucose concentrations, anthropometry and pictures should be applied. Since relevant baseline parameters regarding body composition are usually not available, prospective research is necessary.

References

1. Danner SA, Carr A, Leonard JM, et al. A short-term study of the safety, pharmacokinetics, and efficacy of ritonavir, an inhibitor of HIV-1 protease. European-Australian Collaborative Ritonavir Study Group. N Engl J Med 1995;333:1528-33.
2. Markowitz M, Saag M, Powderly WG, et al. A preliminary study of ritonavir, an inhibitor of HIV-1 protease, to treat HIV-1 infection. N Engl J Med 1995;333:1534-9.
3. Gulick RM, Mellors JW, Havlir D, et al. Treatment with indinavir, zidovudine, and lamivudine in adults with human immunodeficiency virus infection and prior antiretroviral therapy [see comments]. N Engl J Med 1997;337:734-9.
4. Stein DS, Fish DG, Bilello JA, Preston SL, Martineau GL, Drusano GL. A 24-week open-label phase I/II evaluation of the HIV protease inhibitor MK-639 (indinavir). AIDS 1996;10:485-92.
5. Hammer SM, Squires KE, Hughes MD, et al. A controlled trial of two nucleoside analogues plus indinavir in persons with human immunodeficiency virus infection and CD4 cell counts of 200 per cubic millimeter or less. AIDS Clinical Trials Group 320 Study Team. N Engl J Med 1997;337:725-33.
6. Hogg RS, O'Shaughnessy MV, Gataric N, et al. Decline in deaths from AIDS due to new antiretrovirals. Lancet 1997;349:1294.
7. Rogers PA, Whitmore-Overton SE, Evans BG, Allardice GM, Noone A. Survival of adults with AIDS in the United Kingdom. Commun Dis Rep CDR Rev 1997;7:R93-100.
8. Mouton Y, Alfandari S, Valette M, et al. Impact of protease inhibitors on AIDS-defining events and hospitalizations in 10 French AIDS reference centres. Federation National des Centres the Lutte contre le SIDA. AIDS 1997;11:F101-5.
9. Palella FJ, Delany KM, Moorman AC, et al. Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. N Engl J Med 1998;338:853-860.
10. Meijer van Putten JB. [Protease inhibitors connected to diabetes]. Ned Tijdschr Geneeskd 1997;141:1405-6.

11. Helal A. HIV protease inhibitors and increased bleeding in hemophilia? Can Med Assoc J 1997;156:90, 94-5.
12. Hengel RL, Watts NB, Lennox JL. Benign symmetric lipomatosis associated with protease inhibitors. Lancet 1997;350:1596.
13. Miller MD, Jones E, Yanovski JA, Shankar R, Feuerstein I, Falloon J. Visceral abdominal-fat accumulation associated with use of indinavir. Lancet 1998;351:871-75.
14. Carr A, Samaras K, Burton S, et al. A syndrome of peripheral lipodystrophy, hyperlipidaemia and insulin resistance in patients receiving HIV protease inhibitors. AIDS 1998;12:F51-8.
15. Lo JC, Mulligan K, Tai VW, Algren H, Schambelan M. "Buffalo hump" in men with HIV-1 infection. Lancet 1998;351:867-70.
16. Herry I, Bernard L, Truchis the P, Perronne C. Hypertrophy of the breast in a patient with indinavir. Clin Infect Dis 1997;25:937-8.
17. Lipsky J. Abnormal fat accumulation in patients with HIV-1 infection. Lancet 1998;351:847-8.
18. Carvalho JS, Shinebourne EA. Congenital total lipodystrophy and peripheral pulmonary artery stenosis. Arch Dis Child 1997;77:466.
19. Bjornstad PG, Foerster A, Ihlen H. Cardiac findings in generalized lipodystrophy. Acta Paediatr Suppl 1996;413:39-43.
20. Henry K, Melroe H, Huebsch J, et al. Severe premature coronary artery disease with protease inhibitors. Lancet 1998;351:1328.
21. Gallet B, Pulik M, Genet P, Chedin P, Hiltgen M. Vascular complications associated with use of HIV protease inhibitors. Lancet 1998;351:1958-9.
22. Vittecoq D, Escaut L, Monsuez JJ. Vascular complications associated with use of HIV protease inhibitors. Lancet 1998;351:1959.
23. Behrens G, Schmidt H, Meyer D, Stoll M, Schmidt RE. Vascular complications associated with use of HIV protease inhibitors. Lancet 1998;351:1958.
24. Laurence J. Vascular complications associated with use of HIV protease inhibitors. Lancet 1998;351:1960.
25. Henry K, Melroe H, Huebesch J, Hermudson J, Simpson J. Atorvastatin and gemfibrozil for protease-inhibitor-related lipid abnormalities. Lancet 1998;352:1031-2.
26. Westvik J. Radiological features in generalized lipodystrophy. Acta Paediatr Suppl 1996;413:44-51.
27. Smevik B, Swensen T, Kolbenstvedt A, Trygstad O. Computed tomography and ultrasonography of the abdomen in congenital generalized lipodystrophy. Radiology 1982;142:687-9.
28. Carr A, Samaras K, Chisholm DJ, Cooper DA. Pathogenesis of HIV-1-protease inhibitor-associated peripheral lipodystrophy, hyperlipidaemia, and insulin resistance. Lancet 1998;351:1881-83.
29. Gagnon A, Angel JB, Sorisky A. Protease inhibitors and adipocyte differentiation in cell culture. Lancet 1998;352:1032.

Chapter 3.2

Risk of lipodystrophy is highest after prolonged exposure to stavudine containing antiretroviral treatment.

The ATHENA cohort.

Abstract

Background: Epidemiological studies on the incidence rate of lipodystrophy among HIV-infected patients treated with antiretroviral agents and on risk factors are scanty.

Objective: To estimate the incidence of clinically evident lipodystrophy and to identify risk factors within the Dutch HIV-infected patient population.

Methods: All antiretroviral treatment naïve patients from the nation wide ATHENA cohort in the Netherlands were included upon starting antiretroviral treatment and followed until a clinical diagnosis of lipodystrophy, death or end of study period (December 2000). Cumulative exposure to individual antiretroviral agents and their combinations, immunological and virological response and body mass index (BMI) were calculated.

Results: 1952 patients were included of whom 261 developed lipodystrophy. The incidence rate of lipodystrophy was 6.2 per 100 person years with a four-year cumulative incidence of 25%. Older age, female gender, higher BMI and superior immunological and virological response were associated with an increased risk of lipodystrophy. Patients who had used stavudine had the highest risk of lipodystrophy. Indinavir was identified as a risk factor as well, but appeared to play a minor role compared to stavudine. The lowest risk was observed with zidovudine/lamivudine regimens especially if combined with nevirapine.

Conclusion Antiretroviral treatment including stavudine, treatment response, age and female gender are associated with an increased risk of lipodystrophy. To reduce the risk of lipodystrophy, regimens with zidovudine and lamivudine may be preferred.

Introduction

The lipodystrophy syndrome is a widely recognized adverse effect of antiretroviral treatment (1-3), although consensus on an adequate definition of the syndrome has not been reached yet. It is generally accepted that the lipodystrophy syndrome comprises changes in body fat distribution consisting of subcutaneous fat loss with or without central fat accumulation (1, 4, 5). These phenotypic changes are often accompanied by hyperlipidemia and disturbances in glucose metabolism (6). The patho-physiological processes underlying these signs and symptoms, however, are incompletely understood.

Previous studies and case reports have attributed the lipodystrophy syndrome to HIV-protease inhibitors alone (in particular indinavir) (3, 4, 7-16), to nucleoside analogue reverse transcriptase inhibitors (stavudine in particular) (8, 16-19) and to immune reconstitution and metabolic stress relieve effects (19, 20). Most of the studies, however, had important limitations because of either cross-sectional designs or small patient numbers not allowing adequate analysis of individual antiretroviral agents and specific combinations of antiretroviral agents. High prevalences (10-80%) (8, 12, 21, 22), potential cardio-vascular risks (23) and uncertainty about the reversibility of the syndrome prompt further research into the patho-physiological pathway and risk factors (24, 25).

In the present study we examined risk factors for the lipodystrophy syndrome in the antiretroviral treatment naïve subset of the ATHENA cohort, with particular emphasis on individual antiretroviral agents and combinations of antiretroviral agents.

Patients and methods

Setting

The present study was performed in the ATHENA cohort of treated HIV-infected patients in the Netherlands. Patients were included in the ATHENA cohort if their treatment at least included an HIV-protease inhibitor, a non-nucleoside reverse transcriptase inhibitor or one of the newer nucleoside analogue reverse transcriptase inhibitors such as lamivudine and stavudine, and gave written informed consent. All 22 Dutch hospitals providing treatment to HIV-infected patients participate in the ATHENA project, which effectively started in May 1998. At the time of this analysis 3377 patients had entered the ATHENA cohort representing about 75% of the estimated treated HIV-infected patient population in the Netherlands.

Patients are followed at approximately three-monthly intervals (26). Data for the ATHENA project are collected from the medical records on standardised forms by trained research nurses and treating physicians. This is done retrospectively for the period prior to

consent and prospectively thereafter and continues to date. The resulting database contains information on gender, age, route of HIV-transmission, height and weight. Start and stop dates as well as dose frequency of any antiretroviral medication and prophylactic treatment against opportunistic infections, reasons for stopping such treatments, date of onset and resolution of HIV-related diseases, CD4-cell counts, plasma HIV-1 RNA load and abnormal laboratory values are all recorded in a standardised manner. Information on adverse events in the ATHENA database comprises all events, which lead to a change in antiretroviral treatment and a number of pre-specified adverse events, among which lipodystrophy. No distinction, however, is made regarding the presence of fat accumulation and/or lipoatrophy. In addition, physicians are requested to report all remarkable clinical events and laboratory abnormalities. On site data monitoring takes place at regular intervals on 10% of completed study forms. In addition, central data verification through automated database consistency checks is performed, the resulting queries of which are resolved by local research nurses and physicians. For the present analysis, we used data, which had been monitored and verified before December 2000. The study period lasted from July 1, 1996 until the date of last data extraction on December 15, 2000.

Cohort definition

For the purpose of our analysis, patients had to have used at least one of the newly available antiretroviral agents (ie. abacavir, adefovir, amprenavir, combivir, delavirdine, efavirenz, indinavir, lamivudine, lopinavir, loviride, nelfinavir, nevirapine, ritonavir, saquinavir and stavudine) after July 1, 1996. Cohort entry was defined as the date of first intake of one of these antiretroviral agents. Patients who had used any antiretroviral agent before cohort entry or before July 1, 1996 were excluded in order to generate a cohort of antiretroviral naïve patients and limit the effect of survival bias. In addition, we excluded all patients for whom treatment dates were missing, who had a diagnosis of lipodystrophy before cohort entry or for whom there was no follow-up available. Follow-up lasted from the date of cohort entry until the occurrence of lipodystrophy, last ATHENA update or death, whichever came first.

Exposure definition

As the main risk factor for lipodystrophy, we considered the type of antiretroviral agents used and cumulative exposure time to individual antiretroviral agents and combinations. Since lipodystrophy is probably not an on/off phenomenon we assumed an induction plus latency period of more than six months and therefore accepted exposure to antiretroviral agents or regimens if the cumulative time on a certain agent or regimen exceeded six months. The analysis of antiretroviral treatment combinations was limited to all regimens

including zidovudine and lamivudine (AZT/3TC-based regimens), stavudine and lamivudine (d4T/3TC-based regimens), zidovudine and didanosine (AZT/DDI-based regimens) or stavudine and didanosine (d4T/DDI-based regimens).

In addition, we examined age, gender, calculated body mass index (kg/m^2), change in weight over the last six months, baseline CD4 cell count, change in CD4 cell count and HIV-1 RNA load as potential risk factors for lipodystrophy.

Outcome

Although there is no global consensus on the definition of the lipodystrophy syndrome HIV-specialized physicians are mostly able to easily recognize the more severe syndrome, which comprises overt loss of subcutaneous fat and/or central fat accumulation irrespective of metabolic changes. Therefore, we confined the outcome to a clinical diagnosis of lipodystrophy as made by the treating physician. No differentiation was made between the various forms of lipodystrophy, such as lipoatrophy, fat accumulation or a mixed syndrome. The date of onset of first symptoms as reported by the physicians was defined as the index date.

Analysis

Incidence rates of lipodystrophy were calculated by dividing the total number of cases of lipodystrophy by the total amount of follow-up time or by the total cumulative exposure time to specific antiretroviral regimens, irrespective of the agents used at diagnosis of lipodystrophy. Ninety-five percent confidence intervals (95%CI) were estimated assuming a normal distribution. The cumulative incidence of lipodystrophy was estimated by means of Kaplan-Meier survival analysis. In order to examine the effect of calendar time, we stratified for calendar time by calculating cumulative incidences per calendar year among persons commencing treatment in that calendar year.

Factors associated with lipodystrophy were analyzed by means of univariate and multivariate Cox-regression analysis. In order to adjust for calendar time and differences between hospitals, all analyses were stratified for the period of cohort entry and hospital, the results of which were presented as pooled relative risks with 95%CI. The influence of partially retrospective data collection was examined. Factors that could alter the risk of lipodystrophy over time, such as exposure to individual antiretroviral agents or specific combinations, and change in body mass index, weight and CD4 cell counts were entered into the model as time dependent co-variates.

Results

At the time of this analysis, the ATHENA population comprised 3377 eligible patients. For our study, we excluded 1374 patients because of previous treatment with antiretroviral agents, 47 patients because of missing treatment dates, two patients because of a diagnosis of lipodystrophy before cohort entry and two because of missing follow-up. The final study cohort consisted of 1952 patients, who entered the study cohort between July 1, 1996 and September 30, 2000. The median date of cohort entry was October, 28 1997 (Inter Quartile Range [IQR]: Feb 28, 1997-Nov 4, 1998).

The baseline characteristics of the study cohort are summarized in table 1. In brief, 15% was female, the median age was 37 years, the median weight was 70 kg, the median HIV-1 RNA load was 4.97 log copies/mL, and the median CD4 cell count was 230 cells/mm^3. The most frequently used antiretroviral treatment combination at cohort entry was zidovudine combined with lamivudine and indinavir.

Table 1 Baseline characteristics of the study cohort comprising HIV-infected patients who started their first antiretroviral regimen (n=1952)

Characteristic at cohort entry		N	(%)
Age		37	[32-45]
Female gender		293	(15)
CDC-C disease		502	(26)
HIV-transmission [n=1806]	Homosexual	1207	(67)
	Heterosexual	452	(25)
	Intra-venous drug use	103	(6)
	Other	44	(2)
Weight (kg) [n=1748]		70	[63-79]
BMI (kg/m2) [n=1712]		22	[20-25]
CD4 baseline (cells/mL) [n=1478]		236	[90-380]
Log HIV-1 RNA (copies/mL) [n=1708]		5.0	[4.5-5.4]
PI upon cohort entry		1602	(82)
First antiretroviral regimen	AZT/3TC/IDV	330	(17)
	AZT/3TC/RTV	223	(11)
	AZT/3TC/NFV	203	(10)
	AZT/3TC/SQV	157	(8)
	d4T/3TC/IDV	112	(6)
	AZT/3TC/NVP	105	(5)
	d4T/3TC/SQV/RTV	89	(5)
	Other Φ	733	(38)

ARV= antiretroviral medication; CDC-C= Centers for Disease Control and Prevention disease classification for HIV class C; PI= HIV-protease inhibitor
AZT= zidovudine; 3TC= lamivudine; d4T= stavudine; IDV= indinavir; SQV= saquinavir; RTV= ritonavir; NFV= nelfinavir; SQV/RTV= SQV combined with RTV; NVP= nevirapine; NNRTI= non-nucleoside analogue reverse transcriptase inhibitor
Φ Comprises 97 different antiretroviral treatments

Incidence rates of lipodystrophy

The total follow-up time of the study cohort was 4208 person years with a median follow-up of 2.2 years (inter quartile range [IQR]: 1.3-3.2). During this period, 261 cases of lipodystrophy were identified. Other reasons for end of follow-up (right censoring) other than last data retrieval were death (n=84) and loss to follow-up (n=4). The incidence density of lipodystrophy accumulated to 6.2 per 100 person years (95%CI: 5.5-6.9) with a one-year cumulative incidence of 2.7% (95%CI: 1.9-3.5), a two-year cumulative incidence of 12.1% (95%CI: 10.5-13.8) and a three-year cumulative incidence of 18.2% (95%CI: 16.0-20.4). The four-year cumulative incidence of lipodystrophy was 24.8% (95%CI: 20.1-29.3) (Figure 1).

Over calendar time, the one-year cumulative incidence of lipodystrophy increased from 1.7% (95%CI: 0.0-4.5) in 1997 to 1.8% (95%CI: 0.0-3.6) in 1998 and 2.5% (95%CI: 0.0-5.3) in 1999. The two-year cumulative incidence increased from 2.0% (95%CI: 0.4-3.7) for patients starting in 1996 to 11.4% (95%CI: 8.2-14.6) for patients starting in 1997 and 11.7% (95%CI: 7.3-16.1) for patients starting in 1998.

Lipodystrophy occurred most frequently among patients who had been exposed to stavudine and was least frequent among patients exposed to zidovudine irrespective of the other antiretroviral agents used during follow-up (Figure 2 upper panel). A relatively high

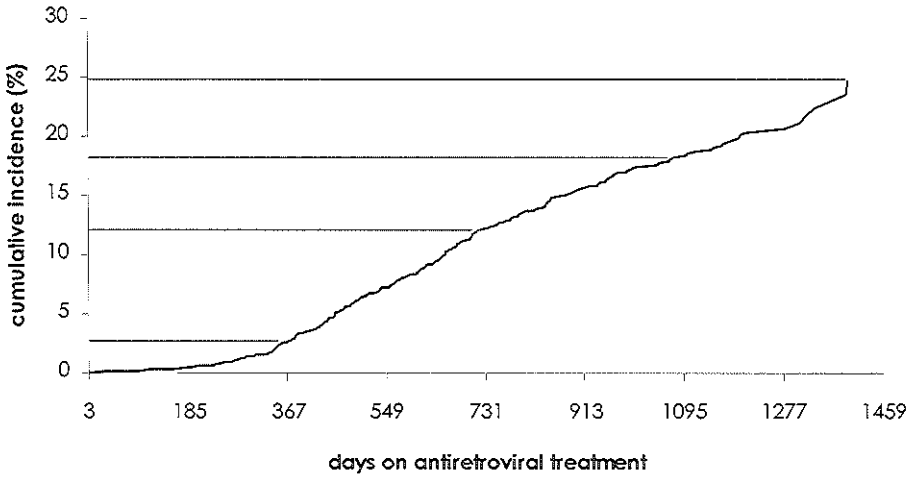

Figure 1 The cumulative incidence of lipodystrophy in a population of newly treated HIV-infected patients (n=1952).
Pale gray lines indicate the one-year, two-year, three-year and four-year cumulative incidence respectively.

incidence was observed among patients exposed to combinations with nevirapine or ritonavir. However, since treatment switches and clustering of antiretroviral treatment combinations are expected to have occurred, the results concerning individual drugs cannot be interpreted adequately in isolation.

Patients used a median of two (IQR: 1-3) antiretroviral treatment combinations during follow-up. Indinavir combined with zidovudine and lamivudine was the most frequently used combination (Table 2). Comparing patients who had used stavudine-based regimens during follow-up with patients who had used zidovudine based regimens showed that the rates of lipodystrophy were consistently higher among patients who had been exposed to stavudine (Figure 2 lower panel). Among the HIV-protease inhibitors the highest incidence rate was observed for patients who had used the ritonavir saquinavir combination.

Table 2 Cumulative exposure to antiretroviral treatment regimens during follow-up of a cohort of previously antiretroviral treatment naïve patients (n=1952)

Antiretroviral regimen		AZT/3TC based	d4T/3TC based	AZT/DDI based	d4T/DDI based
Months of use of	Months	13524	6790	672	1039
IDV	Months	4948	1934	231	368
SQV	Months	2344	715	354	121
RTV	Months	2373	913	41	258
NFV	Months	877	520	17	91
SQV/RTV	Months	1374	703	0	62
SQV/NFV	Months	680	877	22	90
IDV/RTV	Months	57	449	0	2
IDV800/RTV100	Months	240	239	6	7
IDV400/RTV400	Months	94	0	0	0
NVP	Months	142	120	0	0
No with > 6 months exposure*	N	804	439	39	58
IDV	N	281	127	14	22
SQV	N	151	46	20	5
RTV	N	131	52	2	14
NFV	N	107	58	0	4
SQV/RTV	N	40	62	2	4
SQV/NFV	N	4	34	0	0
IDV/RTV	N	16	20	1	0
IDV800/RTV100	N	6	6	0	0
IDV400/RTV400	N	10	10	0	0
NVP	N	63	39	6	7

AZT= zidovudine; 3TC= lamivudine; d4T= stavudine; DDI= didanosine; IDV= indinavir; SQV= saquinavir; RTV= ritonavir; NFV= nelfinavir; SQV/RTV= SQV combined with RTV; IDV/RTV= IDV combined with RTV; IDV800/RTV100= IDV 800 mg combined with RTV 100 mg; IDV400/RTV400= IDV 400 mg combined with RTV 400 mg; NVP= nevirapine

* The sum of individual agents may be higher than the total due to combined use or switching

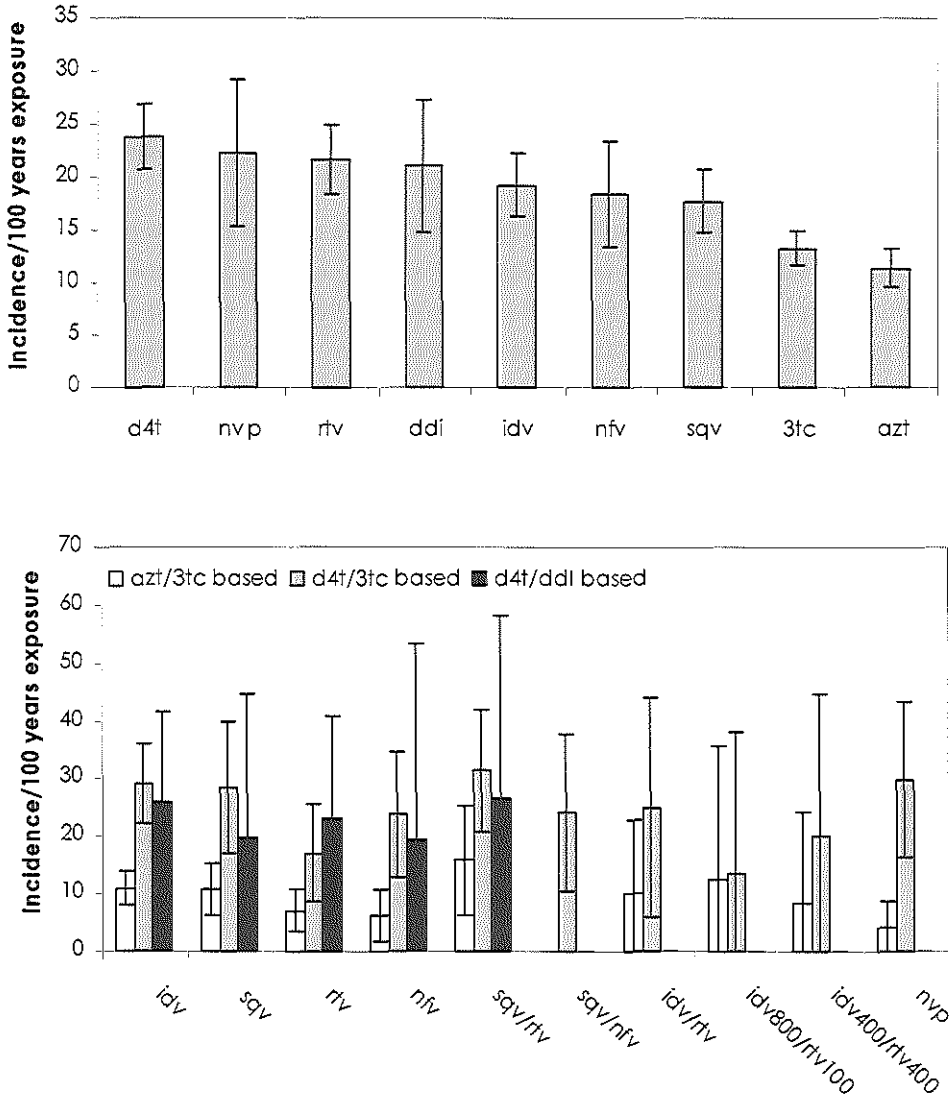

Figure 2 Incidence rates and 95%-confidence intervals of lipodystrophy for individual antiretroviral agents (upper panel) and for combinations of antiretroviral agents (lower panel) among previously untreated antiretroviral treatment patients (n=1952)
3TC= lamivudine; AZT= zidovudine; d4T= stavudine; DDI= didanosine; IDV= indinavir; IDV/RTV= IDV with RTV; IDV800/RTV100= IDV 800 mg with RTV 100 mg; IDV400/RTV400= IDV 400 mg with RTV 400 mg; NFV= nelfinavir; NVP= nevirapine; RTV= ritonavir; SQV= saquinavir; SQV/RTV= SQV with RTV

Risk factors for lipodystrophy

The risk of lipodystrophy increased with older age, female gender, higher body mass index, more weight loss and better immunological and virological response (Table 3). HIV CDC-disease classification and route of HIV-transmission as well as starting with an HIV-protease inhibitor-sparing regimen were not associated with lipodystrophy. Also, no influence of retrospective data collection was observed.

Considering individual antiretroviral agents irrespective of other antiretroviral agents used, the highest risk of lipodystrophy was observed with stavudine and indinavir, whereas the lowest risk was observed with zidovudine and nevirapine (Table 3). After adjustment for all other associated factors, stavudine was the only agent with an increased risk for lipodystrophy, whereas nevirapine was associated with a significantly lower risk.

Table 3 Risk factors for development of clinically evident lipodystrophy (n=261) among patients receiving antiretroviral treatment (n=1952)

Variable [cases for whom data are available if less than 261]	Value cases (n=261)		univariate		multivariateΦ	
	N Median	(%) [IQR]	RR	(95%CI)	RR	(95%CI)
Age (per 10 years)	40	[34-46]	**1.3**	**(1.1-1.4)**	**1.3**	**(0.97-1.6)**
Female gender	62	(24)	**2.0**	**(1.5-2.7)**	1.7	(0.9-3.3)
BMI cohort entry > 22 kg/m² [n=242]	155	(64)	**1.6**	**(1.2-2.0)**	**2.4**	**(1.3-4.3)**
On date of diagnosis						
BMI > 22 kg/m² [n=196]	148	(76)	**1.4**	**(1.02-2.0)**	-	-
Weight change¥ (kg) [n=107]	-0.92	[-2.6-1.5]	**1.1**	**(1.01-1.1)**	1.0	(0.96-1.1)
CD4 increase [n=172]						
<= 20%	29	(17)	1		1	
20-100%	50	(29)	1.0	(0.6-1.6)	1.0	(0.5-2.1)
>100%	93	(54)	**1.6**	**(1.04-2.4)**	1.6	(0.8-3.2)
Log HIV-1 RNA (per c/mL lower) [n=222]	2.6	[1.9-2.6]	**1.2**	**(0.99-1.3)**	-	-
HIV-1 RNA ≤ 500 c/mL	209	(94)	**1.7**	**(1.1-2.6)**	2.0	(0.6-6.4)
per month exposure to♣						
regimens containing AZT	138	(53)	**0.95**	**(0.94-0.97)**	1.0	(0.97-1.1)
regimens containing DDI	35	(13)	1.0	(0.98-1.03)	-	-
regimens containing d4T	180	(69)	**1.1**	**(1.1-1.1)**	**1.1**	**(1.04-1.2)**
regimens containing 3TC	231	(85)	1.0	(0.99-1.02)	-	-
regimens containing IDV	131	(50)	**1.2**	**(1.01-1.03)**	1.0	(0.96-1.03)
regimens containing SQV	110	(42)	1.0	(0.99-1.02)	-	-
regimens containing RTV	134	(51)	1.0	(0.98-1.01)	-	-
regimens containing NFV	43	(16)	1.0	(0.98-1.04)	-	-
regimens containing NVP	31	(12)	**0.95**	**(0.9-0.98)**	**0.8**	(0.7-0.97)

3TC= lamivudine; AZT= zidovudine; BMI= body mass index (weight/height²); CI= confidence interval; d4T= stavudine; DDI= didanosine; IDV= indinavir; NFV= nelfinavir; NVP= nevirapine; PI= HIV-protease inhibitor; RR= relative risk; RTV= ritonavir; SQV= saquinavir

Φ Includes variables that were associated with lipodystrophy according to the univariate analysis (bold prints). Excludes variables that were strongly associated with another variable in the analysis (BMI at index date, log HIV-1 RNA).

¥ Change over six months preceding diagnosis of lipodystrophy

♣ exposure groups are not mutually exclusive as patients may have used multiple antiretroviral agents either sequentially or in combination

Antiretroviral treatment combinations

Since the use of antiretroviral agents was expected to be clustered, we also considered commonly used antiretroviral treatment combinations as opposed to individual agents. This analysis showed a significantly higher risk of lipodystrophy for all combinations with stavudine (Table 4). The confidence intervals of those regimens did not overlap with the confidence intervals for combinations with zidovudine and lamivudine, implying statistically significant different risks of lipodystrophy.

Table 4 Association between antiretroviral treatment combinations and development of clinically evident lipodystrophy (n=261) among patients receiving antiretroviral treatment (n=1952)

	> 6 months exposure vs. no exposure					Per month exposure				
Used regimen$^{\Phi}$	Cases	Univariate		multivariate$^{\clubsuit}$		Cases	Univariate		multivariate$^{\clubsuit}$	
		RR	(95%CI)	RR	(95%CI)		RR	(95%CI)	RR	(95%CI)
AZT/3TC combined with										
IDV	45	0.8	(0.6-1.1)	-	-	65	0.99	(0.98-1.01)		
SQV	21	0.6	(0.4-1.01)	-	-	26	**0.97**	**(0.9-1.00)**	1.0	(0.95-1.1)
RTV	14	**0.4**	**(0.3-0.7)**	1.1	(0.3-3.8)	30	**0.96**	**(0.9-0.99)**	1.0	(0.9-1.1)
NFV	7	0.5	(0.2-1.2)	-	-	13	0.96	(0.9-1.02)		
SQV/RTV	9	0.7	(0.3-1.3)	-	-	14	0.96	(0.9-1.01)		
SQV/NFV	0	-	-	-	-	1	-	-		
IDV/RTV	2	0.7	(0.2-2.7)	-	-	11	0.96	(0.9-1.1)		
IDV800/RTV100	1	-	-	-	-	3	1.03	(0.9-1.2)		
IDV400/RTV400	1	-	-	-	-	7	0.9	(0.7-1.1)		
NVP	3	**0.2**	**(0.1-0.5)**$^{\Psi}$	Not assessable		13	**0.9**	**(0.8-0.9)**	0.7	(0.5-1.1)
D4T/3TC combined with										
IDV	47	**3.0**	**(2.2-4.1)**	**3.0**	**(1.6-5.8)**	61	**1.1**	**(1.04-1.1)**	**1.1**	**(1.01-1.1)**
SQV	17	**2.1**	**(1.3-3.5)**	**7.7**	**(2.0-29.9)**	20	**1.04**	**(1.01-1.1)**	1.05	(0.96-1.2)
RTV	13	1.2	(0.7-2.1)$^{\forall}$	-	-	16	1.01	(0.98-1.1)		
NFV	14	**2.3**	**(1.3-4.1)**	**2.7**	**(0.9-9.2)**	20	**1.1**	**(1.02-1.1)**	1.1	(0.98-1.2)
SQV/RTV	23	**2.0**	**(1.3-3.2)**	**2.9**	**(1.3-6.7)**	31	**1.1**	**(1.02-1.1)**	**1.1**	**(1.03-1.2)**
SQV/NFV	9	1.5	(0.8-2.9)	-	-	9	1.02	(0.97-1.1)		
IDV/RTV	5	**3.6**	**(1.3-9.5)**	2.8	(0.2-32.6)	6	**1.1**	**(1.01-1.2)**	1.1	(0.9-1.4)
IDV800/RTV100	1	-	-	-	-	3	-	-		
IDV400/RTV400	2	2.5	(0.6-10.6)	-	-	3	1.1	(0.91-1.3)		
NVP	13	1.5	(0.9-2.7)	-	-	17	1.02	(0.98-1.1)		
D4T/DDI combined with										
IDV	8	**2.2**	**(1.1-4.5)**	**3.8**	**(1.1-13.6)**	11	**1.03**	**(0.99-1.1)**	1.1	(0.99-1.1)
SQV	2	1.7	(0.4-7.0)	-	-	3	1.1	(0.99-1.1)		
RTV	5	1.7	(0.7-4.2)	-	-	7	1.03	(0.99-1.1)		
NFV	1	-	-	-	-	5	1.1	(0.96-1.2)		
SQV/RTV	2	3.1	(0.8-12.8)	-	-	6	1.1	(1.03-1.1)		
SQV/NFV	1	-	-			1	-	-		

AZT= zidovudine; 3TC= lamivudine; d4T= stavudine; DDI= didanosine; IDV= indinavir; SQV= saquinavir; RTV= ritonavir; NFV= nelfinavir; SQV/RTV= SQV combined with RTV; IDV/RTV= IDV combined with RTV; IDV800/RTV100= IDV 800 mg combined with RTV 100 mg; IDV400/RTV400= IDV 400 mg combined with RTV 400 mg; NVP= nevirapine

$^{\Phi}$ exposure groups are not mutually exclusive as patients may have sequentially used multiple antiretroviral treatment combinations

$^{\forall}$ statistically significant difference from IDV/d4T/3TC

$^{\Psi}$ statistically significant difference from IDV/AZT/3TC

$^{\clubsuit}$ adjusted for age, gender, bmi at baseline, change in weight over past six months, cd4 cell increase, HIV-1 RNA detectability and treatment combinations that were associated with lipodystrophy in the univariate analysis (bold prints)

Distinguishing cumulative exposure to antiretroviral agents into mutually exclusive exposure groups allowed for the direct comparison of different agents and combinations. We hence compared, for example, patients who only used stavudine and never used zidovudine with patients who only used zidovudine but never used stavudine. This analysis showed that saquinavir, ritonavir and nevirapine tended to have a lower risk of lipodystrophy than indinavir (Table 5).

Table 5 Association between mutually exclusive antiretroviral treatment combinations and development of clinically evident lipodystrophy (n=261) among previously antiretroviral treatment naïve patients (n=1952)

> 6 months exposure vs. no exposureΦ		Univariate		multivariate♣	
	cases	RR	(95%CI)	RR	(95%CI)
Exclusively used one of the following key components					
IDV	69	reference		reference	
SQV	11	0.4	(0.2-0.8)	1.0	(0.2-4.6)
RTV	13	0.4	(0.2-0.7)	0.3	(0.1-1.4)
NFV	8	0.6	(0.3-1.4)	0.4	(0.1-2.2)
NVP	2	0.3	(0.1-1.1)	-	-
None	6	0.9	(0.4-2.0)	-	-
Multiple or < 6 months use	152	0.8	(0.6-1.05)	0.6	(0.3-1.04)
Exclusively used one of the following backbones					
AZT	74	reference		reference	
D4T	128	**3.8**	**(2.8-5.1)**	**5.4**	**(2.6-11.5)**
AZT and d4T	21	**1.8**	**(1.04-3.1)**	2.8	(0.7-10.7)
Neither AZT nor d4T	5	1.6	(0.6-4.4)	1.6	(0.2-13.7)
< 6 months use of AZT and or d4T	33	**1.9**	**(1.3-2.8)**	1.7	(0.6-4.7)
Exclusively used one of the following regimensΨ					
AZT/3TC/IDV	28	reference		reference	
AZT/3TC/SQV	12	1.1	(0.5-2.1)	1.4	(0.2-8.0)
AZT/3TC/RTV	7	0.5	(0.2-1.2)	0.6	(0.1-5.4)
AZT/3TC/NFV	1	0.3	(0.03-2.0)	-	-
AZT/3TC/SQV/RTV	3	0.9	(0.3-3.2)	-	-
d4T/3TC/IDV	32	4.6	(2.7-7.7)	4.3	(1.3-13.8)
d4T/3TC/SQV	5	3.4	(1.3-9.0)	90.2	(3.2-2527.1)
d4T/3TC/RTV	4	1.4	(0.5-4.1)	1.2	(0.1-11.8)
d4T/3TC/NFV	7	4.3	(1.7-11.3)	8.1	(1.2-54.9)
d4T/3TC/IDV400/RTV400	1	3.9	(0.5-33.7)	-	-
d4T/3TC/SQV/NFV	7	2.6	(1.1-6.1)	2.7	(0.7-10.9)
d4T/3TC/SQV/RTV	7	2.2	(0.9-5.2)	3.6	(0.7-17.4)
d4T/3TC/NVP	7	1.1	(0.3-4.7)	-	-
d4T/DDI/IDV	9	2.3	(1.1-5.0)	1.8	(0.4-8.7)
otherΨ	32	1.7	(1.03-2.9)	2.1	(0.6-7.4)

AZT= zidovudine; 3TC= lamivudine; d4T= stavudine; DDI= didanosine; IDV= indinavir; SQV= saquinavir; RTV= ritonavir; NFV= nelfinavir; SQV/RTV= SQV combined with RTV; IDV/RTV= IDV combined with RTV; IDV800/RTV100= IDV 800 mg combined with RTV 100 mg; IDV400/RTV400= IDV 400 mg combined with RTV 400 mg; NVP= nevirapine

Φ Patients contribute only to one category of the key components and backbones during follow-up

♣ Adjusted for age, gender, bmi at baseline, change in weight over past six months, cd4 cell increase and HIV-1 RNA detectability, key components and backbone

Ψ There were insufficient data to analyse combinations of AZT/3TC with IDV/RTV, SQV/NFV and NVP, d4T/3TC with IDV800/RTV100 and d4T/ddI combinations with SQV and RTV. These regimens are not included in the 'other' group. The 'other' group contains patients who switched regimens, who used regimens not included in this list or used regimens for less than 6 months. Multivariate results are not adjusted for keycomponent and backbone.

Comparing patients who had used stavudine for more than six months with patients who had only used zidovudine showed that the risk was highest after exposure to stavudine. Direct comparison of treatment combinations showed that compared to regimens containing AZT, 3TC and IDV, regimens containing d4T and 3TC had the highest risk of lipodystrophy. In the multivariate analysis, the observed risk was especially high for d4T, 3TC combinations with saquinavir, nelfinavir and indinavir, but confidence intervals were extreemly high due to the low number of patients.

Discussion

In this population based prospective cohort study of incident users of antiretroviral treatment, we observed an incidence rate of clinically evident lipodystrophy of 6.2 per 100 person years of treatment. Within four years after starting treatment, comprising a wide range of regimens, 25% of patients had developed lipodystrophy.

The risk of lipodystrophy increased with older age, female gender, high body mass index and good immunological and virological response. The results showed a clear association between the prolonged use of any combination with stavudine and lipodystrophy. The lowest risk of lipodystrophy was observed with zidovudine and lamivudine containing regimens, especially if combined with nevirapine. Indinavir appeared to play a minor role compared to stavudine.

Only few cohort studies on incident users of antiretroviral treatment and lipodystrophy have been reported so far. Studies with prevalent cases have been reported but these are highly susceptible to bias. Prevalences of lipodystrophy have been reported but vary widely from 49% to 83% (8, 12, 21, 22, 27) since time on treatment is not always taken into account. Figures on incidence rates of lipodystrophy are scanty (7, 17). Martinez et-al reported an incidence rate of 11.7 per 100 person years (7), whereas Van der Valk et-al reported a cumulative incidence of 17% over two years (17). Differences may be explained by differences in patient populations and assessment of lipodystrophy.

Despite the inconsistencies between studies, other studies also reported that the risk of lipodystrophy increased with older age and was highest in women (7, 8, 19, 27). In addition, most studies agree on a role of antiretroviral treatment in the development of lipodystrophy (7, 8, 18, 19, 27), but not necessarily on the type of antiretroviral agents involved. Many studies reported on individual antiretroviral agents without accounting for the potential risk of clustering between certain agents (7, 18). The sample size of our study was large enough to extensively analyse both individual antiretroviral agents and antiretroviral treatment combinations. We therefore believe our results convincingly show

that among the antiretroviral agents studied, stavudine is the most important independent risk factor for lipodystrophy. Indinavir also acted as an independent risk factor, but the effect was less pronounced than with stavudine. The fact that the results were adjusted for immunological and virological response argues against speculations that lipodystrophy is a pure immune recovery effect or metabolic stress relieve effect, although interaction between these effects and treatment effects cannot be excluded (19). The association between weight loss in the last six months and lipodystrophy might reflect increased recognition of lipodystrophy among patients with weight loss.

An important limitation of our study is the lack of objective measurements of lipodystrophy and discrimination between lipoatrophy and fat accumulation, and the absence of an accepted definition of the lipodystrophy syndrome. The recently presented validated algorithm, including results from DEXA scans, anthropometric measurements and laboratory assessments (28) could not be applied to the ATHENA cohort. It has, however, been reported previously that there is 98% concordance between patient self-report and physical examination (21). The amount of misclassification of lipodystrophy may therefore have been small. Furthermore, we have no reason to believe that misclassification of the outcome, if any, was differential especially since we stratified for hospital and calendar time. Importantly, our results are consistent with other studies, which did identify an association between the use of stavudine and lipo-atrophy and the use of indinavir and fat-accumulation (16, 18, 19, 27), even though we did not differentiate between various types of lipodystrophy. Finally, our data produced similar relative risks for gender and age as previous studies, suggesting little influence of our case definition on the actual associations.

In 1996 the cumulative incidence of lipodystrophy was lower than later in calendar time, which probably is the result of underreporting or under-recognition bias shortly after the introduction of the new antiretroviral agents when lipodystrophy was not even known. Subsequent awareness may have led to delayed reporting of early cases of lipodystrophy. This might have caused an underestimation of the actual incidence rate of lipodystrophy. Since we did anticipate a calendar time effect we dealt with this in the analyses of risk factors by stratification for calendar time. In doing so, the analyses effectively compared cases of lipodystrophy with non-cases who started the first antiretroviral regimen within a three month window of the case and had the same length of follow-up. Thus calendar time is unlikely to have disturbed our results.

In conclusion, in this previously antiretroviral treatment naïve patient population clinically evident lipodystrophy occurred at a rate of 6.2 cases per 1000 years of follow-up. Apart from older age, female gender, high body mass index, and good immunological and virological response, exposure to stavudine and to a lesser extent exposure to indinavir were

independent risk factors for lipodystrophy. Patients exposed to zidovudine and lamivudine-containing regimens, combined with nevirapine in particular, had the lowest risk of lipodystrophy. The risk of developing lipodystrophy with the use of other regimens including other non-nucleoside reverse transcriptase inhibitors, such as efavirenz, boosted HIV protease inhibitor combinations, regimens solely composed of nucleoside analogues, or nucleoside-sparing regimens remains to be determined.

References

1. Carr A. HIV protease inhibitor-related lipodystrophy syndrome. Clin Infect Dis 2000;30 Suppl 2:S135-42.
2. Carr A, Cooper DA. Images in clinical medicine. Lipodystrophy associated with an HIV-protease inhibitor. N Engl J Med 1998;339:1296.
3. Hengel RL, Watts NB, Lennox JL. Benign symmetric lipomatosis associated with protease inhibitors. Lancet 1997;350:1596.
4. Miller MD, Jones E, Yanovski JA, Shankar R, Feuerstein I, Falloon J. Visceral abdominal-fat accumulation associated with use of indinavir. Lancet 1998;351:871-75.
5. Carr A, Samaras K, Chisholm DJ, Cooper DA. Abnormal fat distribution and use of protease inhibitors. Lancet 1998;351:1736.
6. van der Valk M, Bisschop PH, Romijn JA, Ackermans MT, Lange JM, Endert E, et al. Lipodystrophy in HIV-1-positive patients is associated with insulin resistance in multiple metabolic pathways. AIDS 2001;15:2093-100.
7. Martinez E, Mocroft A, Garcia-Viejo MA, Perez-Cuevas JB, Blanco JL, Mallolas J, et al. Risk of lipodystrophy in HIV-1-infected patients treated with protease inhibitors: a prospective cohort study. Lancet 2001;357:592-8.
8. Heath KV, Hogg RS, Chan KJ, Harris M, Montessori V, O'Shaughnessy MV, et al. Lipodystrophy-associated morphological, cholesterol and triglyceride abnormalities in a population-based HIV/AIDS treatment database. AIDS 2001;15:231-9.
9. Herry I, Bernard L, de Truchis P, Perronne C. Hypertrophy of the breasts in a patient treated with indinavir. Clin Infect Dis 1997;25:937-8.
10. Toma E, Therrien R. Gynaecomastia during indinavir antiretroviral therapy in HIV infection. AIDS 1998;12:681-2.
11. Caeiro J-P, Visnegarwala F, Rodriguez-Barradas MC. Gynaecomastia associated with indinavir therapy. Clin Infect Dis 1998;27:1539-40.
12. Carr A, Samaras K, Burton S, Law M, Freund J, Chisholm DJ, et al. A syndrome of peripheral lipodystrophy, hyperlipidaemia and insulin resistance in patients receiving HIV protease inhibitors. AIDS 1998;12:F51-8.
13. Carr A, Samaras K, Chisholm DJ, Cooper DA. Pathogenesis of HIV-1-protease inhibitor-associated peripheral lipodystrophy, hyperlipidaemia, and insulin resistance. Lancet 1998;351:1881-83.

14. Striker R, Conlin D, Marx M, Wiviott L. Localized adipose tissue hypertrophy in patients receiving human immunodeficiency virus protease inhibitors. Clin Infect Dis 1998;27:218-20.
15. Tsiodras S, Mantzoros C, Hammer S, Samore M. Effects of protease inhibitors on hyperglycemia, hyperlipidemia, and lipodystrophy: a 5-year cohort study. Arch Intern Med 2000;160:2050-6.
16. Mallal SA, John M, Moore CB, James IR, McKinnon EJ. Contribution of nucleoside analogue reverse transcriptase inhibitors to subcutaneous fat wasting in patients with HIV infection. AIDS 2000;14:1309-16.
17. van der Valk M, Gisolf EH, Reiss P, Wit FW, Japour A, Weverling GJ, et al. Increased risk of lipodystrophy when nucleoside analogue reverse transcriptase inhibitors are included with protease inhibitors in the treatment of HIV-1 infection. AIDS 2001;15:847-55.
18. Saint-Marc T, Partisani M, Poizot-Martin I, Rouviere O, Bruno F, Avellaneda R, et al. Fat distribution evaluated by computed tomography and metabolic abnormalities in patients undergoing antiretroviral therapy: preliminary results of the LIPOCO study. AIDS 2000;14:37-49.
19. Lichtenstein KA, Ward DJ, Moorman AC, Delaney KM, Young B, Palella FJ, Jr., et al. Clinical assessment of HIV-associated lipodystrophy in an ambulatory population. AIDS 2001;15:1389-98.
20. Aldeen T, Wells C, Hay P, Davidson F, Lau R. Lipodystrophy associated with nevirapine-containing antiretroviral therapies. AIDS 1999;13:865-7.
21. Carr A, Samaras K, Thorisdottir A, Kaufmann GR, Chisholm DJ, Cooper DA. Diagnosis, prediction, and natural course of HIV-1 protease-inhibitor-associated lipodystrophy, hyperlipidaemia, and diabetes mellitus: a cohort study. Lancet 1999;353:2093-9.
22. Saint-Marc T, Partisani M, Poizot-Martin I, Bruno F, Rouviere O, Lang JM, et al. A syndrome of peripheral fat wasting (lipodystrophy) in patients receiving long-term nucleoside analogue therapy. AIDS 1999;13:1659-67.
23. Hadigan C, Meigs JB, Corcoran C, Rietschel P, Piecuch S, Basgoz N, et al. Metabolic abnormalities and cardiovascular disease risk factors in adults with human immunodeficiency virus infection and lipodystrophy. Clin Infect Dis 2001;32:130-9.
24. Hatano H, Miller KD, Yoder CP, Yanovski JA, Sebring NG, Jones EC, et al. Metabolic and anthropometric consequences of interruption of highly active antiretroviral therapy. AIDS 2000;14:1935-42.
25. Ruiz L, Negredo E, Domingo P, Paredes R, Francia E, Balague M, et al. Antiretroviral treatment simplification with nevirapine in protease inhibitor-experienced patients with hiv-associated lipodystrophy: 1-year prospective follow-up of a multicenter, randomized, controlled study. J Acquir Immune Defic Syndr 2001;27:229-36.
26. Borleffs JC, Danner SA, Lange JM, van Everdingen JJ. [CBO guidelines 'Antiretroviral therapy in the Netherlands']. Ned Tijdschr Geneeskd 2001;145:1585-9.
27. Bogner JR, Vielhauer V, Beckmann RA, Michl G, Wille L, Salzberger B, et al. Stavudine versus zidovudine and the development of lipodystrophy. J Acquir Immune Defic Syndr 2001;27:237-44.
28. Carr A, group for the HIV lypodystrophy case definition study group. An objective case definition of HIV lipodystrophy. In: 9th Conference on Retroviruses and Opportunistic Infections; 2002; Seattle; abstract 31.

Chapter 4

Effects of intolerance on treatment continuity

Chapter 4.1

Substituting nevirapine for protease inhibitors because of intolerance

Short report

Many patients fail Highly Active Anti-Retroviral Therapy (HAART) because of poor compliance, which is a consequence of the complexity of the dosing regimens, the large volumes of pills to be taken and the side effects associated with the use of protease inhibitors (PIs). The most commonly reported side effects of PIs include nausea, vomiting, diarrhoea, urological complaints and lipodystrophy (1, 2). Several studies have shown that 'step down- strategies' as an easier maintenance therapy fail to preserve viral suppression (3-5).

Nevirapine (NVP) has demonstrated anti-HIV-1 efficacy with a less complex dosing regimen and a more favourable safety profile than PIs in antiretroviral therapy naïve patients (6). Rash seems the most important side effect. In view of its favourable efficacy and safety profile, substituting NVP for PI might be a good alternative as maintenance therapy after PI induction treatment.

To investigate this strategy, we retrospectively reviewed data on all patients whose PI containing regimen was switched to a NVP containing regimen because of PI-intolerance (reasons listed in Table 1) between July 1997 and September 1998. All patients had HIV-1 RNA levels below the detection limit (<500 copies/mL, Roche Amplicor) at the time of switch. Of 300 patients on HAART in our institute, 41 (14%) patients (29 men and 12 women) had changed therapy. Their mean age was 41±10 years and of them twelve (29%) had CDC C3 disease classification. As part of their first HAART-regimen, 18 patients used indinavir, 13 ritonavir, seven a ritonavir-saquinavir combination and three used saquinavir. Viral loads had decreased from a mean of 4.97±0.52 log copies/mL to undetectable levels within a mean of 17±17 weeks (range 3-91) after start of HAART. The average CD4 cell count had increased by approximately 200 cells/mL at the time of switch. Before changing to NVP, eight patients tried another PI.

Table 1 shows reasons for PI discontinuation, treatment duration and subsequent virological, immunological and clinical outcomes. Following the switch to NVP, gastro-intestinal complaints improved considerably in 22 out of 26 patients. Although lipodystrophy improved in some patients according to the patient's self report, body appearance remained abnormal in all six during a median follow-up period of 24 (range 15-45) weeks. Urological complaints subsided. Glucose regulation improved in the two hyperglycaemic patients. Four patients (10%) developed a rash on NVP treatment. Two patients discontinued NVP within four weeks because of side effects, one because of rash and one because of persistent nausea and vomiting. HIV-1 RNA remained undetectable in all patients who

continued NVP treatment but two, who experienced an increase to 1340 copies/mL and 18700 copies/mL after 56 and 15 weeks of NVP treatment, respectively.

Table 1 Reasons for stopping PI and effects of changing to NVP

Assessment	N= 41	End of PI treatment	On NVP treatment
Main reason for switch			
Gastro-intestinal complaints	N (%)	26 (63)	4 (10)
Lipodystrophy	N (%)	6 (15)	6 (15)
Urological complaints	N (%)	2 (5)	0 (0)
Hyperglycaemia	N (%)	2 (5)	2 (5)
Patient request	N (%)	3 (7)	-
Unknown	N (%)	2 (5)	-
Weeks of therapy	Mean (SD)	49 (31)	36 (19)
	Median (range)	47 (5–102)	30 (8–87)
CD4 count (cells/mL)	N	32	38
	Mean (SD)	439 (227)	518 (265)#
	Median (range)	435 (90–900)	440 (70–1230)
HIV-1 RNA (copies/mL)	N	41	39
	N<500 (%)	41 (100)	37 (95)

Wilcoxon signed ranks test for paired samples (CD4 count at end of PI treatment versus last CD4 cell count during NVP treatment, n=29): p<0.001

In this case-study, we show that switching from a PI to a NVP containing anti-HIV-1 regimen diminished most side effects without loss of viral suppression in 37 of 39 patients during a median follow-up period of 30 weeks (range 8-87). We suggest that NVP in combination with two NRTIs has potential as a long-term maintenance treatment after successful induction therapy with a PI containing regimen. The favourable risk profile and the less complex treatment regimen should enhance adherence and promote long-term effectiveness, which is an emerging issue in anti-HIV-1 treatment to date. It seems worthwhile to further investigate the role of NVP as part of a maintenance therapy in a controlled setting and with ultra-sensitive HIV-1 RNA measurement techniques.

References

1. Carr A, Samaras K, Chisholm DJ, Cooper DA. Pathogenesis of HIV-1-protease inhibitor-associated peripheral lipodystrophy, hyperlipidaemia, and insulin resistance. Lancet 1998, 351:1881-83.
2. Deeks SG, Smith M, Holodniy M, Kahn JO. HIV-1 protease inhibitors. A review for clinicians. JAMA 1997, 277:145-53.
3. Pialoux G, Raffi F, Brun-Vezinet F, et al. A randomized trial of three maintenance regimens given after three months of induction therapy with zidovudine, lamivudine, and indinavir in previously untreated HIV-1-infected patients. Trilege (Agence Nationale de Recherches sur le SIDA 072) Study Team. N Engl J Med 1998, 339:1269-76.

4. Havlir DV, Marschner IC, Hirsch MS, et al. Maintenance antiretroviral therapies in HIV infected patients with undetectable plasma HIV RNA after triple-drug therapy. AIDS Clinical Trials Group Study 343 Team. N Engl J Med 1998, 339:1261-8.
5. Reijers MH, Weverling GJ, Jurriaans S, et al. Maintenance therapy after quadruple induction therapy in HIV-1 infected individuals: Amsterdam Duration of Antiretroviral Medication (ADAM) study. Lancet 1998, 352:185-90.
6. Montaner JS, Reiss P, Cooper D, et al. A randomized, double-blind trial comparing combinations of nevirapine, didanosine, and zidovudine for HIV-infected patients: the INCAS Trial. Italy, The Netherlands, Canada and Australia Study. JAMA 1998, 279:930-7.

Chapter 4.2

Low risk of treatment failure one year after substituting nevirapine for human immunodeficiency virus (HIV)-protease inhibitors among HIV-infected patients with viral suppression.

The ATHENA cohort.

Abstract

Information on the risk of treatment failure after switching from human immunodeficiency virus (HIV)-protease inhibitors (PI) to nevirapine (NVP) while having successful viral suppression is scanty. We compared the one-year risk of treatment failure between patients switching from the first PI-containing antiretroviral regimen to NVP (NVP-group) and patients switching to second-line PI (PI-group) in the ATHENA-cohort (n=2470) while HIV-1 RNA was <500 copies/mL. Treatment failure was defined as HIV-1 RNA twice >500 or once >10,000 copies/mL or treatment discontinuation for any reason.

There were 446 eligible patients, 125 and 321 patients in the NVP-group and PI-group respectively. Adjusted for other risk factors, the NVP-group had a five-fold (95%CI:0.1-0.4) lower risk of treatment failure than the PI-group, predominantly because of lower discontinuation rates. In presence of successful viral suppression switching to NVP as opposed to second-line PIs was superior with regarding the likelihood of maintaining the same treatment.

Introduction

Treatment of human immunodeficiency virus (HIV)-1 infection with Highly Active Antiretroviral Therapy (HAART) containing an HIV-protease inhibitor (PI) may be troubled by large quantities of capsules, stringent intake schedules, food restrictions, drug-drug interactions and numerous adverse drug reactions (1, 2) . These factors may interfere with long-term adherence and therefore lead to premature viral rebound and the selection of resistant viral variants. Various strategies for maintenance treatment have proven to be ineffective in a number of studies (3-5) . Moreover, the benefits of switching between PIs are limited, possibly due to the presence of class specific problems (6) .

Nevirapine (NVP) is a non-nucleoside analogue reverse transcriptase inhibitor (NNRTI) that has demonstrated potent anti-HIV efficacy in combination with two nucleoside analogue reverse transcriptase inhibitors (NRTIs) (7, 8) . Combination therapy of NVP with two NRTIs is relatively easy and has shown few adverse effects, although potentially serious rashes and liver toxicity have been reported (8) .

Patients who have been treated successfully with a PI-containing HAART regimen may benefit from the less complex treatment regimen with NVP, which should help improve long-term adherence, and thereby the chance of sustained antiviral efficacy. This hypothesis has been subject of a number of studies so far, most of which lacked a comparison arm, studied small patient numbers or had a short follow-up period (9-12) . As a consequence the information regarding the effectiveness and safety of NVP for patients with viral suppression is insufficient so far.

In order to gain more insight into the consequences of substituting NVP for PIs during viral suppression under everyday circumstances, we conducted an observational cohort analysis within the Dutch nationwide ATHENA cohort of patients treated for their HIV-infection. In this cohort with detailed data on HIV-treatment and treatment outcome we determined the one-year risk of treatment failure after switching from PIs to NVP as opposed to switching to a second-line PI regimen (PItoN study cohort).

Materials and methods

Setting

We conducted our study within the ATHENA cohort, which is a nationwide observational cohort in the Netherlands. The ATHENA cohort includes HIV-infected patients who are treated with one or more of the new antiretroviral drugs that became available in the Netherlands in July 1996 and thereafter (ie. all PIs, all NNRTIs and the newer NRTIs such as

lamivudine and stavudine). The source population comprises HIV-infected patients in follow-up at the 22 hospitals that provide treatment to HIV-infected patients in the Netherlands and willing to give written informed consent. The ATHENA project has been approved by the local Ethics Committees of all centres.

According to the national guidelines for HIV-treatment in the Netherlands, patients are seen at approximately three-monthly intervals for regular follow-up (13) . Data for the ATHENA cohort are collected on standardised forms, retrospectively from medical records until patient entry into the cohort, and prospectively thereafter by trained research nurses and treating physicians. The resulting ATHENA database, which is updated approximately every six months, contains information on gender, age, hospital, height and weight. Start and stop dates as well as dose frequency of every antiretroviral medication and prophylactic treatment against opportunistic infections, primary reason for stopping such treatments as classified by the treating physician, dates of onset and resolution of HIV-related diseases, CD4 cell counts, plasma HIV-1 RNA load and abnormal laboratory values are recorded. Information on adverse events comprises all events that lead to a change in antiretroviral treatment and a number of pre-specified adverse events such as neuropathy, lipodystrophy and hepatitis. Available data on plasma HIV-1 RNA result from three different quantitative assays, Amplicor (Roche Diagnostics, Branchburg, NJ, USA), NucliSens (Organon Teknika, Boxtel, Nederland) or Quantiplex (Chiron Diagnostics Corporation, East Walpole, MA, USA). At the time of data extraction for the analysis in March 2000 data were available for 2470 patients.

PItoN study cohort

The source population for our study involved all patients in the ATHENA database using a PI-containing first antiretroviral regimen during the study period. The study period ran from 1 May 1997, when the compassionate use program for NVP became effective, until March 2000. Patients entered the PItoN study cohort upon switching the PI component of the first-line antiretroviral regimen (HAART 1) while HIV-1 RNA was below 500 copies/mL. Changes of ritonavir (RTV) formulation were not considered as a switch because of a nation-wide change to a new formulation. Any other change of the PI component, including dose frequency change other than standard initial dose escalations, addition or removal of a PI and change of saquinavir (SQV) formulation, was considered as a switch. The date of cohort entry was defined as the start date of the second HAART regimen (HAART 2). We excluded all patients for whom we did not have an HIV-1 RNA assessment within at least three months prior to the start of HAART 2 or who interrupted treatment for more than seven days in order to ensure the presence of viral suppression at the start of HAART 2. The resulting PItoN study cohort was divided into two treatment groups based on the use of NVP or PIs in

HAART 2. Switches to other NNRTIs, such as efavirenz, were not studied separately in this study since NVP was the only registered NNRTI in the Netherlands at the time of this analysis. Switches to triple NRTI-regimens were not studied either as the primary interest of our study was to compare switching to NVP with switching between PIs.

Outcome

The primary endpoint of the study was the proportion of patients experiencing treatment failure within one year after switching to HAART 2. Treatment failure was defined as either virological failure or discontinuation of HAART 2 for any reason. Virological failure was defined as plasma HIV-1 RNA levels above 500 copies/mL on two consecutive measurements or a single plasma HIV-1 RNA assessment of more than 10,000 copies/mL. Discontinuation of HAART 2 was defined as either another treatment switch according to the above description, discontinuation of HAART 2 or death. Notably switching NRTIs only was not considered as a treatment switch.

As secondary endpoints we studied time to treatment failure, the effect of substituting NVP versus substituting a new HIV-protease inhibitor and versus other types of switch, the effect of the most common switches at the level of individual antiretroviral agents, the course of CD4 cell counts and the occurrence of clinical adverse events. Follow-up for the study lasted from the date of study entrance until the completion of one year, last update of data collection or treatment failure, whichever occurred first.

Co-variates

As potential determinants of treatment failure and potential confounding factors we considered demographic factors, such as age, gender, country of birth, route of HIV-transmission and CDC-disease classification at the time of switch (14) . In addition, we considered treatment history prior to HAART 1 (categorised as either naive or experienced to NRTIs), duration of HAART 1, duration of viral suppression prior to switch (defined as the last uninterrupted period of HIV-1 RNA below 500 copies/mL), prior history of virological rebound (defined as HIV-1 RNA at least once above 500 copies/mL after suppression below 500 copies/mL), CD4 cell count at the time of switch, type of antiretrovirals used in HAART 1 and 2, calendar time (expressed as months since start study period) and the reason for switch. Reasons for switch were categorised by the treating physician as: intolerance, patient request, failure (the latter in the context of the ATHENA study being defined as either increase in viral load, decrease in CD4 cell count or disease progression), pharmacological indication, other with specification and unknown.

Analysis

Differences in baseline characteristics between the PI-group and NVP-group were tested by means of the Pearson's Chi-square test or Fisher's exact test for categorical variables. The Student's t-test was used for continuous variables unless non-normally distributed in which case we used the Mann-Whitney-U test. The one-year hazard for treatment failure was estimated by means of Kaplan Meier survival analysis. Crude comparisons regarding the risk of treatment failure between the HAART 2 groups were made both with the log-rank test and univariate Cox-regression analysis. Risk factors for treatment failure were identified by means of univariate and subsequent multivariate Cox-regression analysis. The multivariate model included all factors from the univariate analysis that were associated with treatment failure at a p-value of <0.1. Time to failure was calculated from the date of cohort entry to the first date of HIV-1 RNA above 500 copies/mL or discontinuation of HAART 2, whichever occurred first. Results were expressed as % of patients or relative risk (RR) with 95%-confidence intervals (95%CI). The course of CD4 cell counts until treatment failure or end of follow-up was analysed by means of a General Linear Model (GLM) for repeated measurements adjusted for the baseline CD4 cell count. The presence and resolution of adverse events was described.

Statistical significance was accepted at a two-sided p-value of 0.05. All analyses were performed with SPSS statistical package for Windows version 8.0.

Results

Cohort

The source population in the ATHENA database consisted of 2434 patients who ever used a PI before March 2000. Of these, 1716 patients were ineligible for our study cohort because of: an NNRTI in HAART 1 (n=126), continuation of HAART 1 (n=738), HIV-1 RNA above 500 copies/mL (n=637) and change of HAART 1 before 1 May 1997 (n=215). We further excluded those with more than seven days between HAART 1 and HAART 2 (n=510) and absence of a baseline HIV-1 RNA measurement (n=129), resulting in a final cohort of 446 patients.

The final study cohort consisted of 125 (28%) patients for whom PIs were replaced by NVP (NVP-group) and 321 (72%) patients who had a change of the PI component (PI-group, Table 1). The most common switches included switches from SQV hard gel capsule (hgc) to SQVhgc combined with RTV, from one indinavir (IDV) dose frequency to another IDV dose frequency (n=42, 9%), from RTV, SQVhgc combined with RTV or from IDV to NVP (n=42, 9%) and from IDV or RTV to nelfinavir (Table 2).

Table 1 Type of treatment change of the first human immunodeficiency virus (HIV)-protease inhibitor containing antiretroviral treatment in presence of viral suppression

Treatment change[a]	N	(%)
NVP-group		
Changed PI into NVP	125	(28.0)
PI-group		
Changed to another type of PI	117	(26.2)
Removed one PI, maintaining one of two PIs	7	(1.6)
Added one PI	99	(22.2)
Changed SQV formulation	22	(4.9)
Changed dosage and/or dose frequency	76	(17.0)
Total	446	(100)

NVP= nevirapine; PI= HIV-protease inhibitor; SQV= saquinavir

Table 2 The most common substitutions for the first human immunodeficiency virus (HIV)-protease inhibitor in presence of viral suppression

HIV-protease inhibitor / NNRTI component			
HAART 1	HAART 2	N	(%)
SQVhgc	SQVhgc/RTV	53	(12)
IDV	IDV	42	(9)
RTV	NVP	42	(9)
SQVhgc/RTV	NVP	34	(8)
IDV	NVP	25	(6)
IDV	NFV	25	(6)
RTV	NFV	21	(5)
IDV	IDV/RTV	20	(4)
RTV	SQVhgc/RTV	19	(4)
Other		165	(37)

HAART= highly active antiretroviral therapy; IDV= indinavir; NFV= nelfinavir; NVP= nevirapine; RTV= ritonavir; SQVhgc= saquinavir hard gel capsule

Patient characteristics at the time of switch of HAART 1 (baseline) are presented in table 3. The NVP-group was more often antiretroviral treatment-naive prior to HAART 1, had longer successful viral suppression prior to switch, more often had a concomitant change in NRTIs, switched later in calendar time and more often had intolerance and patient request as reasons for switch than the PI-group. Furthermore, the NVP-group used more stavudine (d4T) and less didanosine (ddI) in HAART 1 than the PI-group, and the NVP-group more often had RTV and SQV combined with RTV but less SQV in HAART 1.

Table 3 Patient characteristics of the PItoN study cohort (n= 446)

Characteristic	N (%)	Switch to NVP (n=125)		PI (n=321)[b]		P-valuea
Age	Median (IQR)	40	(35-48)	39	(33-46)	Ns
Gender	Male	110	(88.0)	264	(82.)	Ns
	Female	15	(12.0)	57	(17.8)	
Country of birth	Netherlands	83	(66.9)	233	(73.7)	Ns
	Other	41	(33.1)	83	(26.3)	
Route of transmission	MSM	80	(67.8)	204	(67.3)	Ns
	intravenous drug use	3	(2.5)	20	(6.6)	
	Other	35	(29.7)	79	(26.1)	
CDC-classification	C	30	(24.0)	78	(24.3)	Ns
	A or B	95	(76.0)	243	(75.7)	
Naive start HAART 1	Yes	97	(77.6)	189	(58.9)	<0.001
	No	28	(22.4)	132	(41.1)	
Weeks on HAART 1	Median (IQR)	66	(38-92)	64	(30-87)	Ns
Weeks <=500 copies/mL	Median (IQR)	47	(21-82)	37	(15-66)	0.008
Prior virological failure	Yes	17	(14.7)	61	(22.4)	Ns
	No	99	(85.3)	211	(77.6)	
CD4 at switch (cells/µL)	Median (IQR)	440	(300-600)	420	(300-600)	Ns
NRTIs changed	Yes	32	(25.6)	36	(11.2)	<0.001
	No	93	(74.4)	285	(88.8)	
Months since 1 May 1997)	Median (IQR)	20	(16-23)	14	(10-18)	<0.001
Reason for switch HAART 1	Intolerance	62	(49.6)	89	(27.7)	<0.001
	Patient request	38	(30.4)	66	(20.6)	
	Pharmacological indication	3	(2.4)	36	(11.2)	
	Failure[d]	3	(2.4)	15	(4.7)	
	Other[e]	11	(8.8)	83	(25.8)	
	Unknown	8	(6.4)	32	(10.0)	
HIV-protease inhibitor before switch	NFV	5	(4.0)	6	(1.9)	<0.001
	IDV	25	(20.0)	104	(32.4)	
	SQVhgc	10	(8.0)	77	(24.0)	
	SQVsgc	2	(1.6)	2	(0.6)	
	RTV	42	(33.6)	66	(20.6)	
	IDV/RTV	6	(4.8)	2	(0.6)	
	SQVhgc/NFV	1	(0.8)	14	(4.4)	
	SQVsgc/NFV	0	(0)	10	(3.1)	
	SQVhgc/RTV	34	(27.2)	38	(11.8)	
	other	0	(0)	2	(0.6)	
NRTIs before switch	AZT/3TC	60	(48.0)	151	(47.0)	0.042
	D4T/3TC	44	(35.2)	117	(36.4)	
	d4T	10	(8.0)	9	(2.8)	
	AZT/ddI	2	(1.6)	14	(4.4)	
	d4T/ddI	2	(1.6)	12	(3.7)	
	other	3	(2.4)	15	(4.7)	
	none	4	(3.2)	3	(0.9)	

3TC= lamivudine; AZT= zidovudine; d4T= stavudine; ddC= zalcitabine; ddI= didanosine; HAART= Highly Antiretroviral Therapy; IDV= indinavir; IQR= Inter Quartile Range; MSM= men having sex with men; NFV= nelfinavir; NRTI= nucleoside analogue reverse transcriptase inhibitor; NVP= nevirapine; PI= HIV-protease inhibitor; RTV= ritonavir; SQVhgc= saquinavir hard gel capsule; SQVsgc= SQV soft gel capsule

a Pearson Chi-square or Fisher's exact test for categorical variables and Mann-Whitney-U for continuous variables; Ns: p>0.05

b PI-group consisted of nelfinavir (22%), indinavir (20%), saquinavir hard gel capsule (4%), saquinavir soft gel capsule (3%), ritonavir (3%), indinavir/ritonavir (9%), saquinavir hard gel capsule/nelfinavir (1%), saquinavir soft gel capsule/nelfinavir (7%), saquinavir hard gel capsule/ritonavir (30%), saquinavir soft gel capsule/ritonavir (1%), other (2%)

c Percentages of treatment group HAART 2

d Failure while HIV-1 RNA<500 copies/mL (ie. low CD4 cell count and/or clinical progression and/or HIV-1 RNA detectable with ultra sensitive HIV-1 RNA assay).

e Including convenience (7% resp.12%), protocol directed (2% resp.9%) and other (0% resp.4%).

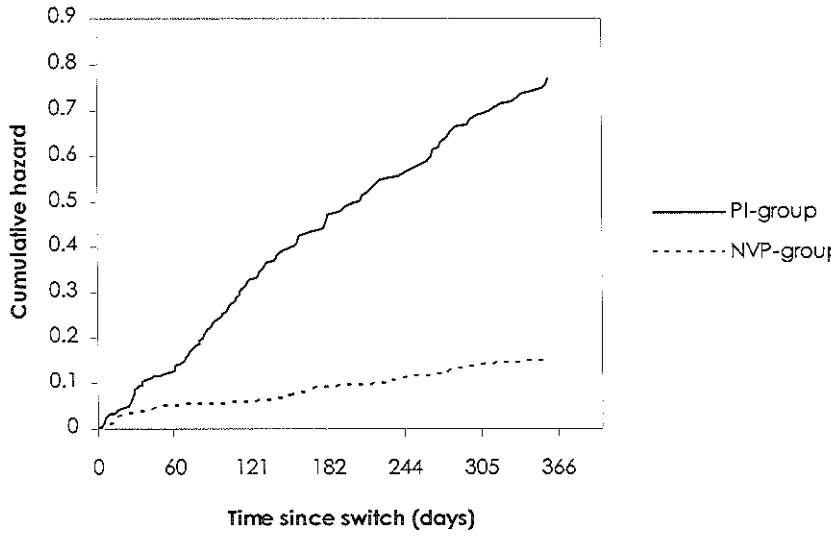

Figure 1 Cumulative hazard of treatment failure after substituting the first human immunodeficiency virus (HIV)-protease inhibitor (PI)
Patients who had switched to a second-line HIV-protease inhibitor (PI-group) under successful viral suppression (solid line) had a significantly higher risk of treatment failure (virological failure or treatment discontinuation) than patients, who had switched to nevirapine (NVP-group) under the same circumstances (dotted line) (log-rank p<0.001).

Treatment failure

Within one year after changing HAART 1, 187 (42%) patients experienced treatment failure (Figure 1). The one-year cumulative hazard of treatment failure was 0.2 (95%CI: 0.1-0.2) and 0.8 (95%CI: 0.7-0.9) for the NVP-group and PI-group respectively, resulting in a RR of 0.2 (95%CI: 0.1-0.3) for the NVP-group versus the PI-group (Table 4). The median time to treatment failure within the first year after switch was 22 (IQR: 5-35) and 17 (IQR: 9-30) weeks for the NVP-group and PI-group, respectively.

Regarding the causes of treatment failure, 27 (6%) patients failed because of virological failure, including 11 patients with more than 10,000 HIV-1 RNA copies/mL. In total, 160 (36%) patients failed because of discontinuation of HAART 2. We observed significantly fewer treatment discontinuations (crude RR: 0.2, 95%CI: 0.1-0.3) and a trend toward less virological failure (crude RR: 0.4, 95%CI: 0.2-1.2) in the NVP-group as compared to the PI-group (Table 4). For both groups the primary reason for HAART 2 discontinuation was intolerance (50% and 48% of the discontinuations in the NVP-group and PI-group respectively). These included two deaths in the NVP-group. One male patient with a history

of cardiac disease died at the age of 69 because of heart failure. The cause of death of the other patient could not be retrieved as neither HIV-related events nor adverse events had been reported for this 41-year old man.

Other characteristics associated with treatment failure were: intra-venous drug use as route of HIV-transmission, antiretroviral treatment experience before HAART 1, d4T/ddI in HAART 1 or HAART 2, shorter duration of viral suppression before switch, absence of a change in NRTIs and switch later in calendar time. The use of RTV or a RTV/SQV combination in HAART 1 was associated with less treatment failure on the subsequent regimen. Many of the above factors were not equally distributed across the NVP-group and PI-group at baseline. After adjustment for all of these factors, including the reason for switch HAART 1 in a multivariate analysis, substitution of NVP for PIs relative to other changes of the PI-component remained, however, associated with a lower risk of treatment failure with an adjusted RR of 0.3 (95%CI: 0.1-0.5) (Table 4).

Restricting the outcome to virological failure and switches due to intolerance with right censoring of the other switches, produced similar results as analyses with the primary outcome (Table 4). Separating virological failure and treatment switches, however, indicated that differences between the NVP-group and PI-group were mainly attributable to treatment discontinuations as the risk of virological failure was not significantly different between the NVP-group and PI-group in the multivariate analysis. Stratification for NRTI experience prior to HAART 1 showed that the favourable effect of NVP was more pronounced among NRTI treatment naive patients. The adjusted RRs of treatment failure were relatively constant when comparing the NVP-group to individual types of switch (ie. change to another type of PI, continuation of one of two PIs, addition of one PI, change of SQV formulation and change of dose and/or frequency), ranging from 0.1 (95%CI: 0.03-0.2) to 0.3 (95%CI: 0.1-0.6).

Similar to the univariate analysis, the lowest risk of treatment failure was observed after switches from RTV to NVP (adjusted RR: 0.2, 95%CI: 0.1-0.6) when compared to switching from SQVhgc to SQVhgc with RTV (the most frequent switch) (Table 4). The highest risk was observed after switches from IDV to IDV with RTV (adjusted RR: 3.6, 95%CI: 1.6-8.2).

Among patients who used IDV in HAART 1, switching to NVP was associated with a 5-fold lower risk of treatment failure than switching to another PI-containing regimen (adjusted RR: 0.2, 95%CI:0.04-0.6). Similar results were found for patients coming from a regimen with RTV (adjusted RR: 0.2, 95%CI: 0.03-0.9) or a regimen of SQVhgc with RTV (adjusted RR: 0.1, 95%CI: 0.01-0.7).

Table 4 The relative risks of treatment failure within one year after substituting the first human immunodeficiency virus (HIV)-protease inhibitor

	Crude		Adjusted[a]	
	RR	95%CI	RR	95%CI
NVP-group versus PI-group				
Overall	0.2	(0.1-0.3)	0.2	(0.1-0.4)
ART experienced patients	0.4	(0.2-0.8)	0.3	(0.1-0.96)
ART naive patients	0.1	(0.1-0.3)	0.1	(0.1-0.3)
Outcome HAART 2[b]				
Virological failure + Treatment switch due to intolerance	0.2	(0.1-0.4)	0.3	(0.1-0.6)
Virological failure	0.4	(0.2-1.2)	0.8	(0.2-3.0)
Treatment switch for any reason	0.2	(0.1-0.3)	0.2	(0.1-0.3)
NVP-group versus				
Changing to another type of PI	0.2	(0.1-0.4)	0.3	(0.1-0.6)
Removing one PI	0.1	(0.04-0.3)	Too few	-
Adding one PI	0.2	(0.1-0.3)	0.1	(0.03-0.2)
Changing SQV formulation	0.2	(0.1-0.4)	0.1[c]	(0.02-0.4)
Changing the PI dose frequency	0.2	(0.1-0.4)	0.1	(0.05-0.4)
Most common switches				
SQVhgc to SQVhgc/RTV	1	Reference	1	Reference
IDV to new dose frequency IDV	1.2	(0.7-2.1)	0.9[c]	(0.4-2.0)
RTV to NVP	0.1	(0.05-0.4)	0.2[c]	(0.1-0.6)
SQVhgc/RTV to NVP	0.2	(0.1-0.6)	0.4[c]	(0.1-1.5)
IDV to NVP	0.3	(0.1-0.8)	0.3[c]	(0.1-0.98)
IDV to NFV	0.8	(0.4-1.7)	0.7[c]	(0.3-1.7)
RTV to NFV	0.4	(0.2-1.03)	0.4[c]	(0.1-1.2)
IDV to IDV/RTV	2.2	(1.2-4.1)	3.6[c]	(1.6-8.2)
RTV to SQVhgc/RTV	1.9	(1.00-3.7)	2.4[c]	(1.03-5.6)
Other	0.9	(0.6-1.5)	1.2[c]	(0.6-2.4)

95%CI= 95% confidence interval; HAART= Highly Antiretroviral Therapy; IDV= indinavir; NFV= nelfinavir; NVP= nevirapine; PI= HIV-protease inhibitor; RR= relative risk; RTV= ritonavir; SQVhgc= saquinavir hard gel capsule

a Adjusted for route of HIV-transmission, type of PIs in HAART 1, nucleoside analogue reverse transcriptase inhibitors (NRTIs) in HAART 1 and HAART 2, duration of viral suppression before HAART 2, change in NRTIs yes or no, time of switch, reason for switch and either adjusted or stratified for treatment experience prior to HAART 1

b Calculated with right censoring of the alternative outcome(s)

c Adjusted for all variables mentioned in note a except for type of PI in HAART 1

Adverse events

Before switching to HAART 2 adverse events had been reported for 91 (73%) patients in the NVP-group and 207 (65%) in the PI-group (p=0.094). After switch respectively 43 (47%) and 96 (46%) patients in the NVP-group and PI-group failed to achieve resolution of these events or died within the available follow-up period. For 19 (21%) patients in the NVP-group and 38 (18%) patients in the PI-group part of the events resolved. Twenty-nine (32%) patients in the NVP-group and 73 (35%) patients in the PI-group had complete resolution of all events. The most frequently reported new adverse event after switching was lipodystrophy as diagnosed by the treating physician, occurring among 13% and 11% of patients in the NVP-group and PI-group respectively. Only 2 (2%) patients in the NVP-group and 5 (2%) in the PI-

group experienced dermatological adverse effects. There were no reports of toxic (non-viral) hepatitis or severe rash on NVP leading to discontinuation of NVP.

Immunology

CD4 cell counts continued to increase in both groups after start of HAART 2 (Figure 2). No statistically significant difference in CD4 cell counts was observed between the NVP-group and PI-group at any time point (GLM for repeated measurements, p=0.088).

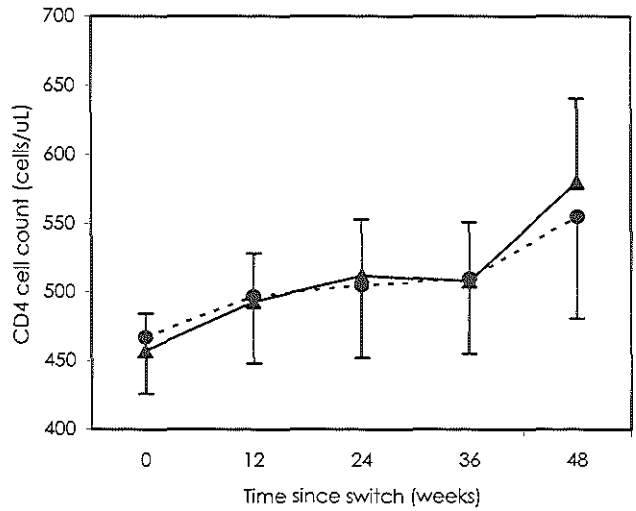

Figure 2 Course of CD4 cell counts after substituting the first human immunodeficiency virus (HIV)-protease inhibitor (PI)
No difference in CD4 cell counts was observed between patients who switched to a second-line HIV-protease inhibitor (PI-group, solid line) and patients who switched to nevirapine (NVP, dotted line) while having successful viral suppression (General Linear Model for repeated measurements: p>0.05). Mean CD4 cell counts (lines) and 95%-confidence intervals (bars) are plotted.

Discussion

In this study under everyday circumstances it was observed that substituting NVP for PIs during viral suppression was three-fold more likely to result in sustained virus suppression and maintenance of the new treatment regimen than substituting for any alternative PI or PI combination. This was found after adjustment for the imbalance in other determinants of treatment failure (ie. the route of HIV-transmission, type of antiretrovirals used in HAART 1 and HAART 2, concomitant changes of NRTIs, duration of viral suppression prior to switch, and

calendar time of switch) and reasons for switch. The differences were most pronounced among patients who were fully treatment-naive before their first PI and were mainly attributable to the lower number of treatment discontinuations on NVP.

By using the database derived from the ATHENA project we were able to substantiate previous findings in a larger cohort of patients with a more substantial follow-up period (9-12). The available data allowed us to compare switching to NVP with switching to second-line PIs in a multivariate analysis including multiple predictors of treatment failure. Moreover, this cohort allowed us to explore different types of switch within the class of PIs, including switches at the level of individual antiretroviral agents, which indicated that switching to NVP was superior to all types of PI switches included in this study. For adequate analysis of the effects on virological failure, probably a larger sample size is necessary as the rate of virological failure was generally low.

With respect to adverse events the NVP-group and PI-group appeared equally successful in resolving events, although there were two deaths in the NVP-group. We were unable to exclude NVP as the cause of one of these deaths. No other serious adverse effects, such as severe hepatitis or skin rash leading to treatment discontinuation had been reported. The incidence of skin rashes in the NVP-group was 2%, whereas we anticipated see skin rashes in at least 5% of patients in this group (7, 8). The absence of severe skin reactions and severe clinically overt liver toxicity to NVP (15-17) might point at a potentially lower risk of these events among patients with viral suppression than among patients with high viral loads as has been speculated before (18). On the other hand potential underreporting in the ATHENA project may be responsible for the low incidences of skin rashes and hepatitis observed in our study.

Our study was not primarily aimed at assessing the differences in adverse events after switch. Nevertheless, despite the comparable number of adverse events in both groups, the number of discontinuations in the NVP-group was significantly lower, suggesting relatively better tolerance of NVP-based second-line regimens. On the basis of the available data on the ATHENA cohort it was not possible to examine improvement of lipodystrophy as described elsewhere (19).

Possible limitations of observational studies concern potential bias and confounding. Since we did not have data on the actual severity of adverse effects, we cannot exclude the effect of confounding due to the absence or presence of a simpler alternative (or a new class of antiretrovirals) to the regimen on which intolerance is experienced. The observed difference in the rate of treatment discontinuations might partially reflect different switching behaviour in both treatment groups. If this bias indeed

plays a role, the results imply that the presence of an alternative in addition to the presence of adverse effects is a risk factor for treatment switches.

We aimed to create a homogeneous study cohort with respect to the reasons for changing therapy by limiting the cohort to patients with viral suppression at the time of switch. As a result, the reasons for changing to a certain regimen were assumed to be unrelated to the risk of treatment failure. Yet there were important differences between patients who substituted NVP for PIs and patients who switched to a second-line PI. For example, the proportion of patients treated with NRTIs prior to starting the first PI was lower in the NVP-group. NVP probably is prescribed less frequently to NRTI-experienced patients since they have a potential for viral resistance to NRTIs as a result of which they are less likely to have a sustained virological response to NVP (20-22). Furthermore, the duration of the first PI-containing regimen was longer and the type of antiretrovirals used in the first regimen was different. The risk profile for virological failure of patients who substituted NVP for PIs therefore seemed more favourable. We were, however, able to adjust for those factors that were associated with both the treatment group and treatment failure and for the reason for treatment switch in a multivariate analysis.

Finally, we compared switching to NVP with a variety of switches between PIs. The types of switch used as a reference included switching to another type of PI, switching to a dual PI regimen or switching from dual to a single PI regimen, changing SQV formulation and changing the PI dose frequency. The resulting treatment regimens were believed to be effective in earlier documented studies (23-28). However, in order to exclude the potential negative influence of any of these switches, we also compared switching to NVP with each single type of switch and individual type of PI. These sub-analyses did show similar results across all types of switch.

In conclusion, this study showed that under everyday circumstances, second-line PIs had a poorer performance than NVP with respect to tolerability in particular, as reflected in a higher rate of treatment discontinuations. For patients who have achieved viral suppression on the first HAART regimen, replacing PIs by NVP appears to be safe in terms of treatment endurance and therefore may improve the likelihood of long-term adherence and viral suppression. Further studies are needed to explore the benefits of switching to other NNRTIs such as efavirenz.

References

1. d'Arminio Monforte A, Cozzi Lepri A, Rezza G, et al. ft I.CO.N.A.Study Group. Insights into the reasons for discontinuation of the first highly active antiretroviral therapy (HAART) regimen in a cohort of antiretroviral naive patients. AIDS 2000;14:499-507
2. Friedland GH, Williams A. Attaining higher goals in HIV treatment: the central importance of adherence. AIDS 1999;13 Suppl 1:S61-72
3. Reijers MH, Weverling GJ, Jurriaans S, et al.. Maintenance therapy after quadruple induction therapy in HIV-1 infected individuals: Amsterdam Duration of Antiretroviral Medication (ADAM) study. Lancet 1998;352:185-90
4. Pialoux G, Raffi F, Brun-Vezinet F, et al.. A randomized trial of three maintenance regimens given after three months of induction therapy with zidovudine, lamivudine, and indinavir in previously untreated HIV-1-infected patients. Trilege (Agence Nationale de Recherches sur le SIDA 072) Study Team. N Engl J Med 1998;339:1269-76
5. Havlir DV, Marschner IC, Hirsch MS, et al.. Maintenance antiretroviral therapies in HIV infected patients with undetectable plasma HIV RNA after triple-drug therapy. AIDS Clinical Trials Group Study 343 Team. N Engl J Med 1998;339:1261-8
6. Bini T, Testa L, Chiesa E, et al.. Outcome of a second-line protease inhibitor-containing regimen in patients failing or intolerant of a first highly active antiretroviral therapy. J Acquir Immune Defic Syndr 2000;24:115-22
7. D'Aquila RT, Hughes MD, Johnson VA, et al.. Nevirapine, zidovudine, and didanosine compared with zidovudine and didanosine in patients with HIV-1 infection. A randomized, double-blind, placebo-controlled trial. National Institute of Allergy and Infectious Diseases AIDS Clinical Trials Group Protocol 241 Investigators. Ann Intern Med 1996;124:1019-30
8. Montaner JS, Reiss P, Cooper D, et al.. A randomized, double-blind trial comparing combinations of nevirapine, didanosine, and zidovudine for HIV-infected patients: the INCAS Trial. Italy, The Netherlands, Canada and Australia Study. JAMA 1998;279:930-7
9. Dieleman JP, Gyssens IC, Sturkenboom MCJM, Niesters HGM, van der Ende ME. Substituting nevirapine for protease inhibitor because of intolerance. AIDS 1999;13:1423-4
10. Barreiro P, Soriano V, Blanco F, Casimiro C, de la Cruz JJ, Gonzalez-Lahoz J. Risks and benefits of replacing protease inhibitors by nevirapine in HIV-infected subjects under long-term successful triple combination therapy. AIDS 2000;14:807-12
11. Raffi F, Bonnet B, Ferre V, et al.. Substitution of a Nonnucleoside Reverse Transcriptase Inhibitor for a Protease Inhibitor in the Treatment of Patients with Undetectable Plasma Human Immunodeficiency Virus Type 1 RNA. Clin Infect Dis 2000;31:1274-8
12. De Luca A, Baldini F, Cingolani A, et al.. Benefits and risks of switching from protease inhibitors to nevirapine with stable background therapy in patients with low or undetectable viral load: a multicentre study. AIDS 2000;14:1655-6
13. Borleffs JJC, Danner SA, Boer K, et al., on behalf of The Dutch Association of Treating Phycisians in AIDS and the National AIDS Therapy Evaluation Centre (NATEC). [Guidelines for HIV-suppressing therapy 1998]

14. Revision of HIV classification codes. MMWR Morb Mortal Wkly Rep 1988;36:821
15. Pollard RB, Robinson P, Dransfield K. Safety profile of nevirapine, a nonnucleoside reverse transcriptase inhibitor for the treatment of human immunodeficiency virus infection. Clin Ther 1998;20:1071-92
16. Cattelan AM, Erne E, Salatino A, Trevenzoli M, Carretta G, Meneghetti F, Cadrobbi P. Severe hepatic failure related to nevirapine treatment. Clin Infect Dis 1999;29:455-6
17. Clarke S, Harrington P, Condon C, Kelleher D, Smith OP, Mulcahy F. Late onset hepatitis and prolonged deterioration in hepatic function associated with nevirapine therapy. Int J STD AIDS 2000;11:336-7
18. Wit FWNM. Experience with nevirapine in previously treated HIV-1-infected individuals. Antiviral Therapy 2000;5:257-66
19. Martinez E, Conget I, Lozano L, Casamitjana R, Gatell JM. Reversion of metabolic abnormalities after switching from HIV-1 protease inhibitors to nevirapine. AIDS 1999;13:805-10
20. Jorgensen LB, Katzenstein TL, Gerstoft J, Mathiesen LR, Pedersen C, Nielsen C. Genotypic and phenotypic nevirapine resistance correlates with virological failure during salvage therapy including abacavir and nevirapine. Antivir Ther 2000;5:187-94
21. Gilbert PB, Hanna GJ, De Gruttola V, et al.. Comparative analysis of HIV type 1 genotypic resistance across antiretroviral trial treatment regimens. AIDS Res Hum Retroviruses 2000;16:1325-36
22. Khanna N, Klimkait T, Schiffer V, et al., ft Swiss HIV Cohort Study. Salvage therapy with abacavir plus a non-nucleoside reverse transcriptase inhibitor and a protease inhibitor in heavily pre-treated HIV-1 infected patients. AIDS 2000;14:791-9
23. Deeks SG, Smith M, Holodniy M, Kahn JO. HIV-1 protease inhibitors. A review for clinicians. JAMA 1997;277:145-53
24. Bardsley-Elliot A, Plosker GL. Nelfinavir: an update on its use in HIV infection. Drugs 2000;59:581-620.
25. Burger DM, Hugen PW, Aarnoutse RE, et al.. A Retrospective, Cohort-Based Survey of Patients Using Twice-Daily Indinavir + Ritonavir Combinations: Pharmacokinetics, Safety, and Efficacy. J Acquir Immune Defic Syndr 2001;26:218-24.
26. Haas DW, Arathoon E, Thompson MA, et al.. Comparative studies of two-times-daily versus three-times-daily indinavir in combination with zidovudine and lamivudine. AIDS 2000;14:1973-8.
27. Gisolf EH, Jurriaans S, Pelgrom J, et al.. The effect of treatment intensification in HIV-infection: a study comparing treatment with ritonavir/saquinavir and ritonavir/saquinavir/stavudine. Prometheus Study Group. AIDS 2000;14:405-13
28. Figgitt DP, Plosker GL. Saquinavir soft-gel capsule: an updated review of its use in the management of HIV infection. Drugs 2000;60:481-516.

Chapter 4.3

Determinants of recurrent toxicity-driven switches of highly active antiretroviral therapy.

The ATHENA cohort.

Abstract

Background: Toxicity is the most important reason for premature switching of highly active antiretroviral therapy (HAART). In order to optimise the benefit-risk ratio of HAART, guidelines for toxicity-management are needed.

Objective: We conducted an observational cohort study to estimate the incidence and identify determinants of toxicity-driven switches on second-line HAART after having switched first-line HAART despite successful viral suppression.

Methods: We selected all patients from the ATHENA-cohort (n=2470), who switched the initial HIV-protease inhibitor (PI) containing HAART while plasma HIV-1-RNA was ≤ 500 copies/mL (n=775). One-year cumulative incidences of subsequent toxicity-driven switches and adjusted relative risks (RR) for potential determinants were calculated.

Results: The one-year cumulative incidence of toxicity-driven switches of the second regimen was 24% (95%CI:21-28), mostly because of gastro-intestinal toxicity and neuropathy. Those who had switched the first HAART due to toxicity, were at an increased risk of a recurrent toxicity-driven switch (RR:2.5, 95%CI:1.7-3.5). Switching from PI to nevirapine while continuing the other antiretrovirals was more protective against a subsequent switch because of further toxicity than changing to another PI-containing regimen (RR:0.2, 95%CI:0.1-0.6).

Conclusions: As for first-line HAART, toxicity remains responsible for the majority of switches during second-line HAART. Prior switching for toxicity increases the risk of having to switch the subsequent regimen for toxicity, but this risk is reduced when switching to nevirapine rather than to an alternative PI. The latter should be taken into account when designing toxicity-management guidelines.

Introduction

The burden of toxicity resulting from highly active antiretroviral therapy (HAART) is of concern, as it constitutes a threat to sustained success of HIV treatment (1, 2). The high frequency of toxicity (3, 4) results in a high rate of treatment switches (5-7). In fact, 44-58% of switches on initial HAART regimens and 56% of switches on subsequent regimens have been attributed to toxicity, which makes toxicity the most important reason for premature switching of antiretroviral treatment (6, 8, 9). The impact of toxicity on the benefit-risk ratio of HIV treatment may become even more pronounced as sustained virological suppression becomes more feasible due to the availability of potent antiretroviral regimens (10-12).

Obviously, risk factors for developing HAART-associated toxicity include the specific antiretroviral drugs used. (10). However, underlying conditions and patient characteristics, including pharmacokinetic parameters such as high drug absorption, low distribution volume and low metabolic clearance, may cause increased susceptibility to toxic effects of certain classes of drugs (13-16). Accordingly, patients who switch first-line HIV-protease inhibitor (PI)-containing treatment because of toxicity are at an increased risk of a similar switch on a subsequent PI-containing regimen (8). As yet there is no information to suggest that switching to another class of drugs can reduce this risk. In addition, information on the influence of other factors on the risk of recurrent toxicity-driven switches is scanty, despite the high incidence of such switches.

We conducted the present study within the ATHENA cohort to estimate the incidence of treatment switches because of toxicity in a population of patients who had switched first-line PI-containing HAART despite successful viral suppression. In order to obtain more insight into toxicity-driven switches, we identified determinants of the renewed need to switch treatment.

Patients and methods

Setting

The ATHENA-cohort is a multi-centre clinical cohort of HIV-infected individuals in the Netherlands, who are or have been treated with one or more antiretroviral medications that became generally available as of July 1996 (lamivudine, stavudine, the PIs and non-nucleoside reverse transcriptase inhibitors). All 22 Dutch hospitals that provide treatment to HIV-infected patients participate in this cohort study, with approval of their local ethical committees. Patient enrolment, for which informed consent is obtained, effectively started in May 1998 and continues to date.

According to the national guidelines for HIV-treatment in the Netherlands, patients receiving antiretroviral treatment visit the outpatient clinic at approximately three-monthly intervals for regular follow-up (17). Data for the ATHENA cohort are abstracted by treating physicians and trained research assistants from medical records onto standardised forms. This is done retrospectively for the period prior to having obtained participants' informed consent, and prospectively thereafter. The resulting database contains anonymous information on patient characteristics (eg. age, gender, date of birth, date of death, height and weight), HIV-infection (eg. HIV-type, HIV-related events, route of HIV-transmission, CD4-cell count and plasma HIV-1 RNA load), detailed treatment data (eg. antiretroviral and prophylactic medications against opportunistic infections, dose frequencies, start and stop dates and reasons for treatment switch) and adverse events. Adverse events recorded include certain drug-associated events, which are pre-printed on the data collection forms (e.g. hepatitis, lipodystrophy, nephrolithiasis, neuropathy) and which are recorded at the discretion of the treating physician. In addition other events resulting in a treatment switch, as well as laboratory values outside pre-specified ranges are recorded. Reasons for treatment switch are categorised into mutually exclusive groups, consisting of toxicity (referring to the adverse event(s)), treatment failure (increase in viral load and/or decrease in CD4 cell count, and/or clinical disease progression), patient request, pharmacological indication, dose escalation, other (with specification) and unknown. Treating physicians are requested to indicate a single primary reason for the switch.

Study cohort

The source population for the present study comprised all patients who started a first HAART regimen, consisting of at least one HIV-protease inhibitor (PI) and two nucleoside analogue reverse transcriptase inhibitors (NRTIs) or the combination of ritonavir and saquinavir, with or without stavudine as used in the Prometheus study (18). The current study cohort included all patients from the source population who had a switch of their first PI-containing HAART regimen without having virological failure, in order to select a population of patients for whom toxicity was of a potential concern. To achieve this we excluded patients in whom plasma HIV-1 RNA at the time of treatment switch was above 500 copies/mL. In addition, we excluded patients for whom treatment data were missing, no information on a subsequent regimen was available, or whose treatment was interrupted for more than seven days, in order to reduce the chance of an increased viral load being present at the start of the second-line HAART regimen.

The date of switch of the first HAART regimen was considered as the baseline date for the current cohort study. Switches were defined as any change in composition of the

regimen, dose adjustments other than the standard dose escalations (coded as dose escalation) or adjustments in frequency of administration of drugs.

The primary outcome measure was a toxicity-driven switch, defined as a switch of the second-line regimen due to toxicity, as indicated by the treating physician. A secondary outcome measure was switch of the second regimen for reasons other than toxicity. Follow-up lasted from the date of switch of the first HAART regimen until the date of switching the second regimen, the end of the one-year period, or the end of follow-up, whichever came first. A one-year period was chosen to ensure an equal follow-up time for various regimens.

Statistical analysis

The cumulative incidence of a switch, while on the second-line HAART regimen was estimated by means of Kaplan Meier survival analysis. The proportion of patients with HIV-1 RNA > 500 copies/mL at the end of follow-up was calculated. Risk factors for toxicity-driven switches were analysed by means of univariate and multivariate Cox-regression analysis. Factors included into the analysis were: calendar time of switch, route of HIV-transmission, Centre for Disease Control and Prevention (CDC) classification of HIV-disease (classified as C and non-C) (19), antiretroviral treatment experience prior to the first HAART, duration of the first HAART, CD4-cell count at switch, reason for switch and the type of treatment switch. As proxies for underlying conditions or characteristics we included age, gender, nationality (classified as Dutch or non-Dutch), body mass index and lean body mass. All factors that were univariately associated with toxicity-driven switch at a p-value of 0.1 were included in the multivariate analysis. Subsequently we excluded variables from the multivariate analysis in a stepwise manner if the p-value was above 0.1. Results were expressed as relative risks (RR) with 95%-confidence intervals (95%CI).

All analyses were performed using SPSS® for Windows® version 9.01 as the statistical software package.

Results

Among the 2470 patients in the ATHENA database who were available for analysis, 2096 (85%) used at least one PI with two NRTIs or a Prometheus-trial regimen (18) as the first HAART regimen, and 1573 (75%) of these patients had ever switched this regimen. From the population of switchers we excluded 696 (45%) patients because of an HIV-1 RNA level above 500 copies/mL, 9 (0.6%) patients because of missing treatment data, 41 (3%) patients because there was no information on a subsequent regimen, and 52 (3%) patients because of a treatment interruption of more than seven days. Thus, our final cohort consisted of 775

patients who entered the study between June 1996 and December 1999. The baseline characteristics of the study cohort are summarized in Table 1.

Table 1 Baseline characteristics of the study cohort of patients who switched a first PI-containing HAART regimen without having virological failure (n=775)

Characteristic		Study cohort (n=775)[a]		Toxicity-driven switch second HAART (n=142)	
		N	(%)[b]	N	(%)[b]
Gender	Male	659	(85)	113	(85)
	Female	116	(15)	29	(15)
Age	Median (IQR)	40	(34-47)	39	(34-47)
Nationality	Dutch	633	(86)	112	(85)
	Other	101	(14)	20	(15)
Route of HIV-transmission	MSM	509	(66)	84	(59)
	Other	266	(34)	58	(41)
CDC-C disease classification	Yes	272	(35)	49	(34)
	No	503	(65)	93	(66)
ARV naïve at start first HAART	Yes	398	(51)	62	(44)
	No	377	(49)	80	(56)
Duration first HAART (months)	Median (IQR)	7.8	(3.4-15.9)	5.9	(3.0-14.7)
CD4-cell count at change ($x10^6$/L)	Median (IQR)	365	(195-550)	370	(180-558)
Reason for switch first HAART[c]	Toxicity	270	(35)	77	(54)
	Failure[d]	51	(7)	7	(5)
	Patient request	116	(15)	13	(9)
	Pharmacological indication	92	(12)	14	(10)
	Other (specified)	157	(20)	20	(14)
	Unknown	89	(12)	11	(8)
Type of switch					
NRTI unchanged	PI(s) to other PI(s)	185	(24)	44	(31)
	PI(s) to nevirapine	89	(12)	4	(3)
	Dose/frequency PI changed	108	(14)	15	(11)
	Other change of PI(s)	32	(4)	9	(6)
	PI(s) unchanged[e]	169	(22)	28	(20)
NRTI changed	PI(s) to other PI(s)	40	(5)	10	(7)
	PI(s) to nevirapine	24	(3)	6	(4)
	Dose/frequency PI changed	13	(2)	1	(1)
	Other change of PI(s)	11	(1)	2	(1)
	PI(s) unchanged	104	(13)	23	(16)

ARV= antiretroviral; CDC-C= CDC disease classification C; HAART= highly active antiretroviral therapy; IQR= Inter Quartile Range; MSM= men having sex with men; NRTI= nucleoside analogue reverse transcriptase inhibitor; NNRTI= non-nucleoside analogue reverse transcriptase inhibitor; PI= HIV-protease inhibitor

a Within one year 373 patients had switched the second regimen, 142 of whom for toxicity reasons

b Percentage of column total

c A single reason for change had to be chosen from the following: toxicity, patient request, treatment failure (immunological,and/or virological,and/or or clinical), pharmacological indication, other (with specification) and unknown.

d Patients may have switched because of failure while HIV-1 RNA was below 500 copies/mL

e Includes dose frequency changes of NRTIs and treatment interruptions shorter than 7 days

The median date of cohort entry, i.e. the date of having switched a first-line HAART regimen, was March 1998. The majority of patients were homosexual males of Dutch nationality with a median age of 40 years. Most patients had asymptomatic or mildly symptomatic HIV-disease with a median CD4 cell count of 365 cells/mm^3. Approximately half of the patients were antiretroviral treatment-experienced prior to initiating their first HAART. The most frequent reason for switching the first HAART regimen (with HIV-1 RNA ≤ 500 copies/mL) as indicated by the treating physician was toxicity and the most frequent type of treatment switch was replacement of PIs by another PI without changing the NRTIs. The various PIs used in HAART regimens are summarized in table 2. The most common PI and NRTI-combinations in both the first and second regimens were indinavir with zidovudine and lamivudine (data not shown). The only NNRTI used in second-line regimens was nevirapine (n=122, Table 1).

Table 2 PIs or NNRTIs used in HAART regimens by patients who switched a first PI-containing HAART regimen without having virological failure (n=775)

PI or NNRTI used	First HAART		Second HAART	
	N	(%)	N	(%)
IDV	248	(32)	186	(24)
RTV	182	(24)	84	(11)
SQV	212	(27)	124	(16)
NFV	18	(2)	66	(9)
IDV/RTV	7	(1)	20	(3)
SQV/RTV	84	(11)	114	(15)
SQV/NFV	21	(3)	21	(3)
NVP	0	(0)	113	(15)
Other	3	(0.4)	13	(2)
No PI or NNRTI	0	(0)	34	(4)

IDV= indinavir; NFV= nelfinavir; NNRTI= non-nucleoside analogue reverse transcriptase inhibitor; NVP= nevirapine; PI= HIV-protease inhibitor; RTV= ritonavir; SQV= saquinavir

Incidence of toxicity-driven switches of the second HAART regimen

Within one year 373 (48%) patients had a switch of the second regimen, with toxicity being the most common reason (n=142) (Tables 1 and 3). Table 3 summarizes the incidence of the different reasons for switching second-line HAART, as well as the detectability of HIV-1 RNA (below or above 500 copies/mL) at the end of follow-up. The one-year cumulative incidence of toxicity-driven switch on second-line HAART was 24% (95%CI: 21-28). The overall one-year cumulative incidence of switching a second-line HAART regimen was 53%, indicating that approximately half of the switches were attributable to toxicity. Switching treatment for toxicity usually did not coincide with virological failure.

Table 3 Reasons for switch of the second HAART regimen with one-year cumulative incidences and last HIV-1 RNA among patients who had switched the first HAART regimen without having virological failure (n=775)

Reason for switch of the second HAART	Total	HIV-1 RNA at end of follow-up[a]						One-year cumulative incidence[b]	
		Missing		≤500 copies/mL		>500 copies/mL			
		N	(%)	N	(%)	N	(%)	%	(95%CI)
Not switched	n=402	1	(0)	352	(88)	49	(12)	-	-
Total switched	N=373	45	(12)	259	(69)	69	(18)	53	(49-56)
Switched due to[c]:									
Toxicity	n=142	17	(12)	113	(80)	12	(8)	24	(21-28)
Failure	n=53	9	(17)	5	(9)	39	(74)	11	(8-13)
Patient request	n=40	3	(8)	30	(75)	7	(18)	8	(5-10)
Pharmacological indication	n=24	2	(8)	21	(88)	1	(4)	5	(3-7)
Other	n=76	6	(8)	64	(84)	6	(8)	14	(11-17)
Unknown	n=38	8	(21)	26	(68)	4	(11)	7	(5-9)

95%CI= 95%-confidence interval; HAART= highly active antiretroviral therapy

a Follow-up ended at last data delivery, end of one-year period or switch of the second regimen, whichever came first

b Figures have been estimated by means of Kaplan Meier survival analysis

c Within one year 373 patients had switched the second regimen due to various reasons. Physicians have to indicate the primary reasons for switch as toxicity, patient request, treatment failure (immunological, virological or clinical), pharmacological, other (with specification) and unknown.

In a sub-group analysis among patients who had switched the first HAART regimen due to toxicity (n=270), 77 (representing more than 10% of the current study cohort) subsequently had another toxicity-driven switch. The one-year cumulative incidence of such subsequent toxicity-driven switches was 37% (95%CI: 30-44), indicating a higher risk of switching for toxicity for patients who had previously changed treatment likewise as a result of toxicity compared to patients who had not (Figure 1).

Factors associated with toxicity-driven switches of the second HAART regimen

After univariate analysis and stepwise multivariate analysis, four factors were identified which were associated with toxicity-driven switching of second-line HAART (Table 4). First, we observed a risk reduction over calendar time. Second, women appeared to have a higher risk of toxicity-driven switches than men. Similarly, patients who switched the first HAART regimen due to toxicity relative to patients who switched due to other reasons had a 2.5-fold increased risk (95%CI: 1.7-3.5) for toxicity-driven switch of the second regimen. Finally, among the various types of switch, switching from PI to nevirapine without changing the NRTIs appeared to be protective against a subsequent switch for toxicity reasons (adjusted RR: 0.2, 95%CI: 0.1-0.6). Conversely, switching from PI to nevirapine with a concomitant change of NRTIs was not protective. Within the sub-group of patients who previously switched first HAART due to toxicity (Table 4, column 3), switching to nevirapine without changing the NRTIs was equally protective, whereas switching to nevirapine and concomitantly changing NRTIs tended to increase the risk.

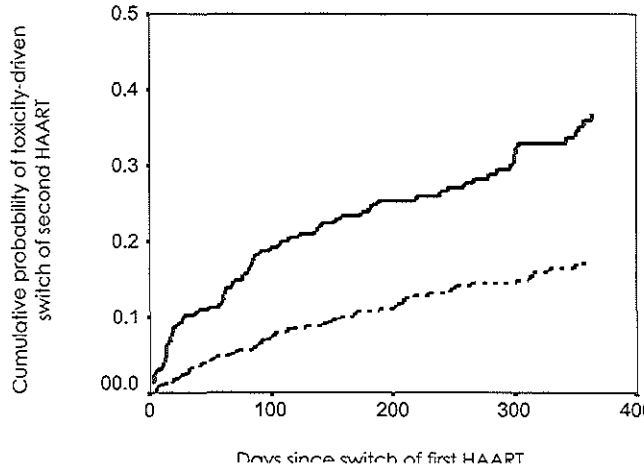

Figure 1 Probability of toxicity-driven switches of the second HAART regimen in relation to the reason for having switched the first HAART regimen
Figure shows the probability of toxicity-driven switches of the second HAART regimen according to the reason for having switched the first HAART regimen. Patients who switched the first regimen due to toxicity (solid line) had a higher probability than patients who switched the first regimen for other reasons (broken line).

Table 4 Risk factors for toxicity-driven switch on the second HAART regimen for patients who had switched the first HAART regimen without having virological failure (n=775)

Risk factor			Multivariate[a]		First HAART switched for toxicity (n=270)	
			RRadj	95%CI	RRadj	95%CI
Calendar time of switching first HAART		Per month	0.98	(0.9-0.99)	0.97	(0.9-1.0)
Gender		Male	1		1	
		Female	1.7	(1.1-2.6)	1.6	(0.9-3.0)
Switch first HAART due to toxicity		No	1			
		Yes	2.5	(1.7-3.5)	-	-
Type of switch						
NRTI unchanged	PI(s) to other PI(s)		1		1	
	PI(s) to nevirapine		0.2	(0.1-0.6)	0.2	(0.1-0.8)
	Dose/frequency PI changed		0.8	(0.4-1.5)	1.1	(0.4-2.9)
	Other change of PI		1.4	(0.7-2.8)	1.4	(0.6-3.5)
	PI(s) unchanged		0.8	(0.5-1.2)	1.0	(0.5-2.0)
NRTI changed	PI(s) to other PI(s)		0.9	(0.4-1.8)	0.9	(0.4-2.9)
	PI(s) to nevirapine		1.5	(0.6-3.7)	2.6	(0.9-7.3)
	Dose/frequency PI changed		0.3	(0.0-1.8)	0.4	(0.1-2.9)
	Other change of PI(s)		0.8	(0.2-3.2)	1.4	(0.3-5.9)
	PI(s) unchanged		0.7	(0.4-1.2)	0.8	(0.4-1.5)

95%CI= 95% confidence interval; ARV= antiretroviral; freq.= frequency; HAART= highly active antiretroviral therapy; MSM= men having sex with men; NRTI= nucleoside reverse transcriptase inhibitor; NNRTI= non-nucleoside reverse transcriptase inhibitor; PI= HIV-protease inhibitor; RR= relative risk; RRadj= adjusted relative risk

a Adjusted for all other variables in the table, which were all associated with switch of the second regimen due to toxicity at a p-value of 0.1 in the univariate analysis

Body mass index and lean body mass as well as other baseline characteristics, such as nationality, route of HIV-transmission, antiretroviral treatment experience prior to first HAART and duration of first HAART did not significantly influence the incidence of toxicity-driven switch on the second regimen (data not shown). Stratification by hospital, in order to explore whether results may have been influenced by being under the care of a specific treatment centre, did not alter the conclusions (data not shown).

Types of toxicity leading to toxicity-driven switches

Table 5 summarizes the different types of clinical toxicities, which were specified as the reason(s) for switching treatment. No significant difference in the distribution of toxicities was observed between initial and subsequent HAART regimens. For both the first and second regimen, the most common types of toxicity resulting in a treatment switch were gastro-intestinal events and peripheral neuropathy.

Table 5 Types of toxicity leading to treatment switches on first- and second-line HAART regimens

	First HAART (n=270)		Second HAART (n=142)		Both first and second HAART (n=77)	
Event description[a]	N	(%)[b]	N	(%)[c]	N	(%)[d]
Gastro-intestinal	100	(37)	53	(37)	19	(36)
Peripheral neuropathy	34	(13)	17	(12)	6	(35)
Haematological	30	(11)	11	(8)	3	(27)
Renal	18	(7)	10	(7)	3	(30)
Malaise	18	(7)	9	(6)	1	(11)
Lipodystrophy	14	(5)	6	(4)	0	(0)
Hepatological	11	(4)	8	(6)	1	(13)
Ear Nose and Throat	10	(4)	4	(3)	1	(25)
Hyperlipidemia	8	(3)	2	(1)	0	(0)
Musculo-skeletal	7	(3)	2	(1)	0	(0)
Dermatological	5	(2)	1	(1)	0	(0)
Pancreatitis	3	(1)	3	(2)	2	(67)
Diabetes mellitus	1	(0.4)	1	(1)	1	(100)
Miscellaneous	4	(2)	7	(5)	0	(0)
Missing	27	(10)	16	(11)	4	(25)
Discontinuation due to death	-	(-)	4	(3)	-	(-)

HAART= highly active antiretroviral therapy

a Descriptions are based on the body-system in which the event occurred; patients may have more than one event

b Percentage of patients who switched first HAART due to toxicity (n= 270)

c Percentage of patients who switched second HAART due to toxicity (n= 142)

d Percentage of patients who switched both first and second HAART due to this toxicity (column 3)

After having switched the first HAART due to gastro-intestinal toxicity (n=100), the one-year risk of a recurrent toxicity-driven switch due to gastro-intestinal toxicity was 26% (95%CI: 15-37%), representing a 5.1 (95%CI:2.1-12.1) fold increased risk relative to patients who switched

the first HAART due to other toxicity reasons (n=170). Out of 100 patients who switched the first HAART for gastro-intestinal toxicity reasons, 22 patients switched to NVP without changing NRTIs. Only one of those had a recurrent switch due to gastro-intestinal toxicity on the second regimen, suggesting a general improvement of gastro-intestinal toxicity among patients who switched to NVP.

After having switched the first HAART due to neuropathy, the one-year risk of another toxicity-driven switch due to neuropathy on the second regimen was 21% (95%CI: 5-37%) yielding a RR of 12.8 (95%CI: 3.2-51.3) compared to patients who switched the first regimen due to other toxicity reasons. The small number of patients did not allow a distinction between the types of switch regarding the risk of neuropathy driven switches on the second regimen.

Differentiating between those who concomitantly changed NRTIs during the first switch and those who did not, resulted in relative risks for a recurrent switch due to neuropathy on the second regimen of 8.2 (95%CI: 1.5-45.1) and 19.2 (95%CI: 1.7-212.7) for these two groups of patients respectively. These results suggest that changing the NRTIs along with PIs can reduce the risk of a neuropathy driven switch. The fact that recovery from neuropathy is a time consuming process might explain that the increased relative risk did not disappear.

Discussion

In this cohort study, more than half of the patients who switched initial treatment for reasons other than virological failure again switched treatment within one year. Toxicity accounted for approximately half of these second switches and mostly involved gastro-intestinal events and peripheral neuropathy. In line with previous reports, patients who switched a first HAART regimen for reasons of toxicity had a greater than two-fold increased risk of a subsequent switch because of toxicity (8). This implies that a subgroup of patients may exist, including at least 10% of all patients, who are particularly prone to the development of HAART-associated toxicity. There was a slight reduction in risk of toxicity-driven switch over calendar time, which may reflect improved toxicity management as the experience of a physician evolves.

The proportion of toxicity-driven switches has previously been reported to be high for both first-line (26% within one year) (5, 6) and second-line PI-containing HAART regimens (33% during a median of 483 days) (8). These findings however were based on the first regimen only and a relatively small single-centre patient population. In a much larger and multi-centre patient sample, we observed a 24% one-year cumulative incidence of

switching second-line regimens for toxicity. This figure may be slightly higher than for the entire population on second-line HAART, given that patients were selected who had a prior switch of treatment in the absence of virological failure, and therefore may have been at potentially higher risk of a subsequent toxicity-driven switch. However, selecting such a population allowed us to investigate various types of switch including switches between classes of antiretrovirals, without the choice of subsequent regimens being influenced to any important extent by concerns of antiviral potency and cross-resistance between drugs.

An interesting observation was that switching to nevirapine, without concomitantly switching the NRTIs, was associated with a five-fold lower risk of a subsequent toxicity-driven switch. The favourable effect of switching to nevirapine has been described before, albeit in small series of patients with a limited follow-up or a limited reference group (20-23). Unfortunately, we were not able to study the effect of switching to other NNRTIs, such as efavirenz and delavirdine, since these drugs were not licensed in the Netherlands during the period covered by our current analysis. Remarkably, combining the switch to nevirapine with a change in concomitant NRTIs, annihilated the advantageous effect of switching to nevirapine, possibly indicating the additional toxicity of the newly introduced NRTIs. None of the other types of switch, including switching between different PIs and changing NRTIs at the same time, significantly influenced the risk of a subsequent toxicity-driven switch.

No associations were found between lean body mass or body mass index and the incidence of treatment switch for toxicity, suggesting little influence of distribution volume on toxicity risk for any of the drugs. Toxicity-driven switches occurred independently of patient nationality, which might suggest that cultural differences do not play a major role, but this association needs further substantiation. Women seemed to be more susceptible to toxicity. This may be attributable to sex-dependent pharmacokinetics or adherence leading to higher plasma concentrations of PIs in particular (24). Alternatively, women may be more sensitive to toxicity or report toxicity more easily than men, resulting in a reporting bias.

Since this was an observational cohort study, results may have been confounded. For example, as guidelines for switching primarily focus on virological failure rather than toxicity (12, 17, 25), the decision to switch and how to switch was dependent of the treating physicians. They were also asked to indicate the primary reason for switching. As a consequence the observed effects for the type of switch might in fact have been confounded by physician's preference. However, stratification by hospital did not alter the results, which to some extent reduces the likelihood of physician confounding. As we were able to adjust for the potential confounding effects of other determinants for toxicity-driven switches, the effect of residual confounding is assumed to be minimal.

In conclusion, toxicity continues to be a cause of major concern in the treatment of HIV-1 infection. It was deemed responsible for the majority of recurrent treatment switches among patients who had initially switched first-line HAART while having reasonable virus suppression, particularly among patients who had also switched their first-line HAART for toxicity. Switching to nevirapine rather than to an alternative PI, without concomitantly switching the NRTIs, was associated with a significantly lower risk of again having to switch the new regimen for toxicity, even in patients with prior toxicity. These findings may provide guidelines for how to minimize the risk of renewed toxicity when managing patients with toxicity during PI-based HAART.

References

1. Vanhove GF, Schapiro JM, Winters MA, Merigan TC, Blaschke TF. Patient compliance and drug failure in protease inhibitor monotherapy. JAMA 1996,276:1955-1956.
2. Friedland GH, Williams A. Attaining higher goals in HIV treatment: the central importance of adherence. AIDS 1999,13 Suppl 1:S61-72.
3. Flexner C. HIV-protease inhibitors. N Engl J Med 1998,338:1281-1292.
4. Deeks SG, Smith M, Holodniy M, Kahn JO. HIV-1 protease inhibitors. A review for clinicians. JAMA 1997,277:145-153.
5. Ledergerber B, Egger M, M. O, et al. Clinical progression and virological failure on highly active antiretroviral therapy in HIV-1 patients: a prospective cohort study. Lancet 1999,353:863-868.
6. d'Arminio Monforte A, Cozzi Lepri A, Rezza G, et al. Insights into the reasons for discontinuation of the first highly active antiretroviral therapy (HAART) regimen in a cohort of antiretroviral naive patients. AIDS 2000,14:499-507.
7. van Roon EN, Verzijl JM, Juttmann JR, Lenderink AW, Blans MJ, Egberts AC. Incidence of discontinuation of highly active antiretroviral combination therapy (HAART) and its determinants. J Acquir Immune Defic Syndr Hum Retrovirol 1999,20:290-294.
8. Bini T, Testa L, Chiesa E, et al. Outcome of a second-line protease inhibitor-containing regimen in patients failing or intolerant of a first highly active antiretroviral therapy. J Acquir Immune Defic Syndr 2000,24:115-122.
9. Mocroft A, Youle M, Moore A, et al. Reasons for modification and discontinuation of antiretrovirals: results from a single treatment centre. AIDS 2001,15:185-194.
10. Carpenter CC, Cooper DA, Fischl MA, et al. Antiretroviral therapy in adults. Updated recommendations of the International AIDS Society -USA Panel. JAMA 2000,283:381-390.
11. Gazzard P and Moyle M on behalf of the BHIVA Guidelines Writing Committee. 1998 Revision to the British HIV Association guidelines for antiretroviral treatment of HIV seropositive individuals. Lancet 1998,352:314-316.
12. Anonymous. British HIV Association guidelines for antiretroviral treatment of HIV seropositive individuals. BHIVA Guidelines Co-ordinating Committee. Lancet 1997,349:1086-1092.

13. Lin JH, Chiba M, Chen IW, Nishime JA, Vastag KJ. Sex-dependent pharmacokinetics of indinavir: in vivo and in vitro evidence. Drug Metab Dispos 1996,24:1298-1306.
14. Gatti G, Di Biagio A, Casazza R, et al. The relationship of ritonavir plasma levels and side effects: implications for therapeutic drug monitoring. AIDS 1999,13:2083-2089.
15. Gatti G, Vigano A, Sala N, et al. Indinavir pharmacokinetics and parmacodynamics in children with human immunodeficiency virus infection. Antimicrob Agents Chemother 2000,44:752-755.
16. Dieleman JP, Gyssens IC, van der Ende ME, de Marie S, Burger DM. Urological complaints in relation to indinavir plasma concentrations in HIV-infected patients. AIDS 1999,13:473-478.
17. Borleffs JJC, Danner SA, Boer K, et al. [Guidelines for HIV-suppressing therapy 1998]. In.; 1998.
18. Gisolf EH, Jurriaans S, Pelgrom J, et al. The effect of treatment intensification in HIV-infection: a study comparing treatment with ritonavir/saquinavir and ritonavir/saquinavir/stavudine. Prometheus Study Group. AIDS 2000,14:405-413.
19. Human immunodeficiency virus (HIV) infection codes. Official authorized addendum. ICD-9-CM (Revision No. 1). Effective January 1, 1988. MMWR Morb Mortal Wkly Rep 1987,36 Suppl 7:1S-20S.
20. Dieleman JP, Gyssens IC, Sturkenboom MCJM, Niesters HGM, Ende MEv. Substituting nevirapine for protease inhibitor because of intolerance. AIDS 1999,13:1423-1424.
21. De Luca A, Baldini F, Cingolani A, et al. Benefits and risks of switching from protease inhibitors to nevirapine with stable background therapy in patients with low or undetectable viral load: a multicentre study. AIDS 2000,14:1655-1656.
22. Raffi F, Bonnet B, Ferre V, et al. Substitution of a Nonnucleoside Reverse Transcriptase Inhibitor for a Protease Inhibitor in the Treatment of Patients with Undetectable Plasma Human Immunodeficiency Virus Type 1 RNA. Clin Infect Dis 2000,31:1274-1278.
23. Barreiro P, Soriano V, Blanco F, Casimiro C, de la Cruz JJ, Gonzalez-Lahoz J. Risks and benefits of replacing protease inhibitors by nevirapine in HIV-infected subjects under long-term successful triple combination therapy. AIDS 2000,14:807-812.
24. Burger DM, Koopmans PP, Brinkman K. Influence of gender on indinavir pharmacokinetics. Sixth European Conference on Clinical Aspects and Treatment of HIV-Infection. Hamburg 1997.
25. Carpenter CC, Fischl MA, Hammer SM, et al. Antiretroviral therapy for HIV infection in 1998: updated recommendations of the International AIDS Society-USA Panel. JAMA 1998,280:78-86.

Chapter 5

General Discussion

The overall objective of this thesis was to provide more insight into the risk of adverse effects during treatment with HIV-protease inhibitors under everyday circumstances. Within the scope of this objective the incidence of renal complications of the HIV-protease inhibitor indinavir were studied as well as the risk factors and consequences. Also, the incidence of and risk factors for clinically diagnosed lipodystrophy, an alleged adverse effect of HIV-protease inhibitor treatment, were examined. In addition, we investigated whether switching to an HIV-protease inhibitor-sparing regimen might be favorable in terms of regimen consistency and viral suppression. Finally, we evaluated the impact of treatment intolerance on treatment continuity.

In this chapter, the main findings and limitations of the thesis will be discussed and the results will be put into perspective. First, however, relevant developments in the five years following the introduction of the HIV-protease inhibitors are described.

Five-years of experience with HIV-protease inhibitor treatment

In July 1996, the HIV-protease inhibitors were introduced to the Dutch market at an unprecedented speed. Therefore, extensive research on safety and effectiveness had to be done in the post-marketing setting. Typical problems of HIV-protease inhibitor treatment, which were expected to jeopardise treatment success included complex treatment schedules, drug interactions via cytochrome P450 (1-3) and abundance of adverse effects (4-6).

Treatment success

Notwithstanding the anticipated problems, the new antiretroviral treatment modalities involving HIV-protease inhibitors turned HIV-infection into a manageable chronic condition as illustrated by a dramatic decrease in HIV-related morbidity and mortality (7-9). Similar trends were observed in the Dutch treated HIV-infected population described in the final ATHENA report (10). As there is, however, no prospect of eradication of HIV with currently available antiretroviral agents, treatment needs to be life-long (11-16). In fact, HIV-infection continues to be a potentially life-threatening condition as disease progression can occur rapidly if treatment is sub-optimal or plasma drug concentrations are sub-therapeutic (17-19).

Changes in treatment schedules

Waning adherence is a known problem for chronic treatments (20-22). A sub-analysis within the ATHENA cohort showed that only half of the patients fully adhere to treatment instructions (23). Until the arrival of easier treatment regimens, professional support tailored to the individual patients' needs is probably the best safeguard against poor adherence (24).

Most changes within the currently available treatment modalities have been directed at treatment simplification and toxicity reduction. An important development regarding treatment simplification has been the use of the cytochrome P450 dependence of HIV-protease inhibitor clearance for pharmacokinetic manipulation of plasma drug levels, such as the boosting of indinavir or saquinavir plasma levels by ritonavir (25-35). Hence, regular treatment schedules can be given twice daily instead of three times daily and many food restrictions can be dropped.

The arrival of non-nucleoside analogue reverse transcriptase inhibitors, like nevirapine and efavirenz, in 1997 provided further means for treatment simplification (36, 37). These agents exert strong antiretroviral activity (38, 39) but generally have longer half-lives than HIV-protease inhibitors allowing for twice daily or even once daily dosing.

Interactions between HIV-protease inhibitors and concomitant drugs

A high impact of drug interactions with HIV-protease inhibitor treatment was expected because of the involvement of the cytochrome P450 system in the metabolism of HIV-protease inhibitors (1, 4, 40, 41). Tabulated lists of potentially hazardous combinations and contra-indicated medications often based on theoretical interactions were developed to assist treating physicians in choosing the right treatment combinations.

In due course, however, the importance of interactions decreased with the reduced need for concomitant treatment (9). Moreover, growing expertise with HIV-protease inhibitor plasma concentration measurements facilitated the 'safe' use of concomitant therapies in which the cytochrome P450 system might play a role.

Adverse effects reported during the use of HIV-protease inhibitors

Regarding the safety of HIV-protease inhibitors, a large number of published case-reports about adverse effects illustrated some of the risks involved in using HIV-protease inhibitors (Table). Several observational studies showed a major impact of adverse effects on treatment continuity (42-46).

A safety issue, which received much attention in the literature, was renal complications of indinavir treatment. Pre-registration studies had recognized nephrolithiasis as a characteristic adverse effect of indinavir (47, 48), but the severity and frequency of the complications appeared to be different under everyday circumstances (49-52).

Probably the most important finding during post-marketing of HIV-protease inhibitors was the lipodystrophy syndrome and its associated disorders, such as cardiovascular complications (53, 54). Clinical trials had failed to identify this adverse effect despite its high prevalence. This was at variance with the perception of experts at the introduction in July 1996, when many had the opinion that all adverse reactions of HIV-protease inhibitors were already known, and illustrates the importance of post-marketing surveillance.

A likely explanation for the fact that lipodystrophy was not detected in clinical trials is its gradual onset and relatively long induction and latency period. Although lipodystrophy showed a clear temporal association with the introduction of HIV-protease inhibitors, a definite causal link has not yet been established.

Table Adverse effects of HIV-protease inhibitors reported in case reports and case series in the literature

Category	adverse effects descriptions	suspected antiretroviral drugs involved	Relevant co-factor	references
Musculo-skeletal	Acute monoarthritis, arthalgia+cryoglobulinemia	indinavir, HIV-protease inhibitors	hepatitis C	(79, 80)
	Osteonecrosis, osteoporosis, pathological fractures	HIV-protease inhibitors, saquinavir, indinavir+lamivudine+stavudine		(81-85)
	Frozen shoulder	indinavir, indinavir+lamivudine+stavudine		(86-88)
Breasts	Breast hypertrophy, gynaecomastia	indinavir, HIV-protease inhibitors, HIV-protease inhibitors+stavudine, indinavir, saquinavir		(89-95)
Fat metabolism	Buffalo hump	HIV-protease inhibitors		(96, 97)
	Adipose tissue hypertrophy, lipemia retinalis, lipodystrophy, lipomatosis, protease paunch, pseudo Cushing's disease, striae, subcutaneous lipomas	HIV-protease inhibitors, indinavir, ritonavir		(53, 69, 70, 72, 73, 98-108)
Metabolic	Diabetes or glucose abnormalities, hypertriglyceremia, hypercholesterolemia, ketoacidosis, mitochondrial and peroxisomal dysfunction	HIV-protease inhibitors, nelfinavir, indinavir, ritonavir, saquinavir		(109-121)
Cardiovascular	Angiolipomatosis, bradycardia, coronary artery disease, intestinal infarction, myocardial infarction, sudden cardiac death, other vascular complications	indinavir, nelfinavir, HIV-protease inhibitors, ritonavir		(122-133)
Coagulation	Haemolysis, haemorrhage, platelet dysfunction, thrombocytopenia	indinavir, amprenavir, HIV-protease inhibitors, ritonavir, nelfinavir, saquinavir	Warfarin	(134-146)
Urological	Crystalluria, urolithias and nephrolithiasis, interstitial nephritis, renal atrophy	Indinavir		(55-59, 63, 147-168)
Renal	acute renal failure, anuria	indinavir, ritonavir, ritonavir+saquinavir		(60-62, 64, 65, 169-175)
Hepatotoxicity	anoxic hepatic necrosis, toxic hepatitis, portal vein thrombosis	indinavir, ritonavir+saquinavir, ritonavir+zidovudine, ritonavir		(176-183)
Hypersensitivity	rash, Stevens-Johnson syndrome, urticaria	HIV-protease inhibitors, indinavir, nelfinavir		(184-190)
Immune reconstitution	Exacerbation viral hepatitis, MAC infection activation, progressive multifocal leukoencephalopathy	HIV-protease inhibitors, indinavir+lamivudine+stavudine, indinavir, ritonavir+lamivudine+stavudine		(191-197)
Nails	Ingrown toenails, paronychia	indinavir, lamivudine		(198-202)
Pancreatitis	Acute pancreatitis	nelfinavir, ritonavir		(203-205)
Miscellaneous PIs	Alopecia, Graves' disease, carpal tunnel syndrome, hyperaestesia, iron overload, panniculitis, respiratory failure, uveitis, vitritis	HIV-protease inhibitors	CMV	(206-214)
	Galactorrhoea+hyperprolactinaemia	HIV-protease inhibitors, nevirapine		(215)

Category	adverse effects descriptions	suspected antiretroviral drugs involved	Relevant co-factor	references
	Cheilitis, focal mycobacterial lymphadenitis, hair loss, mood changes, leucocytoclastic vasculitis, maculopapular eruption, porphyria, uveitis	Indinavir		(216-222)
	Neuropathy, normalisation von Willebrand, oesophagitis	Nelfinavir		(223-225)
	Herpes Zoster, hypermenorrhea, maculopapular eruption, myasthenia gravis	Ritonavir		(226-229)
	Gout	Ritonavir+didanosine		(230)
	Mucosal Kaposi's sarcoma, renin-angiotensin inhibition	Ritonavir+saquinavir	Overdose	(231, 232)
	Acute paronoid reaction, photosensitivity	saquinavir		(233, 234)

ddI= didanosine; IDV= indinavir; NFV= nelfinavir; NVP= nevirapine; PI= HIV-protease inhibitor; RTV= ritonavir; SQV= saquinavir

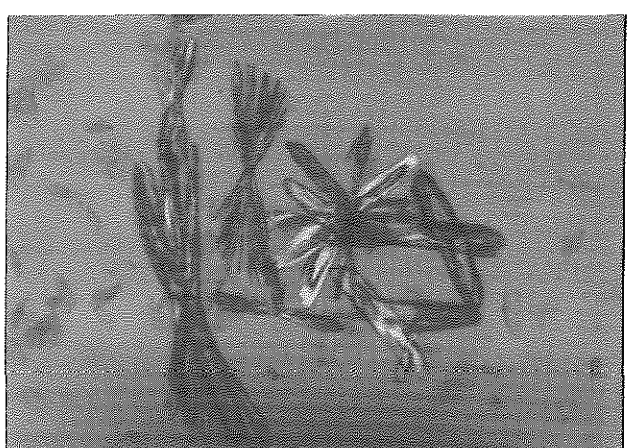

Figure 1 Indinavir crystal retrieved from urine

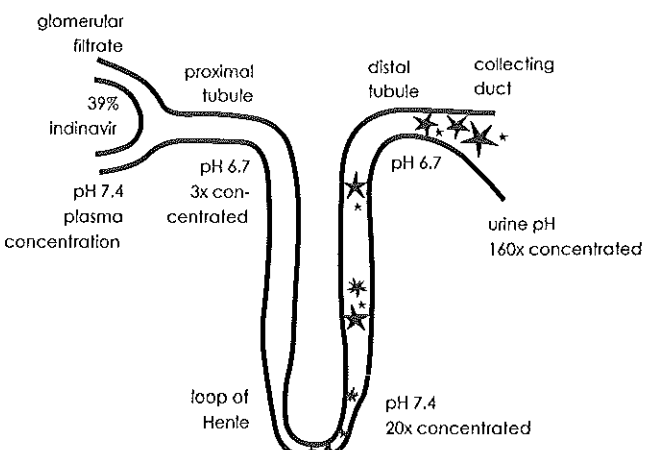

Figure 2 The pattern according to which normal physiological conditions in the renal system vary

Main findings of this thesis

Indinavir associated renal complications (chapter 2)

Renal complications form an important treatment-limiting effect of indinavir (47). Apart from causing treatment switches, forms of renal injury, such as interstitial nephritis (55-57), renal atrophy (58, 59), renal failure (60-66) or, as described in this thesis (chapter 2.1), papillary necrosis (67) may have serious consequences (chapter 2.1). The onset of indinavir nephrotoxicity can be insidious particularly in children. Two out of five children, treated with indinavir at the Sophia children's hospital in Rotterdam and presenting with persistent leukocyturia without overt symptoms, appeared to have congested kidneys associated with renal cortex atrophy on ultrasound (68). Leukocyturia disappeared and renal structures recovered rapidly upon indinavir withdrawal.

The first nephrotoxicity study (chapter 2.2) involved a small case series of patients with urological complaints from the Erasmus university Medical Centre. Plasma indinavir concentrations of patients with complaints were on average 2.6-fold higher than plasma indinavir concentrations of patients without complaints from the University Hospital Nijmegen. Hence, it was suggested that indinavir-associated urological complaints are associated with high indinavir plasma concentrations

The second study (chapter 2.3) describes a population-based cohort study carried out within the ATHENA cohort, which confirmed that urological symptoms are specific for indinavir. The incidence of urological symptoms (renal colic, flank pain, gross hematuria, renal failure, nephritis) among indinavir using patients was 8.3% within the first year of treatment, which is twice as high as reported in clinical trials (47). Risk factors for urological symptoms during indinavir treatment were: viral suppression (indicative of adequate or high indinavir plasma concentrations), low lean body mass (indicative of small distribution volume), indinavir dosing regimen of $\geq$ 1000 mg bid (indicative of high Cmax), and high environmental temperatures (proxy for reduced urine output due to excessive sweating). These results are consistent with a concentration dependent solubility problem of indinavir causing indinavir crystal formation (Figure 1).

The third study (chapter 2.4) had an experimental design and was conducted to obtain more insight into the solubility problem underlying indinavir-associated renal complications. The renal sites at which indinavir solubility is exceeded and crystals may arise was determined by simulating the normal physiological conditions in the renal system in *in vitro* crystallisation experiments (Figure 2).

The risk of indinavir crystallisation appeared highest under the conditions encountered in the loop of Henle. Assuming absence of re-absorption and active secretion of indinavir in proximal tubuli, indinavir crystals might already occur in the loop of Henle at

plasma concentrations of 8 mg/L, which is the average Cmax reached with indinavir regimens of 800 mg tid. As the conditions in the loop of Henle are not substantially influenced by water intake, increasing water intake (ie. diluting urine) as recommended for patients with indinavir nephrotoxicity, might be insufficient, in particular for patients with high indinavir plasma concentrations. Therefore, beyond the present recommendations, plasma concentration-guided dose adjustment is worth considering in the future.

We performed prospective systematic monitoring of renal parameters to study the prevalence of renal abnormalities among adults (chapter 2.5) and children (chapter 2.6) treated with indinavir. These studies demonstrated that indinavir nephrotoxicity can have an insidious onset and may develop into sub-clinical renal failure. In the adult population, who had been using indinavir for more than a year, the prevalence of nephrotoxicity presenting as sub-clinical persistent sterile leukocyturia was 25%. The cumulative incidence of persistent leukocyturia in children was 53% in approximately three years. Leukocyturia was associated with high plasma indinavir concentrations, which confirms the findings of our previous studies. Urine supersaturation may cause indinavir crystal formation and a subsequent inflammatory reaction. Although leukocyturia disappeared instantly upon indinavir discontinuation, the long-term risks of persistent leukocyturia during indinavir continuation are unknown at present.

The lipodystrophy syndrome (chapter 3)

Lipodystrophy is both a remarkable and disturbing adverse effect of antiretroviral treatment, which was unknown at the time of licensing the HIV-protease inhibitors (chapter 3.1). The syndrome was initially attributed to HIV-protease inhibitors in particular (54, 69-73) owing to the temporal association between HIV-protease inhibitor introduction and development of lipodystrophy. Later studies, however, added stavudine as a potential causal agent (74) or even attributed the effect on fat distribution to alleviation of metabolic stress. A complicating factor for studies on causes of lipodystrophy, is the long induction and latency period during which patients may have received multiple treatment regimens.

Because of its alleged but unproven association with HIV-protease inhibitors, we performed a retrospective cohort study within the ATHENA cohort (chapter 3.2). This study aimed to identify risk factors for lipodystrophy, taking into consideration treatment regimens and changes of regimens. Stavudine-containing HAART regimens rather than indinavir-containing regimens were identified as the most important risk factor for lipodystrophy. Factors such as age, gender, baseline body mass index and immunological and virological response also appeared to play a role. Among the HIV-protease inhibitors, indinavir appeared to be most evidently associated with lipodystrophy, but HIV-protease inhibitors in general seemed to play a minor role. In conclusion, the data do not confirm a major role for

HIV-protease inhibitors in the development of lipodystrophy and allocate a more important role for the nucleoside-analogue reverse transcriptase inhibitor, stavudine. These data provide new clues for underlying (multi-factorial) causes of lipodystrophy and could help to direct further (prospective) research both *in vivo* and *in vitro*.

Adverse effects and treatment switches (chapter 4)

Inadequate adherence to antiretroviral treatment regimens is a cause of concern. Apart from the risk of waning motivation after long-term treatment, complex schedules, large quantity of pills, diet restrictions and bothersome or hazardous adverse effects continuously jeopardise adherence to HAART. Efforts to find suitable treatment regimens tailored to individual patients' needs or regimens with generally more favourable properties should help to improve adherence and treatment success. Unlike for virological failure (75-77), guidelines on strategies for other reasons for switching are absent. We examined the impact of switches performed in daily practice on treatment continuity and the impact of intolerance-driven switches on treatment continuity.

Antiretroviral regimens with non-nucleoside analogue reverse transcriptase inhibitors such as nevirapine and efavirenz, are relatively simple and exert few adverse effects despite strong anti-HIV activity. These agents might offer a solution for the problems encountered with HIV-protease inhibitors.

An initial case series from the Erasmus universtity Medical Centre indicated that patients who switched to a nevirapine-containing regimen while having a low HIV-1 RNA (<500 copies/mL) were able to maintain viral suppression with less adverse effects (chapter 4.1).

Subsequently, we compared the incidence of treatment discontinuation and virological failure between persons who switched either to nevirapine or to a second-line HIV-protease inhibitor despite viral suppression in the ATHENA cohort (chapter 4.2). This study failed to show a lower rate of adverse effects, but did show a 5-fold lower risk of having a further switch or having virological failure in the nevirapine group. Switches to non-nucleoside analogue reverse transcriptase inhibitors were therefore beneficial for treatment continuity.

In the last study (chapter 4.3), we aimed to identify risk factors for toxicity-driven switches within the ATHENA cohort. The most frequent reason for switching HAART appeared to be gastro-intestinal complaints, such as nausea, vomiting, diarrhoea, and neuropathy whereas lipodystrophy rarely caused treatment switches. In this cohort study we showed that toxicity-driven treatment switches in daily practice often fail to resolve the problems and are soon followed by a further toxicity-driven switch. Switching to another class of

antiretrovirals, such as nevirapine was, however, associated with less further toxicity-driven switches and therefore seems worth considering for toxicity management.

Methodological considerations

The studies in this thesis were performed predominantly with prospective observational data. The advantages and disadvantages of observational studies are well recognised. Observational studies are often relatively cheap and tell us more about the actual use of drugs than clinical trials. Observational studies with ongoing data collection can provide quick clues to acute health care issues. Studies performed with observational data, however, have to deal with complex validity issues such as bias and confounding.

In observational studies with exposure and disease registries, information bias is less likely since the research objective was not known at the time of registry. The original purpose of the registry and conditions for entering the registry, however, may be a source of selection bias. Moreover, the large amount of data is usually achieved at the cost of detail, due to which it may be impossible to adjust completely for confounding factors. It can be extremely difficult to adjust for confounding by indication since the choice of treatment is not random and the underlying reason for giving a certain regimen is usually not registered.

The limitations of each individual study in this thesis have been addressed in the discussion sections of the study reports. Here we describe potential general shortcomings pertaining to the chosen study settings.

The ATHENA cohort

The ATHENA cohort formed the source population for most of our studies. The ATHENA project aimed to recruit all patients starting on one of the new antiretroviral agents, including the HIV-protease inhibitors, non-nucleoside analogue reverse transcriptase inhibitors and nucleoside analogue reverse transcriptase inhibitors. Extensive information on antiretroviral treatment details, CD4 cell count and HIV-RNA load was collected as well as details on adverse effects leading to antiretroviral treatment changes or included in a list of special adverse effects.

The purpose of the ATHENA project was to determine the implications of introducing the new antiretroviral agents in July 1996 for the course of HIV-infection, public health and HIV/AIDS health care as well as the costs and economic benefits. In addition, the resulting database would serve as a source for several other, more specific studies. In order to judge the generalizability and validity of results derived from the ATHENA cohort, the representativeness of the cohort and bias and confounding inherent to the design and set up of the ATHENA project and observational nature of the registry are discussed below.

Validity and generalizability of patient selection

By the end of the year 2000, 3377 patients had been enrolled into the ATHENA cohort. They represented approximately 70-80% of the treated Dutch HIV-infected population (Monitoring of Human Immunodeficiency Virus Type I (HIV-1) Infection in the Netherlands) (10). The selected population may not be entirely representative of the Dutch HIV-infected patient population, since patients had to give written informed consent in order to be included. This might have caused selection toward relatively healthy patients, who were able to read and understand the Dutch or English language and willing to sign consent forms. Apart from limitations in generalizability, omission of certain patient types will lead to selection bias only if it is done differentially for both treatment regimens and outcomes.

Validity and generalizability concerns pertaining to method of data collection

The quality of information on antiretroviral treatment and disease progression is likely to be high as these data are generally recorded very precisely in the medical records. The quality of information on adverse effects, however, may be a potential source of misclassification since case definitions for the diagnoses (special adverse effects) on the ATHENA data collection sheet are absent.

Physicians may fail to record cases that do not fully comply with the listed diagnosis, but prompting of specific diagnoses may also lead to over-reporting. As a consequence some inaccuracy has to be taken into account for incidence and prevalence rate estimates of such outcomes. Furthermore, ambiguous reporting might induce reporting bias in cohort studies and selection bias in case-control studies if it is done differentially for exposure.

Residual confounding remains a point of concern due to absence of information on a number of potentially important confounding factors (eg. smoking, socio-economic status, family history, medical history, concomitant drug use). Finally, time effects should always be taken into account in view of the rapid developments in the area of HIV/AIDS, such as the rapidly changing treatment regimens and the introduction of more sensitive HIV-RNA measurement techniques.

Validity issues in studies performed with the ATHENA cohort

In the nephrotoxicity and lipodystrophy studies, participation rates for the ATHENA cohort might have negatively affected the incidence rate estimates if patients suffering from these conditions would be less likely to participate. Since data on non-participants are not available we cannot verify the extent of a potential bias resulting from selective participation.

Underreporting of adverse effects could have suppressed the estimated incidence rates, whereas diagnostic suspicion might have led to an overestimation. Diagnostic suspicion was likely to be differential over time because of the changing and strong opinions regarding the causes of the adverse effects. For example, nephrotoxicity and lipodystrophy were both considered as a consequence of indinavir in particular. We dealt with this issue by restricting the study population to indinavir users for the nephrotoxicity studies. Stratification for calendar time solved time-dependent diagnostic bias in the lipodystrophy study.

A potential bias we were unable to deal with adequately in the switch studies, was the fact that availability of alternative treatment regimens may be different for different initial regimens. Suitability of alternative regimens depends on treatment history and current treatment. It is tempting to conclude that class switches, such as switches to nevirapine, would benefit all patients with viral suppression on HIV-protease inhibitors. However, since we could not control for underlying reasons for switching to a certain regimen, such as for example the availability of alternatives, our findings should be confirmed in a randomised study.

Perspectives and recommendations

Due to the introduction of HIV-protease inhibitors on the Dutch market, the therapeutic area of HIV/AIDS has changed considerably. Apart from a substantial gain in survival, experience with the new treatment entities grew quickly. The developments of the past five years resulted in a better application of HIV-protease inhibitor containing antiretroviral treatment and a better patient management.

Presently, adverse effects are responsible for the majority of problems, which accompany antiretroviral treatment. These problems will have to be tolerated or dealt with through effective toxicity management and preventative strategies. For this purpose, risk factors and underlying patho-physiological pathways have to be known and potential control measures have to be tested.

In our experience, data from the ATHENA cohort constituted a useful resource for performing (pharmo-) epidemiological studies on indinavir nephrotoxicity and on lipodystrophy. Many other adverse effects might be studied this way.

Future studies on indinavir nephrotoxicity

Following the work of this thesis and considering literature reports, a number of issues regarding indinavir nephrotoxicity remain to be elucidated in order to develop strategies, which may adequately deal with this adverse effect.

Therapeutic Drug Monitoring and guided dosing adjustment might be worthwhile as an additional control measure for patients with abnormal renal parameters. First, however, relevant pharmacokinetic parameter(s) will have to be identified. This is especially interesting in view of the new 'ritonavir boosted indinavir regimens', which generate either a higher Cmax or lower Cmax with higher area under the curve (AUC). Based on information about relevant pharmacokinetic parameters derived from studies on the association between indinavir nephrotoxicity and individual pharmacokinetic parameters, it may be possible to identify patients at risk of nephrotoxicity early in treatment. Moreover, it may be possible to test the hypothesis that indinavir crystals occur already at plasma indinavir concentrations of 8 mg/L.

HIV-protease inhibitors are given at standard dosing regimens. Experience with indinavir showed that this might cause overdosing and excess adverse effects in some patients. Future studies on the impact of dose adjustments on adverse effects and desired effects may teach us that dose frequencies should rather be tailored to individual patients in order to prevent unnecessary negative effects.

Future studies on lipodystrophy

This thesis had no intention of determining the causes of lipodystrophy since this is a subject, which cannot be adequately sorted out in an observational database without clear case definitions. Using the observational database of the ATHENA project, however, helped understanding the coherence of potential risk factors for the syndrome even though the underlying mechanism remains unclear. The risk of lipodystrophy was most clearly associated with the use of stavudine and was hardly associated with the use of indinavir or any other HIV-protease inhibitor. This raises the question whether the use of stavudine should be avoided as much as possible.

Ideally, causal factors for lipodystrophy are investigated in a prospective cohort of incident users of antiretroviral regimens, employing objective and systematic lipodystrophy assessments supported by laboratory parameters. By focussing on a comparison between stavudine users and non-users as done in the Prometheus study (74, 78), the sample size of such an elaborate study may be kept manageable.

Treatment continuity

Whereas the degree of switching HIV-treatment due to virological failure is low, the degree of switching due to other reasons appeared to be extremely high. Especially gastro-intestinal intolerance and neuropathy are important causes of treatment switches. Studies in this thesis and the literature are suggestive of a disappointing effect from switches within the class of HIV-protease inhibitors as opposed to switches to other classes. This is in line with

class intolerance rather than with drug specific intolerance. The available data provide a basis for the development of provisional switching guidelines as a control measure for intolerance in patients with viral suppression.

Naturally, before implementation, the effect of guidelines should be analysed in clinical studies. Such studies should preferably be conducted in a randomised setting in order to overcome potential confounding by differential switching due to unknown underlying reasons.

Finally

The studies presented in this thesis reported on the scope of and the risk factors for two important adverse effects of HIV-protease inhibitor containing antiretroviral therapy indinavir nephrotoxicity and lipodystrophy. In addition, the impact of adverse effects on treatment continuity and the impact of treatment switch on treatment continuity were reported. Obviously, there are numerous other adverse effects (eg. gastro-intestinal intolerance, neuropathy, lactate acidosis) which deserve scientific attention due to their treatment limiting effect or severe impact on wellbeing.

In addition, the influence of concomitant medication on the risk of adverse effects remains to be studied. Disentangling the influence of concomitant medication on the risk of virological failure might be equally interesting.

From the perspective of pharmaco-epidemiology, the ATHENA project is unique in the sense that it captures an entire patient population. It may set the stage for other disease areas.

References

1. Taburet AM, Singlas E. Drug interactions with antiviral drugs. Clin Pharmacokinet 1996;30:385-401.
2. Lillibridge JH, Liang BH, Kerr BM, Webber S, Quart B, Shetty BV, et al. Characterization of the selectivity and mechanism of human cytochrome P450 inhibition by the human immunodeficiency virus-protease inhibitor nelfinavir mesylate. Drug Metab Dispos 1998;26:609-16.
3. Hoetelmans RMW, Meenhorst PL, Mulder JW, Burger DM, Koks CHW, Beijnen JH. Clinical pharmacology of HIV protease inhibitors: focus on saquinavir, indinavir, and ritonavir. Pharmacy World & Science 1997;19:159-75.
4. Heylen R, Miller R. Adverse effects and drug interactions of medications commonly used in the treatment of adult HIV positive patients: Part 2. Genitourin Med 1997;73:5-11.
5. Max B, Sherer R. Management of the adverse effects of antiretroviral therapy and medication adherence. Clin Infect Dis 2000;30 Suppl 2:S96-116.
6. Deeks SG, Smith M, Holodniy M, Kahn JO. HIV-1 protease inhibitors. A review for clinicians. JAMA 1997;277:145-53.

7. Ledergerber B, Egger M, M. O, Telenti A, Hirschel B, Battegay M, et al. Clinical progression and virological failure on highly active antiretroviral therapy in HIV-1 patients: a prospective cohort study. Lancet 1999;353:863-8.
8. Mocroft A, Vella S, Benfield TL, Chiesi A, Miller V, Gargalianos P, et al. Changing patterns of mortality across Europe in patients infected with HIV-1. EuroSIDA Study Group. Lancet 1998;352:1725-30.
9. Palella FJ, Delany KM, Moorman AC, Loveless MO, Fuhrer J, Satten GA, et al. Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. N Engl J Med 1998;338:853-60.
10. de Wolf F, Lange JMA, Bossuyt PMM, Dijkgraaf MGW, Burger DM, Nieuwkerk PT, et al. Monitoring of Human Immunodeficiency Virus Type 1 (HIV-1) Infection in The Netherlands: 2001.
11. Ho DD. Toward HIV eradication or remission: the tasks ahead. Science 1998;280:1866-7.
12. Wong JK, Hezareh M, Gunthard HF, Havlir DV, Ignacio CC, Spina CA, et al. Recovery of replication-competent HIV despite prolonged suppression of plasma viremia. Science 1997;278:1291-5.
13. Chun TW, Carruth L, Finzi D, Shen X, DiGiuseppe JA, Taylor H, et al. Quantification of latent tissue reservoirs and total body viral load in HIV-1 infection. Nature 1997;387:183-8.
14. Embretson J, Zupancic M, Ribas JL, Burke A, Racz P, Tenner-Racz K, et al. Massive covert infection of helper T lymphocytes and macrophages by HIV during the incubation period of AIDS. Nature 1993;362:359-62.
15. Finzi D, Hermankova M, Pierson T, al. e. Indentification of a reservoir for HIV-1 in patients on highly active antiretroviral therapy. Science 1997;278:1295-1300.
16. Schrager LK, D'Souza MP. Cellular and anatomical reservoirs of HIV-1 in patients receiving potent antiretroviral combination therapy. JAMA 1998;280:67-71.
17. Witvrouw M, Pannecouque C, Desmyter J, De Clercq E, Andries K. In vitro evaluation of the effect of temporary removal of HIV drug exposure. Antiviral Res 2000;46:215-21.
18. Jardine DK, Tyssen DP, Birch CJ. Effect of protease inhibitors on HIV-1 maturation and infectivity. Antiviral Res 2000;45:59-68.
19. Schrager LK, Fauci AS. Human immunodeficiency virus. Trapped but still dangerous. Nature 1995;377:680-1.
20. Urquhart J. The electronic medication event monitor. Lessons for pharmacotherapy. Clin Pharmacokinet 1997;32:345-56.
21. Flack JM, Novikov SV, Ferrario CM. Benefits of adherence to anti-hypertensive drug therapy. Eur Heart J 1996;17(Suppl A):16-20.
22. Mallion JM, Baguet JP, Siche JP, Tremel F, de Gaudemaris R. Compliance, electronic monitoring and antihypertensive drugs. J Hypertens Suppl 1998;16:S75-9.
23. Nieuwkerk PT, Sprangers MA, Burger DM, Hoetelmans RM, Hugen PW, Danner SA, et al. Limited patient adherence to highly active antiretroviral therapy for HIV-1 infection in an observational cohort study. Arch Intern Med 2001;161:1962-8.
24. Friedland GH, Williams A. Attaining higher goals in HIV treatment: the central importance of adherence. AIDS 1999;13 Suppl 1:S61-72.

25. van Heeswijk RP, Veldkamp AI, Hoetelmans RM, Mulder JW, Schreij G, Hsu A, et al. The steady-state plasma pharmacokinetics of indinavir alone and in combination with a low dose of ritonavir in twice daily dosing regimens in HIV-1-infected individuals. AIDS 1999;13:F95-9.
26. Rockstroh JK, Bergmann F, Wiesel W, Rieke A, Theisen A, Fatkenheuer G, et al. Efficacy and safety of twice daily first-line ritonavir/indinavir plus double nucleoside combination therapy in HIV-infected individuals. AIDS 2000;14:1181-5.
27. Casado JL, Moreno A, Marti-Belda P, Sabido R, Garcia-Arata I, Perez-Elias MJ, et al. Increased Indinavir Levels Using Twice Daily Ritonavir/Indinavir at 100/800mg Improves Virological Response even after Multiple Failure (abstract no. 1170). In: 40th Interscience Conference on Antimicrobial Agents and Chemotherapy; September 2000; Toronto; p. 301.
28. Burger DM, Hugen PW, Aarnoutse RE, Dieleman JP, Prins JM, van Der Poll T, et al. A Retrospective, Cohort-Based Survey of Patients Using Twice-Daily Indinavir + Ritonavir Combinations: Pharmacokinetics, Safety, and Efficacy. J Acquir Immune Defic Syndr 2001;26:218-24.
29. Hsu A, Granneman GR, Cao G, Carothers L, Japour A, El-Shourbagy T, et al. Pharmacokinetic interaction between ritonavir and indinavir in healthy volunteers. Antimicrob Agents Chemother 1998;42:2784-91.
30. Hsu A, Granneman GR, Bertz RJ. Ritonavir. Clinical pharmacokinetics and interactions with other anti-HIV agents. Clin Pharmacokinet 1998;35:275-91.
31. Kempf DJ, Marsh KC, Kumar G, Rodrigues AD, Denissen JF, McDonald E, et al. Pharmacokinetic enhancement of inhibitors of the human immunodeficiency virus protease by coadministration with ritonavir. Antimicrob Agents Chemother 1997;41:654-60.
32. Hugen PWH, Burger DM, ter Hofstede HJM, Koopmans PP, Stek M, Hekster YA, et al. Dose-finding study of a once-daily indinavir/ritonavir regimen. J Acquir Immun Def Syndr 2000;25:236-245.
33. Mallolas J, Blanco JL, Sarasa M, Giner V, Martinez E, Garcia-Viejo MA, et al. Dose-finding study of once-daily indinavir/ritonavir plus zidovudine and lamivudine in HIV-infected patients. J Acquir Immuno Def Syndromes 2000;25:229-235.
34. Kilby JM, Sfakianos G, Gizzi N, Siemon-Hryczyk P, Ehrensing E, Oo C, et al. Safety and pharmacokinetics of once-daily regimens of soft-gel capsule saquinavir plus minidose ritonavir in human immunodeficiency virus-negative adults. Antimicrob Agents Chemother 2000;44:2672-8.
35. van Heeswijk RP, Veldkamp AI, Mulder JW, Meenhorst PL, Lange JM, Beijnen JH, et al. Once-daily dosing of saquinavir and low-dose ritonavir in HIV-1-infected individuals: a pharmacokinetic pilot study. AIDS 2000;14:F103-10.
36. Harris M, Montaner JS. Clinical uses of non-nucleoside reverse transcriptase inhibitors. Rev Med Virol 2000;10:217-29.
37. De Clercq E. The role of non-nucleoside reverse transcriptase inhibitors (NNRTIs) in the therapy of HIV-1 infection. Antiviral Res 1998;38:153-79.
38. Montaner JS, Reiss P, Cooper D, Vella S, Harris M, Conway B, et al. A randomized, double-blind trial comparing combinations of nevirapine, didanosine, and zidovudine for HIV-infected patients: the INCAS Trial. Italy, The Netherlands, Canada and Australia Study. JAMA 1998;279:930-7.
39. Staszewski S. Update on study 006--EFV + AZT + 3TC versus the current 'standard of care' IDV + AZT + 3TC. Int J Clin Pract Suppl 1999;103:10-5.

40. Wood AJJ. Drug therapy: interactions among drugs for HIV and opportunistic infections. N Engl J Med 2001;344:984-96.
41. Eagling VA, Back DJ, Barry MG. Differential inhibition of cytochrome P450 isoforms by the protease inhibitors, ritonavir, saquinavir and indinavir. Br J Clin Pharmacol 1997;44:190-4.
42. Bini T, Testa L, Chiesa E, Adorni F, Abeli C, Castelnuovo B, et al. Outcome of a second-line protease inhibitor-containing regimen in patients failing or intolerant of a first highly active antiretroviral therapy. J Acquir Immune Defic Syndr 2000;24:115-22.
43. Barreiro P, Soriano V, Blanco F, Casimiro C, de la Cruz JJ, Gonzalez-Lahoz J. Risks and benefits of replacing protease inhibitors by nevirapine in HIV-infected subjects under long-term successful triple combination therapy. AIDS 2000;14:807-12.
44. Manfredi R, Chiodo F. Switch of protease inhibitor-containing HAART in routine clinical practice: a four-year prospective observational study. Int J STD AIDS 2001;12:84-8.
45. Mocroft A, Youle M, Moore A, Sabin CA, Madge S, Lepri AC, et al. Reasons for modification and discontinuation of antiretrovirals: results from a single treatment centre. AIDS 2001;15:185-94.
46. van Roon EN, Verzijl JM, Juttmann JR, Lenderink AW, Blans MJ, Egberts AC. Incidence of discontinuation of highly active antiretroviral combination therapy (HAART) and its determinants. J Acquir Immune Defic Syndr Hum Retrovirol 1999;20:290-4.
47. Anonymous. Summary product characteristics Crixivan 400 mg. Product characteristics: Merck Sharp & Dohme ltd.; 1996 29/5/96.
48. Yeh KC, Deutsch PJ, Haddix H, Hesney M, Hoagland V, Ju WD, et al. Single-dose pharmacokinetics of indinavir and the effect of food. Antimicrob Agents Chemother 1998;42:332-8.
49. Kopp JB, Miller KD, Mican JA, Feuerstein IM, Vaughan E, Baker C, et al. Crystalluria and urinary tract abnormalities associated with indinavir. Ann Intern Med 1997;127:119-25.
50. Martinez E, Leguizamon M, Mallolas J, Miro JM, Gatell JM. Influence of environmental temperature on incidence of indinavir-related nephrolithiasis. Clin Infect Dis 1999;29:422-5.
51. Gagnon RF, Tecimer SN, Watters AK, Hatzakis GE, Tsoukas CM. The natural history of leukocyturia associated with indinavir treatment in HIV+ individuals. Am J Nephrol 2000;20:448-54.
52. Gagnon RF, Tecimer SN, Watters AK, Tsoukas CM. Prospective study of urinalysis abnormalities in HIV-positive individuals treated with indinavir. Am J Kidney Dis 2000;36:507-15.
53. Carr A, Cooper DA. Images in clinical medicine. Lipodystrophy associated with an HIV-protease inhibitor [see comments]. N Engl J Med 1998;339:1296.
54. Carr A, Samaras K, Burton S, Law M, Freund J, Chisholm DJ, et al. A syndrome of peripheral lipodystrophy, hyperlipidaemia and insulin resistance in patients receiving HIV protease inhibitors. AIDS 1998;12:F51-8.
55. Sarcletti M, Petter A, Zangerle R. Indinavir and interstitial nephritis [letter]. Ann Intern Med 1998;128:320.
56. Marroni M, Gaburri M, Mecozzi F, Baldelli F. Acute interstitial nephritis secondary to the administration of indinavir [letter]. Ann Pharmacother 1998;32:843-4.
57. Jaradat M, Phillips C, Yum MN, Cushing H, Moe S. Acute tubulointerstitial nephritis attributable to indinavir therapy. Am J Kidney Dis 2000;35:E16.
58. Hanabusa H, Tagami H, Hataya H. Renal atrophy associated with long-term treatment with indinavir. N Engl J Med 1999;340:392-3.

59. Cattelan AM, Trevenzoli M, Naso A, Meneghetti F, Cadrobbi P. Severe hypertension and renal atrophy associated with indinavir. Clin Infect Dis 2000;30:619-21.
60. Berns JS, Cohen RM, Silverman M, Turner J. Acute renal failure due to indinavir crystalluria and nephrolithiasis: report of two cases. Am J Kidney Dis 1997;30:558-60.
61. Chen SC, Nankivell BJ, Dwyer DE. Indinavir-induced renal failure. AIDS 1998;12:440-1.
62. Rietsema WJ. Fever, erythroderma, abdominal pain, and renal failure following initiation of indinavir therapy. Clin Infect Dis 1997;25:1268-9.
63. Perazella MA, Kashgarian M, Cooney E. Indinavir nephropathy in an AIDS patient with renal insufficiency and pyuria. Clin Nephrol 1998;50:194-6.
64. Witte M, Tobon A, Gruenenfelder J, Goldfarb R, Coburn M. Anuria and acute renal failure resulting from indinavir sulfate induced nephrolithiasis. J Urol 1998;159:498-9.
65. Rodriguez-Hernandez MJ, Viciana P, Cordero E, de Alarcon A, Herrero M. Acute renal failure caused by indinavir in a patient with a single functioning kidney. Eur J Clin Microbiol Infect Dis 1999;18:386-7.
66. Perazella MA. Crystal-induced acute renal failure. Am J Med 1999;106:459-65.
67. Dieleman JP, van der Feltz M, Bangma CH, Stricker BH, van der Ende ME. Papillary necrosis associated with the HIV protease inhibitor indinavir. Infection 2001;29:232-3.
68. van Rossum AM, Dieleman JP, Fraaij PL, Cransberg K, Hartwig NG, Gyssens IC, et al. Indinavir-associated asymptomatic nephrolithiasis and renal cortex atrophy in two HIV-1 infected children. AIDS 2001;15:1745-7.
69. Striker R, Conlin D, Marx M, Wiviott L. Localized adipose tissue hypertrophy in patients receiving human immunodeficiency virus protease inhibitors. Clin Infect Dis 1998;27:218-20.
70. Hengel RL, Watts NB, Lennox JL. Benign symmetric lipomatosis associated with protease inhibitors. Lancet 1997;350:1596.
71. Carr A, Samaras K, Thorisdottir A, Kaufmann GR, Chisholm DJ, Cooper DA. Diagnosis, prediction, and natural course of HIV-1 protease-inhibitor-associated lipodystrophy, hyperlipidaemia, and diabetes mellitus: a cohort study. Lancet 1999;353:2093-9.
72. Miller MD, Jones E, Yanovski JA, Shankar R, Feuerstein I, Falloon J. Visceral abdominal-fat accumulation associated with use of indinavir. Lancet 1998;351:871-75.
73. Miller KK, Daly PA, Sentochnik D, Doweiko J, Samore M, Basgoz NO, et al. Pseudo-Cushing's syndrome in human immunodeficiency virus-infected patients. Clin Infect Dis 1998;27:68-72.
74. van der Valk M, Gisolf EH, Reiss P, Wit FW, Japour A, Weverling GJ, et al. Increased risk of lipodystrophy when nucleoside analogue reverse transcriptase inhibitors are included with protease inhibitors in the treatment of HIV-1 infection. AIDS 2001;15:847-55.
75. Carpenter CC, Cooper DA, Fischl MA, Gatell JM, Gazzard BG, Hammer SM, et al. Antiretroviral therapy in adults. Updated recommendations of the International AIDS Society -USA Panel. JAMA 2000;283:381-90.
76. Anonymous. British HIV Association guidelines for antiretroviral treatment of HIV seropositive individuals. BHIVA Guidelines Co-ordinating Committee . Lancet 1997;349:1086-92.
77. Borleffs JJC, Danner SA, Boer K, de Groot R, Kauffmann R, Reiss P, et al. [Guidelines for HIV-suppressing therapy 1998]. National guidelines; 1998.

78. Gisolf EH, Jurriaans S, Pelgrom J, van Wanzeele F, van der Ende ME, Brinkman K, et al. The effect of treatment intensification in HIV-infection: a study comparing treatment with ritonavir/saquinavir and ritonavir/saquinavir/stavudine. Prometheus Study Group. AIDS 2000;14:405-13.
79. Brooks JI, Gallicano K, Garber G, Angel JB. Acute monoarthritis complicating therapy with indinavir. AIDS 2000;14:2064-5.
80. Monsuez JJ, Vittecoq D, Musset L, Alemanni M, Dussaix E, Autran B. Arthralgias and cryoglobulinemia during protease inhibitor therapy in a patient infected with human immunodeficiency virus and hepatitis C virus. Arthritis Rheum 1998;41(4):740-3.
81. Bonfanti P, Grabbuti A, Carradori S, Pusterla L, Parrazini F, Landonio S, et al. Osteonecrosis in protease inhibitor-treated patients. Orthopedics 2001;24:271-2.
82. Franzen C, Salzberger B, Fatkenheuer G. Avascular necrosis of both femoral heads in an HIV-infected patient receiving protease inhibitors. Eur J Med Res 2001;6:83-4.
83. Meyer D, Behrens G, Schmidt RE, Stoll M. Osteonecrosis of the femoral head in patients receiving HIV protease inhibitors. AIDS 1999;13:1147-8.
84. Johns DG, Gill MJ. Avascular necrosis in HIV infection. AIDS 1999;13:1997-8.
85. Guaraldi G, Ventura P, Albuzza M, Orlando G, Bedini A, Amorico G, et al. Pathological fractures in AIDS patients with osteopenia and osteoporosis induced by antiretroviral therapy. AIDS 2001;15:137-8.
86. Grasland A, Ziza JM, Raguin G, Pouchot J, Vinceneux P. Adhesive capsulitis of shoulder and treatment with protease inhibitors in patients with human immunodeficiency virus infection: report of 8 cases. J Rheumatol 2000;27:2642-6.
87. Leone J, Beguinot I, Dehlinger V, Jaussaud R, Rouger C, Strady C, et al. Adhesive capsulitis of the shoulder induced by protease inhibitor therapy. Three new cases. Rev Rhum Engl Ed 1998;65:800-1.
88. Peyriere H, Mauboussin JM, Rouanet I, Rouveroux P, Hillaire-Buys D, Balmes P. Frozen shoulder in HIV patients treated with indinavir: report of three cases. AIDS 1999;13:2305-6.
89. Herry I, Bernard L, de Truchis P, Perronne C. Hypertrophy of the breasts in a patient treated with indinavir. Clin Infect Dis 1997;25:937-8.
90. Lui A, Karter D, Turett G. Another case of breast hypertrophy in a patient treated with indinavir. Clin Infect Dis 1998;26:1482.
91. Peyriere H, Mauboussin JM, Rouanet I, Merle C, Sotto A, Arnaud A, et al. Report of gynecomastia in five male patients during antiretroviral therapy for HIV infection. AIDS 1999;13:2167-9.
92. Schurmann D, Bergmann F, Ehrenstein T, Padberg J. Gynaecomastia in a male patient during protease inhibitor treatment for acute HIV disease. AIDS 1998;12:2232-3.
93. Manfredi R, Calza L, Chiodo F. Gynecomastia associated with highly active antiretroviral therapy. Ann Pharmacother 2001;35:438-9.
94. Toma E, Therrien R. Gynaecomastia during indinavir antiretroviral therapy in HIV infection. AIDS 1998;12:681-2.
95. Donovan B, Bodsworth NJ, Mulhall BP, Allen D. Gynaecomastia associated with saquinavir therapy. Int J STD AIDS 1999;10:49-50.
96. Aboulafia DM, Bundow D. Images in clinical medicine. Buffalo hump in a patient with the acquired immunodeficiency syndrome. N Engl J Med 1998;339:1297.

97. Stocker DN, Meier PJ, Stoller R, Fattinger KE. "Buffalo hump" in HIV-1 infection. Lancet 1998;352:320-1.
98. Eng KT, Liu ES, Silverman MS, Berger AR. Lipemia retinalis in acquired immunodeficiency syndrome treated with protease inhibitors. Arch Ophthalmol 2000;118:425-6.
99. O'Mahony C, Price LM, Nelson M. Lipodystrophy despite anabolic steroids. Int J STD AIDS 1998;9:619.
100. Viraben R, Aquilina C. Indinavir-associated lipodystrophy. AIDS 1998;12:F37-9.
101. Williamson K, Reboli AC, Manders SM. Protease inhibitor-induced lipodystrophy. J Am Acad Dermatol 1999;40:635-6.
102. Wurtz R. Abnormal fat distribution and use of protease inhibitors. Lancet 1998;351:1735-6.
103. Wilson JD, Dunham RJ, Balen AH. HIV protease inhibitors, the lipodystrophy syndrome and polycystic ovary syndrome--is there a link? Sex Transm Infect 1999;75:268-9.
104. Olive A, Salavert A, Manriquez M, Clotet B, Moragas A. Parotid lipomatosis in HIV positive patients: a new clinical disorder associated with protease inhibitors. Ann Rheum Dis 1998;57:749.
105. Mishriki YY. A baffling case of bulging belly. Protease paunch. Postgrad Med 1998;104:45-6.
106. Ebright JR, Stellini MA, Tselis AC. Spinal epidural lipomatosis in a human immunodeficiency virus-positive patient receiving steroids and protease inhibitor therapy. Clin Infect Dis 2001;32:E90-1.
107. Darvay A, Acland K, Lynn W, Russell-Jones R. Striae formation in two HIV-positive persons receiving protease inhibitors. J Am Acad Dermatol 1999;41:467-9.
108. Bornhovd E, Sakrauski AK, Bruhl H, Walli R, Plewig G, Rocken M. Multiple circumscribed subcutaneous lipomas associated with use of human immunodeficiency virus protease inhibitors? Br J Dermatol 2000;143:1113-4.
109. Lee EC, Walmsley S, Fantus IG. New-onset diabetes mellitus associated with protease inhibitor therapy in an HIV-positive patient: case report and review. Cmaj 1999;161:161-4.
110. Visnegarwala F, Krause KL, Musher DM. Severe diabetes associated with protease inhibitor therapy [letter]. Ann Intern Med 1997;127:947.
111. Meyer L, Rabaud C, Ziegler O, May T, Drouin P. Protease inhibitors, diabetes mellitus and blood lipids. Diabetes Metab 1998;24:547-9.
112. Nerad JL, Kessler HA. Hypercholesterolemia in a health care worker receiving thyroxine after postexposure prophylaxis for human immunodeficiency virus infection. Clin Infect Dis 2001;32:1635-6.
113. Paterson DL, Singh N. Exacerbated hyperglycemia associated with nelfinavir. Ann Pharmacother 1998;32:609-10.
114. Allan DA, Behrman AJ. Lipid abnormalities in a healthcare worker receiving HIV prophylaxis. Int J STD AIDS 2001;12:532-4.
115. Echevarria KL, Hardin TC, Smith JA. Hyperlipidemia associated with protease inhibitor therapy. Ann Pharmacother 1999;33:859-63.
116. Sullivan AK, Nelson MR. Marked hyperlipidaemia on ritonavir therapy. AIDS 1997;11:938-9.
117. Sullivan AK, Feher MD, Nelson MR, Gazzard BG. Marked hypertriglyceridaemia associated with ritonavir therapy. AIDS 1998;12:1393-4.
118. Zimhony O, Stein D. Saquinavir-induced hypoglycemia in type 2 diabetes. Ann Intern Med 1999;131:980.

119. Kan VL, Nylen ES. Diabetic ketoacidosis in an HIV patient: a new mechanism of HIV protease inhibitor-induced glucose intolerance. AIDS 1999;13:1987-9.
120. Besson C, Jubault V, Viard JP, Pialoux G. Ketoacidosis associated with protease inhibitor therapy. AIDS 1998;12:1399-400.
121. Stojanov S, Wintergerst U, Belohradsky BH, Rolinski B. Mitochondrial and peroxisomal dysfunction following perinatal exposure to antiretroviral drugs. AIDS 2000;14:1669.
122. Dank JP, Colven R. Protease inhibitor-associated angiolipomatosis. J Am Acad Dermatol 2000;42:129-31.
123. Landovitz RJ, Sax PE. Symptomatic junctional bradycardia after treatment with nelfinavir. Clin Infect Dis 1999;29:449-50.
124. Hayes P, Muller D, Kuchar D. Left main coronary artery disease in a 40-year-old man receiving HIV protease inhibitors. Aust N Z J Med 2000;30:92-3.
125. Sullivan AK, Nelson MR, Moyle GJ, Newell AM, Feher MD, Gazzard BG. Coronary artery disease occurring with protease inhibitor therapy. Int J STD AIDS 1998;9:711-2.
126. Karmochkine M, Raguin G. Severe coronary artery disease in a young HIV-infected man with no cardiovascular risk factor who was treated with indinavir. AIDS 1998;12:2499.
127. Wang L, Molina CP, Rajaraman S. Case report. Intestinal infarction due to vascular catastrophe in an HIV-infected patient. AIDS Read 2000;10:718-21.
128. Eriksson U, Opravil M, Amann FW, Schaffner A. Is treatment with ritonavir a risk factor for myocardial infarction in HIV-infected patients? AIDS 1998;12:2079-80.
129. Carr A, Brown D, Cooper DA. Portal vein thrombosis in patients receiving indinavir, an HIV protease inhibitor. AIDS 1997;11:1657-8.
130. Koppel K, Bratt G, Rajs J. Sudden cardiac death in a patient on 2 years of highly active antiretroviral treatment: a case report. AIDS 1999;13:1993-4.
131. Rajs J, Blaxhult A, Sundelin B. Sudden unexpected death as a consequence of indinavir-induced nephropathy. A case report. Apmis 2000;108:581-3.
132. Behrens G, Schmidt H, Meyer D, Stoll M, Schmidt RE. Vascular complications associated with use of HIV protease inhibitors. Lancet 1998;351:1958.
133. Gallet B, Pulik M, Genet P, Chedin P, Hiltgen M. Vascular complications associated with use of HIV protease inhibitors. Lancet 1998;351:1958-9.
134. Prazuck T, Semaille C, Roques S. Fatal acute haemolysis in an AIDS patient treated with indinavir. AIDS 1998;12:531-3.
135. Watson A. Reversible acute haemolysis associated with indinavir. AIDS 2000;14:465-6.
136. Morrison-Griffiths S, Newman M, O'Mahony C, Pirmohamed M. Haemolytic anaemia associated with indinavir. Postgrad Med J 1999;75:313-5.
137. Kodoth S, Bakshi S, Scimeca P, Black K, Pahwa S. Possible linkage of amprenavir with intracranial bleeding in an HIV-infected hemophiliac. AIDS Patient Care STDS 2001;15:347-52.
138. Stanworth SJ, Bolton MJ, Hay CR, Shiach CR. Increased bleeding in HIV-positive haemophiliacs treated with antiretroviral protease inhibitors. Haemophilia 1998;4:109-14.
139. Teitel J. A side effect of protease inhibitors. Cmaj 1998;158:1129-30.

140. Hagerty SL, Ascher DP. Spontaneous bleeding associated with the use of the protease inhibitor ritonavir in a hemophiliac patient with human immunodeficiency virus infection. Pediatr Infect Dis J 1998;17:929-30.
141. Ginsburg C, Salmon-Ceron D, Vassilief D, Rabian C, Rotschild C, Fontenay-Roupie M, et al. Unusual occurrence of spontaneous haematomas in three asymptomatic HIV-infected haemophilia patients a few days after the onset of ritonavir treatment. AIDS 1997;11:388-9.
142. Hollmig KA, Beck SB, Doll DC. Severe bleeding complications in HIV-positive haemophiliac patients treated with protease inhibitors. Eur J Med Res 2001;6:112-4.
143. Pollmann H, Richter H, Jurgens H. Platelet dysfunction as the cause of spontaneous bleeding in two haemophilic patients taking HIV protease inhibitors. Thromb Haemost 1998;79:1213-4.
144. Durand JM. Indinavir and thrombocytopenia. AIDS 1999;13:148-9.
145. Stein DS, Dunn H, Drusano GL. Acute thrombocytopenia secondary to the administration of the peptidomimetic HIV protease inhibitor MK-639(L735524). AIDS 1996;10:678-80.
146. Darlington MR. Hypoprothrombinemia during concomitant therapy with warfarin and saquinavir. Ann Pharmacother 1997;31:647.
147. Antony SJ. Rapid development of indinavir-induced asymptomatic crystalluria in a human immunodeficiency virus-negative patient. Clin Infect Dis 1998;27:911-2.
148. Godwin TA. HIV/AIDS case histories: indinavir crystalluria. AIDS Patient Care STDS 2001;15:169-71.
149. Hachey DM, Force RW, Schott J, O'Leary-Jepsen E. Indinavir crystalluria in an HIV-positive man. Ann Pharmacother 2000;34:403.
150. Tsao JW, Kogan SC. Images in clinical medicine. Indinavir crystalluria. N Engl J Med 1999;340:1329.
151. Famularo G, Di Toro S, Moretti S, De Simone C. Symptomatic crystalluria associated with indinavir. Ann Pharmacother 2000;34:1414-8.
152. Hamm M, Wawroschek F, Rathert P. Urinary cytology changes in protease inhibitor induced urolithiasis. J Urol 2000;163:1249-50.
153. Bruce RG, Munch LC, Hoven AD, Jerauld RS, Greenburg R, Porter WH, et al. Urolithiasis associated with the protease inhibitor indinavir. Urology 1997;50:513-518.
154. Hermans BP, Materne R, Marot JC, Vandercam B, Van Beers BE, Van Cangh PJ. Indinavir calculi: diagnosis with magnetic resonance urography. Eur Urol 2000;37:634-5.
155. John H, Muller NJ, Opravil M, Hauri D. Indinavir urinary stones as origin of upper urinary tract obstruction. Urol Int 1997;59:257-9.
156. Noble CB, Klein LT, Staiman VR, Neu N, Hensle TW, Berdon WE. Ureteral obstruction secondary to indinavir in the pediatric HIV population. Pediatr Radiol 1998;28:627-9.
157. Schenkman NS. Case no. 3. Right flank pain. Crixivan lithiasis. Tech Urol 1999;5:59-60.
158. Sutherland SE, Reigle MD, Seftel AD, Resnick MI. Protease inhibitors and urolithiasis. J Urol 1997;158:31-3.
159. Ascher DP, Lucy MD. Indinavir sulfate renal toxicity in a pediatric hemophiliac with HIV infection. Ann Pharmacother 1997;31:1146-1149.
160. Martinez F, Mommeja-Marin H, Estepa-Maurice L, Beaufils H, Bochet M, Daudon M, et al. Indinavir crystal deposits associated with tubulointerstitial nephropathy. Nephrol Dial Transplant 1998;13:750-3.

161. Sarcletti M, Zangerle R. Persistent flank pain, low-grade fever, and malaise in a woman treated with indinavir. AIDS Patient Care STDS 1999;13:81-7.
162. Sarcletti M, Petter A, Romani N, Lhotta K, Konig P, Maier H, et al. Pyuria in patients treated with indinavir is associated with renal dysfunction. Clin Nephrol 2000;54:261-70.
163. Lerner LB, Cendron M, Rous SN. Nephrolithiasis from indinavir, a new human immunodeficiency virus drug. J Urology 1998;159:2074-5.
164. Polhemus ME, Aronson NE. Persistent nephrolithiasis after discontinuation of indinavir therapy. Clin Infect Dis 1998;27:1536.
165. Grabe DW, Eisele G, Miller C, Singh J, Stein D. Indinavir-induced nephropathy. Clin Nephrol 1999;51:181-3.
166. Reilly RF, Tray K, Perazella MA. Indinavir nephropathy revisited: a pattern of insidious renal failure with identifiable risk factors. Am J Kidney Dis 2001;38:E23.
167. Tashima KT, Horowitz JD, Rosen S. Indinavir nephropathy. N Engl J Med 1997;336:138-40.
168. Weber L, Campbell H, Gritter NJ. Indinavir-induced nephropathy. Tenn Med 1997;90:286-8.
169. Anglicheau D, Duvic C, Nedelec G. Sudden anuria due to indinavir crystalluria. Nephron 2000;86:364-5.
170. Grunke M, Valerius T, Manger B, Kalden JR, Harrer T. Renal dysfunction in a human immunodeficiency virus-infected patient who was treated with indinavir. Clin Infect Dis 1997;25:1270-1.
171. Chugh S, Bird R, Alexander EA. Ritonavir and renal failure [letter]. N Engl J Med 1997;336:138.
172. Duong M, Sgro C, Grappin M, Biron F, Boibieux A. Renal failure after treatment with ritonavir. Lancet 1996;348:693.
173. Benveniste O, Longuet P, Duval X, Le Moing V, Leport C, Vilde JL. Two episodes of acute renal failure, rhabdomyolysis, and severe hepatitis in an AIDS patient successively treated with ritonavir and indinavir. Clin Infect Dis 1999;28:1180-1.
174. Vigano A, Rombola G, Barbiano di Belgioioso G, Sala N, Principi N. Subtle occurrence of indinavir-induced acute renal insufficiency. AIDS 1998;12:954-5.
175. Witzke O, Plentz A, Schafers RF, Reinhardt W, Heemann U, Philipp T. Side-effects of ritonavir and its combination with saquinavir with special regard to renal function. AIDS 1997;11:836-8.
176. Karras A, Rabian C, Zylberberg H, Hermine O, Duchatelle V, Durand F, et al. Severe anoxic hepatic necrosis in an HIV-1-hepatitis C virus-co-infected patient starting antiretroviral triple combination therapy. AIDS 1998;12:827-9.
177. Matsuda J, Gohchi K. Severe hepatitis in patients with AIDS and haemophilia B treated with indinavir . Lancet 1997;350:364.
178. Brau N, Leaf HL, Wieczorek RL, Margolis DM. Severe hepatitis in three AIDS patients treated with indinavir . Lancet 1997;349:924-5.
179. Jeurissen FJ, Schneider MM, Borleffs JC. Is the combination of hepatitis and indinavir potentially dangerous? . AIDS 1998;12:441-2.
180. Vergis E, Paterson DL, Singh N. Indinavir-associated hepatitis in patients with advanced HIV infection. Int J STD AIDS 1998;9:53.
181. Vandercam B, Moreau M, Horsmans C, Gala JL. Acute hepatitis in a patient treated with saquinavir and ritonavir: absence of cross-toxicity with indinavir [letter]. Infection 1998;26:313.

182. Pai VB, Koranyi K, Nahata MC. Acute hepatitis and bleeding possibly induced by zidovudine and ritonavir in an infant with HIV infection. Pharmacotherapy 2000;20:1135-40.
183. Picard O, Rosmorduc O, Cabane J. Hepatotoxicity associated with ritonavir. Ann Intern Med 1998;129:670-1.
184. Dieleman JP, in 't Veld B, Borleffs JC, Schreij G. Acute respiratory failure associated with the human immunodeficiency virus (HIV) protease inhibitor indinavir in an HIV-infected patient. Clin Infect Dis 1998;26:1012-3.
185. Rijnders B, Kooman J. Severe allergic reaction after repeated exposure to indinavir. Clin Infect Dis 1998;26:523-4.
186. Abraham PE, Sorensen SJ, Baker WH, Cushing HE. Nelfinavir desensitization. Ann Pharmacother 2001;35:553-6.
187. Gajewski LK, Grimone AJ, Melbourne KM, Vanscoy GJ. Characterization of rash with indinavir in a national patient cohort. Ann Pharmacother 1999;33:17-21.
188. Fortuny C, Vicente MA, Medina MM, Gonzalez-Ensenat A. Rash as side-effect of nelfinavir in children. AIDS 2000;14:335-6.
189. Teira R, Zubero Z, Munoz J, Baraia-Etxaburu J, Santamaria JM. Stevens-Johnson syndrome caused by indinavir. Scand J Infect Dis 1998;30:634-5.
190. Demoly P, Messaad D, Trylesinski A, Faucherre V, Fabre J, Reynes J, et al. Nelfinavir-induced urticaria and successful desensitization. J Allergy Clin Immunol 1998;102:875-6.
191. Zylberberg H, Pialoux G, Carnot F, Landau A, Brechot C, Pol S. Rapidly evolving hepatitis C virus-related cirrhosis in a human immunodeficiency virus-infected patient receiving triple antiretroviral therapy. Clin Infect Dis 1998;27:1255-8.
192. Mastroianni CM, Trinchieri V, Santopadre P, Lichtner M, Forcina G, D'Agostino C, et al. Acute clinical hepatitis in an HIV-seropositive hepatitis B carrier receiving protease inhibitor therapy. AIDS 1998;12:1939-40.
193. John M, Flexman J, French MA. Hepatitis C virus-associated hepatitis following treatment of HIV-infected patients with HIV protease inhibitors: an immune restoration disease? AIDS 1998;12:2289-93.
194. Nigro L, Romano F, Tosto S, Zagami A, Bruno S, Nunnari A. Severe hepatitis in a HIV-positive subject under treatment with protease inhibitor. Ital J Gastroenterol Hepatol 1999;31:85-6.
195. Behrens GM, Meyer D, Stoll M, Schmidt RE. Immune reconstitution syndromes in human immunodeficiency virus infection following effective antiretroviral therapy. Immunobiology 2000;202:186-93.
196. Girmenia C, Martino P, Mazzucconi MG, Bizzoni L, Cassone A. HAART and Mycobacterium avium complex in an HIV infected patient with severe factor VII deficiency. Haemophilia 2000;6:116-7.
197. Tantisiriwat W, Tebas P, Clifford DB, Powderly WG, Fichtenbaum CJ. Progressive multifocal leukoencephalopathy in patients with AIDS receiving highly active antiretroviral therapy. Clin Infect Dis 1999;28:1152-4.
198. Heim M, Schapiro J, Wershavski M, Martinowitz U. Drug-induced and traumatic nail problems in the haemophilias. Haemophilia 2000;6:191-4.
199. Alam M, Scher RK. Indinavir-related recurrent paronychia and ingrown toenails. Cutis 1999;64:277-8.

200. Tosti A, Piraccini BM, D'Antuono A, Marzaduri S, Bettoli V. Paronychia associated with antiretroviral therapy. Br J Dermatol 1999;140:1165-8.
201. Dauden E, Pascual-Lopez M, Martinez-Garcia C, Garcia-Diez A. Paronychia and excess granulation tissue of the toes and finger in a patient treated with indinavir. Br J Dermatol 2000;142:1063-4.
202. Sass JO, Jakob-Solder B, Heitger A, Tzimas G, Sarcletti M. Paronychia with pyogenic granuloma in a child treated with indinavir: the retinoid-mediated side effect theory revisited. Dermatology 2000;200:40-2.
203. Di Martino V, Ezenfis J, Benhamou Y, Bernard B, Opolon P, Bricaire F, et al. Severe acute pancreatitis related to the use of nelfinavir in HIV infection: report of a case with positive rechallenge. AIDS 1999;13:1421-3.
204. Mirete G, Masia M, Gutierrez F, Mora A, Escolano C, Maestre A. Acute pancreatitis as a complication of ritonavir therapy in a patient with AIDS. Eur J Clin Microbiol Infect Dis 1998;17:810-1.
205. Perry RC, Cushing HE, Deeg MA, Prince MJ. Ritonavir, triglycerides, and pancreatitis. Clin Infect Dis 1999;28:161-2.
206. Sereti I, Sarlis NJ, Arioglu E, Turner ML, Mican JM. Alopecia universalis and Graves' disease in the setting of immune restoration after highly active antiretroviral therapy. AIDS 2001;15:138-40.
207. Sclar G. Carpal tunnel syndrome in HIV-1 patients: a metabolic consequence of protease inhibitor use? AIDS 2000;14:336-8.
208. Gilquin J, Viard JP, Jubault V, Sert C, Kazatchkine MD. Delayed occurrence of Graves' disease after immune restoration with HAART. Highly active antiretroviral therapy. Lancet 1998;352:1907-8.
209. Colebunders R, De Droogh E, Pelgrom Y, Depraetere K, De Jonghe P. Painful hyperaesthesia caused by protease inhibitors?. Infection 1998;26:250-1.
210. McNabb JC, Cappa JA, Ross JW. Disorders of iron metabolism associated with protease inhibitor therapy. Clin Infect Dis 2001;33:413-4.
211. Popp AI, Armstrong D, Sepkowitz KA. Recurrent panniculitis in a patient receiving protease inhibitor therapy for human immunodeficiency virus infection. Clin Infect Dis 1999;29:936-7.
212. Press N, Montessori V, Bai TR, Montaner J. Respiratory failure associated with the lipodystrophy syndrome in an HIV-positive patient with compromised lung function. Can Respir J 2001;8:279-82.
213. Herbort CP, Chave JP. Cicatrization of cytomegalovirus retinitis following introduction of highly active anti-retroviral therapy: uveitis as a possible indicator of good ocular prognosis. Graefes Arch Clin Exp Ophthalmol 1998;236:795-7.
214. Karavellas MP, Lowder CY, Macdonald C, Avila CP, Jr., Freeman WR. Immune recovery vitritis associated with inactive cytomegalovirus retinitis: a new syndrome. Arch Ophthalmol 1998;116:169-75.
215. Duval X, Larger E, Longuet P, Leport C, Vilde JL. Galactorrhoea, hyperprolactinaemia, and protease inhibitors. Lancet 2001;357:475.
216. Fox PA, Hawkins PA, Staughton RC. Cheilitis in association with indinavir. Sex Transm Infect 2000;76:323-4.
217. Race EM, Adelson-Mitty J, Kriegel GR, Barlam TF, Reimann KA, Letvin NL, et al. Focal mycobacterial lymphadenitis following initiation of protease-inhibitor therapy in patients with advanced HIV-1 disease. Lancet 1998;351:252-5.

218. Harry TC, Matthews M, Salvary I. Indinavir use: associated reversible hair loss and mood disturbance. Int J STD AIDS 2000;11:474-6.
219. Rachline A, Lariven S, Descamps V, Grossin M, Bouvet E. Leucocytoclastic vasculitis and indinavir. Br J Dermatol 2000;143:1112-3.
220. Fung HB, Pecini RA, Brown ST, Gropper CA. Indinavir-associated maculopapular eruption. Pharmacotherapy 1999;19:1328-30.
221. Fox PA, Boag FC, Hawkins DA, Francis N. Acute porphyria following commencement of indinavir. AIDS 1999;13:622-3.
222. Gariano RF, Cooney EL. Uveitis following administration of the protease inhibitor indinavir to a patient with AIDS. Clin Infect Dis 1997;24:529.
223. Grunke M, Kraetsch HG, Low P, Rascu A, Kalden JR, Harrer T. Nelfinavir associated with peripheral neuropathy in an HIV-infected patient. Infection 1998;26:252.
224. Arkel YS, Ku DH, Lake C, Pasquale L, Lam X, Gibson D, et al. A case of type 2B von Willebrand disease reverse to normal when treated with high doses of protease inhibitor. Thromb Haemost 1999;82:1558-9.
225. Hutter D, Akgun S, Ramamoorthy R, Dever LL. Medication bezoar and esophagitis in a patient with HIV infection receiving combination antiretroviral therapy. Am J Med 2000;108:684-5.
226. Miller KD, Piscitelli SC, Davey RT, Jr. Herpes zoster in an HIV-negative man on ritonavir. AIDS Patient Care STDS 2000;14:527-8.
227. Nielsen H. Hypermenorrhea associated with ritonavir. Lancet 1999;353:811-2.
228. Bachmeyer C, Blum L, Cordier F, Launay E, Danne O, Aractingi S, et al. Early ritonavir-induced maculopapular eruption. Dermatology 1997;195:301-2.
229. Saadat K, Kaminski HJ. Ritonavir-associated myasthenia gravis. Muscle Nerve 1998;21:680-1.
230. Mehlhaff DL, Stein DS. Gout secondary to ritonavir and didanosine. AIDS 1996;10:1744.
231. Weir A, Wansbrough-Jones M. Mucosal Kaposi's sarcoma following protease inhibitor therapy in an HIV-infected patient. AIDS 1997;11:1895-6.
232. Duval X, Peytavin G, Fouqueray B, Leport C, Vilde JL. Renin-angiotensin system inhibition in a patient having an overdose of HIV protease inhibitor. AIDS 1999;13:1983-4.
233. Finlayson JA, Laing RB. Acute paranoid reaction to saquinavir. Am J Health Syst Pharm 1998;55:2016-7.
234. Winter AJ, Pywell JM, Ilchyshyn JM, Fearn J, Natin D. Photosensitivity due to saquinavir. Genitourin Med 1997;73:323.

Chapter 6

Summary / samenvatting

Chapter 6.1

Summary

The accelerated registration procedures and abrupt introduction of the HIV-protease inhibitors, which constituted an entirely new generation of medicines, required post-marketing research more than usual. The objective of this thesis was to provide more insight into the safety of HIV-protease inhibitors.

Within this scope we examined the incidence of renal complications of the HIV-protease inhibitor indinavir as well as the risk factors and consequences in chapter 2.

In chapter 2.1 a case of papillary necrosis following an episode of indinavir-associated nephrolithiasis is described as a potential secondary complication of indinavir nephrotoxicity.

Chapter 2.2 describes plasma indinavir concentration of patients with urological complaints in a case series of 17 patients from the Erasmus university Medical Centre compared a reference pharmacokinetic curve of 14 patients without complaints. Plasma concentrations of patients with complaints appeared to be on average 2.6-fold higher than concentrations of reference patients. Hence, an association between urological complaints and plasma indinavir concentrations was confirmed.

In chapter 2.3 the incidence of urological symptoms during treatment with indinavir was determined for the Dutch treated patient population. Within the first year of treatment the cumulative incidence was 8.3%, which was twice as high as in clinical trials. Risk factors for developing urological symptoms were low lean body mass, low HIV-1 RNA, indinavir dosing regimens of $\geq$ 1000 mg bid, and high environmental temperatures all pointing at an indinavir solubility problem and consistent with a central role for plasma indinavir concentrations.

The subsequent study in chapter 2.4 clarified the location at which indinavir crystals might occur in the kidney given the knowledge that the problem underlying indinavir nephrotoxicity is a solubility problem. The loop of Henle was identified as the most likely site for indinavir crystallisation proximal to the collecting ducts where urine concentration is dependent on the hydration status of the body. As opposed to the areas beyond the distal tubuli, probably the most effective way of reducing urine indinavir saturation in the loop of Henle is dose adjustment. Based on *in vitro* indinavir solubility tests it was hypothesized that

plasma indinavir concentrations above 8 mg/mL can already induce the crystallisation process and therefore increase the hazard of nephrotoxicity.

Chapter 2.5 and 2.6 both report the results of an indinavir nephrotoxicity monitoring cohort. In this study, both prevalent and incident users of indinavir were systematically screened for signs of nephrotoxicity in urine and blood. Approximately, 25% of adult patients on indinavir had repeat sterile leukocyturia, which was attributed to indinavir since leukocytes disappeared upon indinavir discontinuation. Sterile leukocyturia was even more prevalent among children on indinavir, who had a cumulative incidence of 53% in 96 weeks. For both adults and children sterile leukocyturia remained largely asymptomatic whereas serum creatinine tended to increase indicating renal failure. Prevalence of sterile leukocyturia was associated with high plasma concentrations of indinavir one hour after ingestion (ie. > 9 mg/L). In conclusion, indinavir treated patients should be monitored systematically for presence of leukocyturia or serum creatinine elevation, even if urological symptoms are absent. With respect to nephrotoxicity management, indinavir dose adjustments may have to be considered.

The subject of the following section was lipodystrophy, a highly prevalent newly identified syndrome with a significant impact, which was believed to be an HIV-protease inhibitor effect. In the first chapter three cases of lipodystrophy are presented each exhibiting different aspects of lipodystrophy. Cases clearly demonstrate prominence of zygoma and veins due to subcutaneous fat loss, abdominal distension due to central fat accumulation and buffalo hump.

In chapter 3.2 the incidence of clinically evident lipodystrophy as diagnosed by treating physicians was determined in the treated HIV-infected patient population in the Netherlands. After four years of treatment 25% of patients had lipodystrophy the incidence of which was 11.7 per 100 patient years. In contrast to many earlier studies, HIV-protease inhibitors only appeared to play a minor part in the development of lipodystrophy. Stavudine was established as the most important risk factor.

Finally, the fourth chapter reports on the differential impact of treatment modifications on subsequent treatment continuity and the impact of intolerance on treatment continuity. First, patients who substituted nevirapine for their HIV-protease inhibitor while HIV-1 RNA was below 500 copies/mL showed sustained virological suppression and good tolerance (chapter 4.1).

In chapter 4.2 patients who switched to nevirapine while HIV-1 RNA was below 500 copies/mL were compared to patients who switched to second-line HIV-protease inhibitors under the same circumstances. Switching to nevirapine appeared to be three times more favorable mainly due to a substantially lower rate of subsequent switches. For patients who

achieved sufficient viral suppression on HIV-protease inhibitors, but experienced problems on this regimen, substitution by nevirapine seems to be a safe switching strategy.

The subsequent study in chapter 4.3 describes the rate of intolerance-driven treatment switches among patients who already underwent a switch during effective viral suppression. Within one year after the first switch, 24% had an intolerance-driven subsequent switch. Gastro-intestinal intolerance and neuropathy were the most important causes of such switches. Beyond the direct causes of intolerance, the most important risk factor for an intolerance-driven switch was having had a previous intolerance-driven switch. Switches to nevirapine were the most favorable. Whether switching to other non-nucleoside reverse transcriptase inhibitors is equally beneficial remains to be investigated.

Shortcomings of the studies in this thesis are addressed in the general discussion in chapter 5 and recommendations for further research are given.

Chapter 6.2

Samenvatting

De versnelde registratie procedures en abrupte introductie van de HIV-proteaseremmers legde een extra grote verantwoordelijkheid bij het zogenaamde fase IV ofwel 'post-marketing' onderzoek. Het was belangrijk om meer informatie te verkrijgen over de effecten van deze, toentertijd, nieuwe generatie geneesmiddelen, teneinde de toepassing ervan te optimaliseren. Het doel van dit proefschrift was om meer inzicht te krijgen in de veiligheid van de HIV-proteaseremmers.

In het kader van dit doel onderzochten we in hoofdstuk 2 de het voorkomen van niercomplicaties bij behandeling met indinavir, alsook de mogelijke risicofactoren en gevolgen.

In hoofdstuk 2.1 wordt allereerst melding gemaakt van papilnecrose na een aan indinavir toegeschreven niersteenaanval.

Vervolgens worden in hoofdstuk 2.2 de concentraties van indinavir in het plasma van 17 patiënten met urologische klachten vergeleken met een controlecurve. Deze controlecurve werd gemaakt aan de hand van het verloop van de concentraties tussen tween indinavir innames bij 14 patiënten zonder urologische klachten. Plasmaconcentraties van patiënten met klachten bleken gemiddeld 2.6 keer hoger te zijn dan die van de controlegroep.

In hoofdstuk 2.3 wordt het voorkomen van klachten aan de urinewegen in de gehele Nederlandse populatie van patiënten op indinavir beschreven. Binnen een jaar na starten van de behandeling had 8.3% van de patiënten tenminste één keer last gehad van urologische problemen, hetgeen twee keer hoger is dan cijfers uit klinische onderzoek voor registratie. Urologische klachten kwamen significant vaker voor bij een lage vetvrije lichaamsmassa, een lage hoeveelheid virus in het bloed, bij een hoge dosering van indinavir en bij hoge buitentemperaturen. Deze risicofactoren passen bij het feit dat indinavir gemakkelijk kristalliseert. Het bevestigt de eerdere bevinding dat indinavirconcentraties een belangrijke rol spelen bij het ontwikkelen van klachten aan de urinewegen.

In het volgende hoofdstuk onderzochten we op welke plaats in de nieren indinavirkristallen als eerste ontstaan. De lis van Henle, een onderdeel van de niertubuli, werd hiervoor de meest aannemelijke plek bevonden. Hierin gaat een groot concentrerend

vermogen samen met een hoge pH, waardoor indinavir kristallen kan gaan vormen. Op deze plek, die voor de verzamelbuizen ligt, is de concentratie van de urine nog onafhankelijk van de vochtbalans van het lichaam en zal het verhogen van de vochtinname weinig effect hebben. Om de verzadiging van indinavir in de urine in de lis van Henle te bestrijden is een dosisaanpassing waarschijnlijk het meest effectief. Op basis van *in vitro* oplosbaarheidstesten met indinavir werd geschat dat wanneer de concentraties in plasma boven 8 mg/mL uitkomen, het kristallisatieproces al wordt geïnduceerd en dat daarmee de kans op niercomplicaties toeneemt.

In hoofdstukken 2.5 en 2.6 worden resultaten gepresenteerd van systematische monitoring van tekenen van nefrotoxiciteit in urine en bloed van respectievelijk volwassenen en kinderen, die behandeld worden met indinavir. Hierin werden zowel patiënten, die al indinavir gebruikten, als nieuwe gebruikers onderzocht. Bijna 25% van de volwassen patiënten op indinavir bleek bij herhaling leukocyten in de urine te hebben zonder de aanwezigheid van infectie. De leukocyten verdwenen onmiddellijk bij het stopzetten van de behandeling. Bij kinderen kwam deze afwijking nog vaker voor. Na twee jaar behandeling had 53% van de kinderen één of meer keren leukocyten in de urine gehad. Hoewel het hebben van leukocyten in de urine vaak niet gepaard ging met fysieke klachten, bleek de nierfunctie van zowel kinderen als volwassenen met deze afwijking achteruit te gaan. Ook het voorkomen van leukocyten de urine bleek samen te hangen met hoge concentraties indinavir in het plasma. Concluderend werd gesteld dat patiënten die worden behandeld met indinavir systematisch dienen te worden onderzocht op leukocyten in de urine en dat hun serum creatinine (een nierfunctietest) regelmatig dient te worden bepaald, onafhankelijk van de aanwezigheid van klachten aan de urinewegen. Dosisaanpassingen zouden overwogen kunnen worden bij het bestrijden van deze vorm van nefrotoxiciteit door indinavir.

Hoofdstuk 3 handelt over lipodystrofie, een zeer frequent voorkomende aandoening in de vetverdeling van het lichaam, die werd toebedeeld aan HIV-proteaseremmers. Eerst worden drie ziektegeschiedenissen beschreven van patiënten met lipodystrofie, die elk verschillende aspecten van het syndroom vertonen. Patiënten vertonen sterke vaattekening als gevolg van onderhuids vetverlies, toegenomen buikomvang door centrale vetophoping en kunnen een zogenaamde 'buffalo hump' ontwikkelen.

In hoofdstuk 3.2 bepaalden we de frequentie van voorkomen van klinisch evidente lipodystrofie, gediagnostiseerd door de behandelend arts, in de Nederlandse behandelde HIV-geïnfecteerde patiëntenpopulatie. Na vier jaar behandeling had 25% van de patiënten lipodystrofie ontwikkeld wat neerkomt op 11.7 gevallen per 100 patient-jaren. In tegenstelling tot vele eerdere onderzoeken bleken HIV-proteaseremmers slechts een

geringe rol te spelen bij het ontstaan van lipodystrofie. Het gebruik van stavudine, een (nuclesoide analoge) reverse transcriptaseremmer, bleek de kans op het krijgen van lipodystrofie het sterkst te bepalen.

Het vierde hoofdstuk beschrijft de effecten van therapiewijzigingen en de effecten van het niet kunnen verdragen van de medicatie op het verloop van verdere behandeling. Eerst wordt een reeks patiënten beschreven, die hun HIV-proteaseremmer lieten vervangen door nevirapine, terwijl de hoeveelheid virus (HIV-RNA) onder de detectiegrens (500 kopiëen/mL) lag. Zij vertoonden aanhoudende virusonderdrukking en konden de nieuwe behandeling goed verdragen (hoofdstuk 4.1).

In hoofdstuk 4.2 wordt een vergelijking gemaakt tussen patiënten, die hun HIV-proteaseremmer vervingen door de (non-nucleoside analoge) reverse transcriptase remmer nevirapine en patiënten, die hun HIV-proteaseremmer vervingen door een nieuwe HIV-proteaseremmer. Hierbij vond de wijzinging plaats tijdens virussuppressie tot onder de standaard detectiegrens van 500 HIV-RNA kopiëen/mL in het bloed. Het overgaan naar nevirapine bleek in deze gevallen drie keer gunstiger, met name doordat er substantieel minder therapiewijzigingen optraden in de periode erna. Concluderend werd gesteld dat voor patiënten met virusonderdrukking op HIV-proteaseremmers, die de behandeling dreigen niet vol te kunnen houden, lijkt het omzetten van de HIV-proteaseremmer in nevirapine een veilig alternatief.

Het volgende onderzoek in hoofdstuk 4.3 beschrijft de frequentie van voorkomen van wijzigingen in de behandeling als gevolg van bijwerkingen. Binnen een jaar na de eerste wijziging had 24% van de patiënten een dergelijke wijziging vanwege bijwerkingen ondergaan. Gastro-intestinale klachten en neuropathie waren de belangrijkste oorzaken hiervoor. Het bleek dat wanneer de HIV-proteaseremmer werd veranderd in nevirapine, het daaropvolgend beloop het gunstigst was. Of veranderingen naar andere vergelijkbare HIV-remmers, zoals bijvoorbeeld efavirenz, even gunstig is kon niet worden onderzocht doordat deze ten tijde van het onderzoek nog niet voorhanden waren.

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About the author

Jeanne Dieleman was born in Nijmegen, the Netherlands, on June 6th, 1968. She graduated from the Anton van Duinkerken college in Veldhoven in 1986. In 1991 she obtained her Master of Science degree in Biomedical Sciences at the Faculty of Medicine of Leiden University. Subsequently, she worked for one year as a Clinical Research Associate (CRA) for EuroCetus BV (Chiron) in Amsterdam and for two and a half years as a CRA and senior CRA for ICON Clinical Research Ltd., Twyford, Winchester, United Kingdom. In 1997 she started the work described in this thesis at the Department of Internal Medicine of the Erasmus MC in Rotterdam. During this period she obtained a Master of Science degree in Epidemiology from the Netherlands Institute for Health Sciences (NIHES) in Rotterdam. She will continue working as a pharmaco-epidemiologist at the Departments of Medical Informatics and Internal Medicine of the Erasmus MC.

List of Publications

Dieleman JP, in 't Veld B, Borleffs JC, Schreij G. Acute respiratory failure associated with the human immunodeficiency virus (HIV) protease inhibitor indinavir in an HIV-infected patient. Clin Infect Dis 1998;26:1012-3.

Dieleman JP, Hillebrand-Haverkort ME, van der Ende ME, Sturkenboom MC, Lange JM, Stricker BH. [Lipodystrophy and 'buffalo hump' during treatment with HIV protease inhibitors]. Lipodystrofie en 'buffalo hump' bij de behandeling met HIV-proteaseremmers. Ned Tijdschr Geneeskd 1998;142:2856-60.

Dieleman JP, Gyssens IC, van der Ende ME, de Marie S, Burger DM. Urological complaints in relation to indinavir plasma concentrations in HIV-infected patients. AIDS 1999;13:473-8.

Dieleman JP, Gyssens IC, Sturkenboom MCJM, Niesters HGM, Ende MEv. Substituting nevirapine for protease inhibitor because of intolerance. AIDS 1999;13:1423-4.

Burger DM, Hugen PW, Aarnoutse RE, Dieleman JP, Prins JM, van Der Poll T, et al. A Retrospective, Cohort-Based Survey of Patients Using Twice-Daily Indinavir + Ritonavir Combinations: Pharmacokinetics, Safety, and Efficacy. J Acquir Immune Defic Syndr 2001;26:218-24.

Bijl M, Dieleman JP, Simoons M, van der Ende ME. Low prevalence of cardiac abnormalities in an HIV-seropositive population on antiretroviral combination therapy. J Acquir Immune Defic Syndr 2001;27:318-20.

Dieleman JP, Salahuddin S, Hsu YS, Burger DM, Gyssens IC, Sturkenboom MC, et al. Indinavir Crystallization Around the Loop of Henle: Experimental Evidence. J Acquir Immune Defic Syndr 2001;28:9-13.

Dieleman JP, van der Feltz M, Bangma CH, Stricker BH, van der Ende ME. Papillary necrosis associated with the HIV protease inhibitor indinavir. Infection 2001;29:232-3.

Salahuddin S, Hsu YS, Buchholz NP, Dieleman JP, Gyssens IC, Kok DJ. Is indinavir crystalluria an indicator for indinavir stone formation? AIDS 2001;15:1079-80.

van Rossum AM, Dieleman JP, Fraaij PL, Cransberg K, Hartwig NG, Gyssens IC, et al. Indinavir-associated asymptomatic nephrolithiasis and renal cortex atrophy in two HIV-1 infected children. AIDS 2001;15:1745-7.

Dieleman JP, Jambroes M, Gyssens IC, Sturkenboom MCJM, Stricker BHCh, Mulder WMC, de Wolf F, Weverling GJ, Lange JMA, Reiss P, Brinkman K, on behalf of the ATHENA Study Group. Determinants of recurrent toxicity-driven switches of highly active antiretroviral therapy. The ATHENA Cohort. AIDS 2002;16:737-45.

Dieleman JP, Sturkenboom MCJM, Jambroes M, Gyssens IC, Weverling GJ, ten Veen JH, Schrey G, Reiss P, Stricker BHCh, on behalf of the ATHENA-study group. Risk factors for urological symptoms in a cohort of users of HIV-protease inhibitor indinavir. The ATHENA-cohort. Arch Intern Med, in press.

van Rossum AMC, Dieleman JP, Fraaij PLA, Cransberg K, Hartwig NG, Burger DM, Gyssens IC, de Groot R. Persistent sterile leukocyturia is associated with impaired renal function in HIV-1-infected children treated with indinavir. Pediatrics, in press.

Dieleman JP, Sturkenboom MCJM, Wit FW, Jambroes M, Mulder JW, ten Veen JH, Juttmann J, Stricker BHCh, Lange JMA, van der Ende ME, on behalf of the ATHENA study group. Low risk of treatment failure one year after substituting nevirapine for human immunodeficiency virus (HIV)-protease inhibitors among HIV-infected patients with viral suppression in a real life setting. The ATHENA cohort. J Infect Dis, in press.