Clinical and pharmacological aspects of induction-maintenance therapy in HIV-1 positive patients: the ADAM study

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The ADAM study

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Chapter 1

Introduction.

The lentiviruses, or human immunodeficiency viruses types 1 and 2 (HIV-1 and HIV-2, respectively), are the causative agents of the acquired immunodeficiency syndrome (AIDS).[1-3] Of these two, HIV-1 is the more predominant and the more aggressive.[4,5] The development of antiretroviral drugs has been focussed exclusively on HIV-1, yet a number of drugs that are active against HIV-1 are also active against HIV-2.[5,6]

The first antiretroviral agent available to treat HIV-1 infection was zidovudine (3'-azido-3'-deoxythymidine), a nucleoside analogue reverse transcriptase (RT) inhibitor.[7] This class of agents needs to be intracellularly metabolised to an active triphosphate, before it can exert its antiretroviral activity. The active triphosphate competes with the natural nucleotide, for incorporation into the growing viral DNA chain by HIV-1 reverse transcriptase, resulting in termination of DNA-chain elongation.[8] In 1987, the use of zidovudine in persons with advanced HIV-1 infection was found to result in a decline of HIV-1 related morbidity and mortality.[9] By 1990, the indication for treatment with zidovudine was extended to patients with less advanced disease.[10,11] In the following years, more nucleoside analogue RT-inhibitors such as didanosine and zalcitabine, became available for the use in HIV-1 infected patients.[12-15] However, the increase in $CD4^+$ T-cells and the clinical benefit of monotherapy was only transient, both in early and late HIV-1 disease.[16-18] In addition to smaller clinical trials indicating virological and immunological benefit of dual therapy over monotherapy,[19-25] two large studies reported in 1995 superior clinical outcome in patients treated with dual therapy.[26,27]

Already from 1990 on, the use of another class of agents, the non-nucleoside RT inhibitors (such as the TIBO-derivates, nevirapine, pyridinones and delavirdine), was explored for the treatment of HIV-1 infection.[28-31] These highly potent agents , directly inhibit the viral reverse transcriptase by binding to it non-competitively.[32] However, a rapid emergence of mutant virus was observed in both in vitro- and phase I/II studies, which was associated with a loss of antiretroviral activity.[30,33-36] This rapid appearance of drug resistance delayed further development of these agents for the use in clinical practice.[30]

A few years later, another class of agents was introduced: the protease inhibitors.[37-39] These agents inhibit viral replication by targeting a different viral protein than the nucleoside analogue and non-nucleoside RT inhibitors. The protease inhibitors, for example saquinavir, indinavir, ritonavir and nelfinavir, bind to a specific site of the HIV-1 protease enzyme, thus inhibiting the production of essential structural and enzymatic components of HIV-1, rendering the virus non-

infectious.[40] Just as the non-nucleoside RT inhibitors, these agents proved to be potent inhibitors of viral replication. However, with the use of these agents in monotherapy or in addition to a failing combination, the development of drug resistance was again reducing antiviral efficacy. Here, the protease inhibitors are however in advantage compared to the non-nucleoside RT inhibitors since a sequential appearance of multiple mutations is required for a decreased drug susceptibility of the virus.[33,41] The use of protease inhibitors in antiretroviral therapy was approved from 1995 on. It lasted till 1996, when both the protease inhibitors and the non-nucleoside RT inhibitors proved their value in triple drug combination therapies, before the non-nucleosides were approved for the use in antiretroviral treatment of HIV-1 infected patients as well.

In the same period that these drugs were evaluated, the assessment of HIV-1 RNA in plasma in addition to the $CD4^+$T-cell count proved to be an important parameter for the progression of HIV-1 disease and treatment response.[42,43] With the availability of this parameter, the efficacy of antiretroviral drug regimens could be evaluated more directly than before.[44]

The limited efficacy of mono- and dual therapies was associated with the appearance of drug resistant viral mutants.[45-47] The rapid replication rate of HIV-1 is resulting in large amounts of virus produced on a daily basis (10^{10} virus particles per day).[48] In addition, the mutation rate of HIV-1 per replication cycle is estimated to be 3.4×10^{-5} mutations per nucleotide.[49] So, with incomplete suppression of viral replication, the occurrence of a point mutation in the HIV-1 genome is quite probable. If, in the presence of an antiretroviral drug, the wild-type virus is less fit than the mutant virus, the latter will eventually predominate.[43,48-50] Since the high replication rate and not the rate of mutation appeared the predominant factor in the development of the variety of mutant virus,[50] a significant reduction of the replication rate by the concomitant use of at least three antiretroviral drugs was suggested to be profitable. Moreover, in the presence of non-cross-resistant agents, the virus would have to mutate simultaneously at several positions in the viral genome to become resistant to the antiretroviral drugs used. A rough approximation of the probability of three simultaneous, random mutations in a viral genome per replication cycle would be 3.9×10^{-10}, with a viral genome consisting of 10^4 nucleotides. This is a very low probability in comparison to a probability of 0.34 if only one mutated nucleotide would suffice for decreased drug-susceptibility of the virus.

Indeed, the virological and immunological efficacy of a three-drug regimen consisting of two nucleoside analogue RT inhibitors with one protease inhibitor or one non-nucleoside RT inhibitor appeared to be superior to mono- or dual therapy regimens.[50-52] Subsequently, it was confirmed that the substantial and durable suppression of viral replication and a lasting rise in $CD4^+$ T cell count observed with the use of triple drug combination therapies, resulted in a greater decrease in morbidity and mortality than during mono- and dual therapy regimens.[51,53-55] In the late nineties, when triple drug combination therapy became standard of care in the Western world, the decrease of HIV-1 related morbidity and mortality has been impressive.[53,56-58]

There are however limitations to the triple drug combination therapies for HIV-1 infection. These multi-drug regimens are more effective but are also more complex and less well tolerated than monotherapy. The daily pill burden of triple combination regimens may be large, and require rigid time schedules and in some cases dietary restrictions. In addition, toxicity frequently occurs and is a reason for discontinuation of the medication in many patients.[59-61] Considering that adherence to medication is a prerequisite for a durable suppression of viral replication,[51] these features of multi-drug regimens in HIV-1 infection are a major drawback to the efficacy of triple drug combinations.[62] Poor compliance and toxicity are not the only factors leading to treatment failure. In cohort studies evaluating the efficacy of triple drug combination regimens, treatment failure appears to be multifactorial. The initial plasma HIV-1 RNA concentration and the $CD4^+$ T-cell count appeared to be predictive for treatment failure in observational cohorts.[59,63,64] Patients with a low HIV-1 RNA concentration or a high $CD4^+$ T-cell count at the start of therapy are more likely to have a virological response in which the plasma HIV-1 RNA concentration declines below the quantification limit of the available assays and is maintained at that level during therapy. In addition, the nadir of the plasma HIV-1 RNA concentration achieved, is a predictor for long-term virological outcome.[65,66] Another factor of importance to drug failure, is the prevalence of drug resistant mutant viruses in pre-treated patients, reducing the chances of virological success of subsequent regimens.[67-69] Furthermore, the pharmacokinetic characteristics of a drug, such as low bio-availability or unfavourable drug interactions, may contribute to treatment failure.[63,70] For example, in observational cohort studies, the use of saquinavir in the hard gelatin capsule formulation, known for its relatively poor bio-availability, was a predictor for treatment failure.[63,71] Considering the several risk factors for treatment failure, it is not surprising, that a considerable proportion of the

patients using a triple drug combination regimen are found to have virological treatment failure.[59] To at least improve compliance and minimise intolerance, more simplified, tolerable treatment regimens are a necessity; however, not at the cost of virological efficacy.

In other chronic infectious diseases, like tuberculosis, the concept of induction-maintenance regimens has proven to be useful in reducing long-term toxicity and improving adherence to therapy.[72] This concept implicates a vigorous treatment of the infection in the first phase, quickly reducing the total load of the infectious agent in the body, and reducing the risk of the emergence of drug resistant mutants. Afterwards, a simpler regimen may suffice for the suppression of the residual microbial load, in which the risk of resistance development is low. The analogy between HIV-1 infection and tuberculosis is only limited. Nevertheless, some observations in antiretroviral therapy trials have supported the idea that induction-maintenance should be feasible for HIV-1 infection. In the Incas study, for example, five antiretroviral-naive patients who had undetectable plasma HIV-1 RNA concentrations using a combination of zidovudine, didanosine and nevirapine, discontinued didanosine, violating the study protocol. In some of these patients sustained suppression of viral replication for more than one year was described during the period of didanosine interruption.[73] In addition, triple drug regimens had shown to rapidly reduce HIV-1 RNA concentrations in blood, lymphoid tissues and cerebrospinal fluid to levels below the detection limit of the available assays in antiretroviral naive patients.[74-77] Together with the observation, that prolonged suppression of viral replication by two agents is more likely in case of a lower rather than a higher baseline viral load,[69,78,79] it seemed worthwhile to investigate the induction-maintenance concept. For this purpose the Amsterdam Duration of Antiretroviral Medication (ADAM) study was designed.

The induction-maintenance concept was tested in antiretroviral naive HIV-1 positive patients, to avoid the possibility of the presence of drug resistant viral mutants, which are less susceptible for the maintenance regimens used.[67,69,80] In the induction phase, a quadruple drug regimen consisting of two nucleoside analogue RT inhibitors (stavudine and lamivudine) and two protease inhibitors (nelfinavir and saquinavir) was used for 26 or 50 weeks. The randomisation to one of the two maintenance regimens or prolongation of the quadruple drug regimen at both week 26 and 50 was restricted to patients with a plasma HIV-1 RNA concentration below the detection limit of an ultrasensitive assay. Maintenance therapy consisted of

stavudine plus nelfinavir or nelfinavir plus saquinavir. A dual nucleoside analogue RT-inhibitor regimen was not used, since generally the intracellular phosphorylation required for intracellular activity of the nucleoside analogue RT inhibitors, is not equally efficient in all infected cells.[81] Van 't Wout et al. found differences in suppression of replication of viruses with different cellular tropism, which is in line with the observed differences in phosphorylation in activated and non-activated cells.[82] The contents of Chapter 2 and 3 address the actual feasibility of the induction-maintenance therapy used in the ADAM study.

Although a favourable pharmacokinetic interaction between nelfinavir and saquinavir was anticipated, the exact pharmacokinetic interaction in the quadruple drug regimen was not known at the time of the study design. Therefore, a full (8-hour) concentration curve of the protease inhibitor combination was assessed in a subset of the patients. The steady state pharmacokinetics of the combination of saquinavir and nelfinavir in the quadruple drug induction regimen are described in Chapter 4. In the Chapters 5 and 6, the efficacy of the induction regimen within the first four weeks of induction therapy and the toxicity of the induction therapy within the first 26 weeks are discussed in relation to the exposure to the used protease inhibitors.

Chapter 7 and 8 are focussing on the sub-studies within the ADAM study. Patients could participate in a quality of life and compliance study. In case of equal virological and immmunological efficacy, it is of interest to find out whether the induction-maintenance strategy adds to the quality of life or facilitates compliance compared to standard treatment strategies. The impact of induction-maintenance therapy on quality of life is highlighted in Chapter 7. Chapter 8 describes the presence of drugs in the cerebrospinal fluid and semen of a subset of the patients participating in the ADAM study. The limited penetration of antiretroviral agents in the central nervous system and the genital tract might contribute to the presence of an anatomical reservoir of HIV-1 in these tissues,[83,84] which could facilitate virological failure during the maintenance phase. As a measure for the drug penetration into these compartments, drug concentrations in the cerebrospinal fluid and semen were assessed in a proportion of the patients. In Chapter 9 the concentrations of stavudine in the plasma and cerebrospinal fluid of patients on quadruple induction therapy are compared with those found in patients using other drug combination regimens.

In the general discussion, Chapter 10, a perspective of induction-maintenance strategies in the current treatment of HIV-1 infected patients is outlined.

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Chapter 2

Maintenance therapy after quadruple induction therapy in HIV-1 infected individuals: Amsterdam Duration of Antiretroviral Medication (ADAM) study.

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Abstract

Background

Highly active antiretroviral therapy (HAART) has led to health benefits for patients infected with HIV-1. However, long-term use of multidrug regimens is difficult to sustain. Simplifying antiretroviral treatment regimens would increase patients' adherence and minimise toxicity. We investigated the feasibility of a strategy of induction therapy followed by maintenance therapy with HAART in a randomised open-label study.

Methods

From March, 1997, we enrolled patients infected with HIV-1 with at least 200 CD4 cells/μL, at least 1000 HIV-1 RNA copies/mL in plasma, and no previous exposure to antiretroviral drugs. After 26 weeks of induction therapy (stavudine, lamivudine, saquinavir, and nelfinavir) patients were randomly allocated maintenance therapy (either stavudine and nelfinavir or saquinavir and nelfinavir) or prolonged induction therapy (if the plasma HIV-1 RNA concentration at weeks 24 and 25 was < 50 copies/mL).

Findings

In February, 1998, we discontinued randomisation after an interim analysis. 62 patients had been enrolled. 39 (91%) of the 43 patients who were followed up for at least 26 weeks had an undetectable plasma HIV-1 RNA concentration at week 16. At week 26, 31 patients were randomly allocated treatment. Of these patients 25 had a total follow-up of at least 36 weeks. At week 36, a higher proportion of patients on maintenance therapy (nine (64%) of 14) had a detectable HIV-1 RNA than patients on prolonged induction therapy (one (9%) of 11, p=0.01). The initial virion-clearance rate during induction therapy was higher in five patients on maintenance therapy with a sustained undetectable plasma HIV-1 RNA concentration than in nine patients with recurrence of a detectable plasma HIV-1 RNA concentration at week 36 (0.35 vs 0.19 per day, respectively; p=0.008).

Interpretation

The induction regimen provided a rapid suppression of viral replication to below 50 copies/mL. However, suppression was not sustained in a considerable number of patients who went onto maintenance therapy. It is currently inadvisable to continue attempts at moving from induction to maintenance therapy in day-to-day practice.

Introduction

With a combination of three or more antiretroviral agents, a durable suppression of viral replication in HIV-1 infection can be achieved.[1-3] This highly active antiretroviral therapy (HAART) has resulted in clinical benefit in terms of prolonged disease-free survival.[4,5] However, for sustained clinical benefit, treatment needs to be used for many years, probably for life. The daily burden of taking the pills involved in triple or quadruple antiretroviral regimens is large. Also, a rigid time schedule with complicated dietary prescriptions may interfere with the patient's daily activities. Even in the knowledge of suffering from a life-threatening disease, strict adherence to therapy is difficult for many patients.[6] Unfortunately, adherence is critical for a durable suppression of viral replication, which itself is a prerequisite of avoidance of the development of viral drug resistance.[3,7] Toxicity, such as lipodystrophy, may also restrict the patient in the chronic use of HAART.[8]

However, HAART has been shown to reduce rapidly the viral burden of plasma, lymphatic tissue, and cerebrospinal fluid,[9-12] suggesting that in the near absence of viral replication, maintenance therapy with less antiretroviral agents might be feasible.

In this, the Amsterdam Duration of Antiretroviral Medication (ADAM) study, we explore the feasibility of HAART with a strategy of induction therapy and then maintenance therapy using a quadruple induction regimen followed by maintenance therapy with two drugs. For the induction phase, we added another protease inhibitor to a standard triple regimen to potentially increase the antiviral efficacy. Nelfinavir increases the oral bioavailability of saquinavir about five fold.[13] For the choice of the maintenance therapy, we considered the combination of two nucleoside reverse transcriptase inhibitors less attractive since antiretroviral activity of the various nucleoside reverse transcriptase inhibitors is highly dependent on cellular enzymes, and differs among cell types.[14] Given the lack of experience with maintenance therapy, we decided to study an approach with a combination of a reverse-transcriptase inhibitor and a protease inhibitor versus a double protease-inhibitor combination. Here we present the preliminary results of maintenance therapy after 6 months' induction therapy.

Methods

Patients

The enrolment of HIV-1 infected patients in this open-label randomised controlled study started in March, 1997, and was ended prematurely on April 6, 1998 (see below). Patients, aged 18 years or more, were eligible if they had at least 200 CD4 cells/μL in peripheral blood, 1000 or more HIV-1 RNA copies/mL in plasma, and if they were antiretroviral naïve. Exclusion criteria were the existence of an active opportunistic infection, active hepatitis C or presence of the hepatitis B surface antigen, breastfeeding or pregnancy, and the use of immunomodulatory drugs or investigational drugs up to 1 month before the start of the study medication. Some haematological signs were also exclusion criteria: haemoglobin of less than 7 mmol/L for men or less than 6.5 mmol/L for women; neutropenia of less than 0.75×10^9/L; aspartate or alanine amino transferase of more than five times upper limit of normal; and serum creatinine of more than 1.5 times upper limit of normal. The study was approved by the institutional review boards of all participating institutions. Informed consent was obtained from all participants.

Study design

All patients started the induction phase with a quadruple therapy consisting of stavudine (d4T, 40 mg twice a day, or 30 mg twice a day if body weight <60 kg), lamivudine (3TC, 150 mg twice a day), saquinavir hard gelatin capsules (saquinavir-HGC, 600 mg three times a day) and nelfinavir (750 mg three times a day, figure 1). When saquinavir soft gelatin capsules (SGC) became available (Nov 1, 1997), all patients on saquinavir-HGC 1800 mg daily switched to saquinavir-SGC 2400 mg daily. Patients were told to take their medication with food.

At week 26, patients with a plasma HIV-1 RNA level below the detection limit of an ultrasense assay (<50 copies/mL) at both week 24 and 25 were randomly allocated prolonged induction therapy or a maintenance regimen: stavudine plus nelfinavir or saquinavir plus nelfinavir. Patients were allocated treatment by a computerised minimisation program, weighting imbalance of allocations according to the CD4 cell count (more or less than 400 cells/μL) and HIV-1 RNA (more or less than 50,000 copies/mL) at screening. Treatment allocation was done in a 2:1:1 ratio of prolonged induction: stavudine plus nelfinavir or saquinavir plus nelfinavir, respectively.

Follow-up

Patients were scheduled to visit the outpatient clinic for clinical assessment and routine laboratory monitoring at the start of treatment and weeks 1, 2, 4, 8, 16, 24, 25 (only plasma HIV-1 RNA concentration assessment) and 26. After randomisation, follow-up of patients on maintenance therapy was scheduled at weeks 27, 28, 32 (only plasma HIV-1 RNA concentration assessment for each follow-up), 36, 48, 60, 72, 84, and 96. Follow-up for patients on prolonged induction therapy was done at weeks 36, 48, 60, 72, 84, and 96. Laboratory monitoring included plasma HIV-1 RNA concentration, and CD4 and CD8 cell count.

HIV-1 RNA plasma concentrations were measured with NASBA and NucliSens HIV-1 RNA QT assays (Organon Teknika, Boxtel, Netherlands). When concentrations declined to less than 400 copies/mL, an ultrasensitive protocol with a quantification limit of 50 copies/mL was used. At week 26 and during further follow-up, the ultrasensitive procedure of the Roche Amplicor assay (Roche Diagnostic Systems, Branchburg, New Jersey, USA) with a variable quantification limit was used (median quantification limit of 24 assays done in plasma: 27 (range 14-79) copies/mL).

After attaining a plasma HIV-1 RNA concentration below the quantification limit of the ultrasense assay (<50 copies/mL), a plasma HIV-1 RNA level above 400 copies/mL at two consecutive occasions was originally described in the protocol as a treatment failure. In case of grade 4 toxicity[15] or grade 3 toxicity with no improvement after temporary discontinuation (maximum 2 weeks), or recurrence of grade 3 toxicity after rechallenge, permanent discontinuation of the study medication was obligatory. After treatment failure or discontinuation of the study medication, further therapy was given at the discretion of the investigator.

Protocol amendment and stopping of randomisation

Results of the TRILÈGE[16] and ACTG 343[17] trials regarding induction therapy then maintenance therapy presented at the fifth National Conference of Retroviruses, led to a premature analysis of the ADAM study. Subsequently, the results from this analysis and the above mentioned trials led to an amendment of the protocol and discontinuation of randomisation at week 26 in February, 1988. During this process, the primary endpoint of the study was changed to a detectable HIV-1 RNA copy number with the ultrasensitive assay at a time beyond 26 weeks of induction therapy. What at first seemed a trivial level of viral escape has proven to be relevant. In several studies[18,19] it has been shown that maintaining viral replication levels

below the detection limit of ultrasensitive assays (20-50 copies/mL plasma) is essential for a durable effect of therapy.

Patients with detectable HIV-1 RNA concentrations of more than 100 copies/mL or more than 400 copies/mL during maintenance therapy on two consecutive occasions were advised to continue with the original quadruple regimen or advised change their therapy to three completely different antiretroviral agents, respectively.

Data analysis

For all patients with a follow-up for at least 26 weeks, plasma HIV-1 RNA concentrations, change in CD4 and CD8 cell count, and occurrence of adverse events during the first 26 weeks of treatment were recorded. The proportion of patients with a plasma HIV-1 RNA level below 50 copies/mL was calculated at each time.

The ability to detect HIV-1 RNA in plasma in the group who had induction therapy followed by maintenance therapy was analysed at week 36. Subsequently, patients on maintenance therapy with and without a detectable plasma concentration of HIV-1 RNA at week 36 were compared for baseline characteristics, the time to an undetectable plasma HIV-1 RNA concentration, the change in CD4 and CD8 cell count, and the initial virion-clearance rate constant in plasma during induction treatment. Statistical comparisons were based on Wilcoxon's rank-sum test.

To calculate the virion-clearance rate constant in plasma an exponential function was used to describe the rate of HIV-1 RNA decline during the first two weeks for each patient. The following function was used to describe the decline of the HIV-1 RNA concentration in plasma (first-order elimination):[20]

$$V_{(t)} = V_{(0)} * e^{(-k*t)}$$

$V_{(t)}$ represents plasma HIV-1 RNA in copies/mL at time t; $V_{(0)}$ represents the baseline plasma HIV-1 RNA concentration; k is the elimination-rate constant (per day), and t is the number of days after the start of treatment. All HIV-1 RNA measurements of 50 copies/mL or more, were used for each patient from the start of treatment until a value of less than 50 copies/mL was reached or until a maximum of 16 days. For each patient the value for k was estimated by least-squares regression analysis ($\ln V_{(t)}$ vs t).

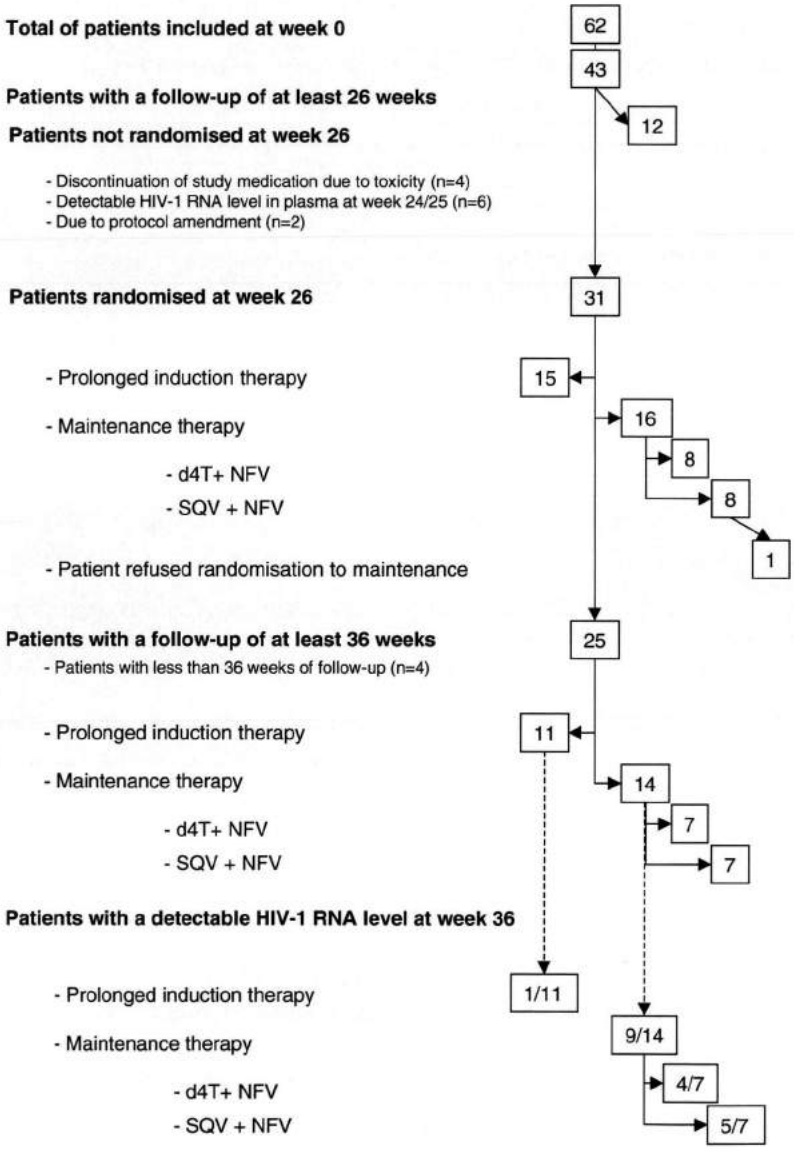

Figure 1 Trial profile.

Results

Patients

A total of 62 patients (59 men, 95%) were enrolled before we stopped randomisation at week 26 (figure 1). The baseline characteristics of 43 patients who were followed up for at least 26 weeks are summarised in table 1. Four of these patients stopped taking the study drugs because of adverse events.

Table 1 Baseline characteristics

Characteristic		Induction therapy[a]	Prolonged Induction therapy[b]	Maintenance therapy[b]
Number of patients		43	11	14
Age, Yrs, mean (SD)		40 (8)	43 (6)	39 (10)
Male, N, (%)		40 (93%)	11 (100%)	12 (86%)
CDC [c]	A	26 (60%)	7 (64%)	9 (65%)
N, (%)	B	15 (35%)	3 (27%)	5 (35%)
	C	2 (5%)	1 (9%)	0
CD4$^+$ cell count, cells/mm^3 median (IQR)		400 (310-510)	420 (310-500)	355 (320-650)
CD8$^+$ cell count, cells/mm^3 median (IQR)		1060 (790-1510)	1070 (930-1760)	950 (770-1510)
HIV-1 RNA, $\log_{10}$ copies/mL median (IQR)		4.53 (4.11-5.04)	4.57 (4.00-5.04)	4.48 (3.85-4.88)

[a]Patients with at least 26 weeks of follow-up.
[b]Patients with at least 36 weeks of follow-up (these patients are included in the 26 weeks follow-up column as well).
[c] According to reference 21.

Induction phase

Plasma HIV-1 RNA concentrations declined from 4.53 $\log_{10}$ copies/mL at baseline to 1.7 $\log_{10}$ copies/mL at week 26 (figure 2A). At week 8, 48 % of the patients had a plasma HIV-1 RNA concentration below 50 copies/mL; at week 16 this proportion had risen to 91%. No treatment failure was observed during the induction therapy according to the protocol definition.

CD4 cell-count response during the first 26 weeks of treatment is shown in figure 2B. The median change from baseline was 200 CD4 cells/μL at week 26. The median CD4 cell count as a percentage of the total lymphocyte count rose from 21.0% at baseline to 29.5% at week 26. A small decrease of the mean CD8 cell count was seen (figure 2C). At week 26, the median change from baseline was -60 CD8 cells/μL.

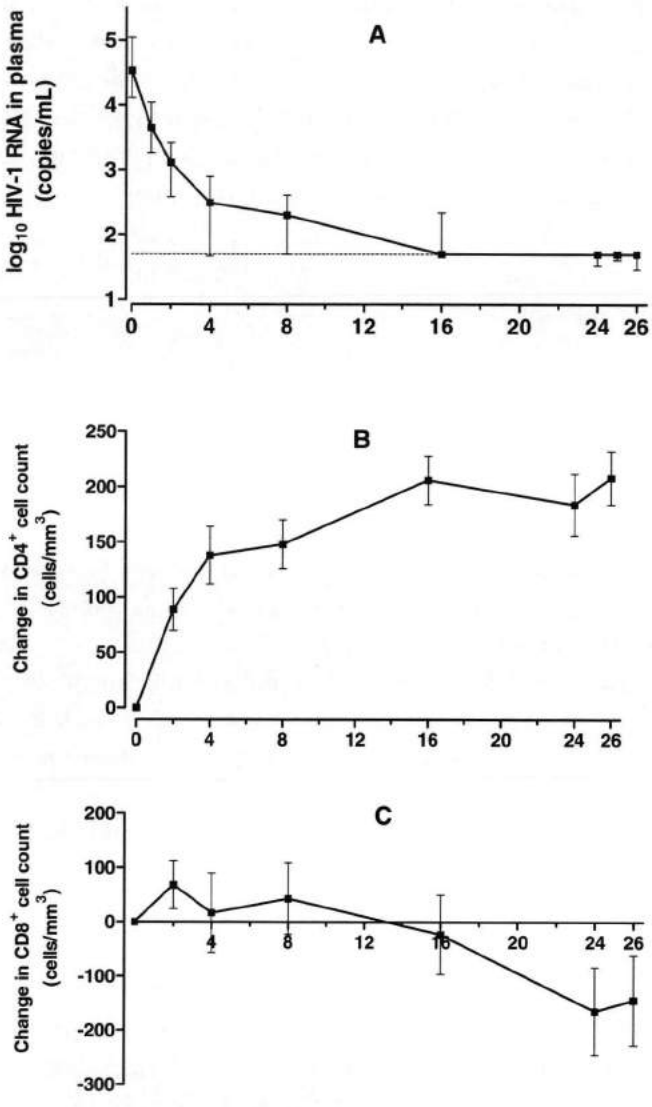

Figure 2 Plasma HIV-1 RNA level, change in $CD4^+$- and $CD8^+$- cell count during induction therapy. Bars represent interquartile range (Panel A) or standard error of the mean (Panel B and C).

Panel A: Median $\log_{10}$ HIV-1 RNA copies/mL in plasma during induction therapy.

Panel B: Mean change in $CD4^+$ cell count during induction therapy.

Panel C: Mean change in $CD8^+$ cell count during induction therapy.

33 of the 43 patients suffered from diarrhoea, mostly loose or watery stools, two or three times a day. Loperamide (2-4 mg a day) was used in half of these patients to relieve the diarrhoea. Only one patient discontinued medication because of diarrhoea (week 8). Mild rises of the liver enzymes occurred in ten patients. In three of these patients, the rise of aspartate- or alanine aminotransferase, alkaline phosphatase, or γ-glutamyl transpeptidase led to discontinuation of the study medication (at week 4, 8 and 16, respectively). Other side-effects reported among the 43 patients were: fatigue in 16 patients, raised triglycerides in 15, headache in 16, and abdominal discomfort in nine.

Maintenance phase

At week 26, 31 of 39 patients still on induction treatment were randomly allocated to stay on induction therapy or to maintenance therapy (figure 1). One patient subsequently refused maintenance therapy. Eight patients were not randomly allocated treatment: six had detectable plasma concentrations of HIV-1 RNA, albeit without treatment failure (maximum plasma HIV-1 RNA concentration was 76 copies/mL); the remaining two were not randomly allocated treatment because randomisation at week 26 was stopped.

11 of the 25 patients randomly allocated to prolonged treatment and 14 of 16 patients switched to maintenance therapy were followed up for at least 36 weeks. Seven of the 14 were given stavudine plus nelfinavir and seven were given saquinavir plus nelfinavir.

Baseline characteristics were similar in both groups of patients (table 1). At week 36, a difference in the proportion of patients with a detectable plasma concentration of HIV-1 RNA was observed between the treatment arms. One (9%) of the 11 patients in the prolonged-induction group had a detectable plasma concentration of HIV-1 RNA by contrast with nine (64%) of 14 patients in the two maintenance arms (Fisher's exact test: $p=0.01$). Five (55%) of these nine patients already had a detectable plasma concentration of HIV-1 RNA at week 32. The numbers of patients with detectable plasma concentrations of HIV-1 RNA were evenly distributed between the two maintenance arms; four (57%) of seven and five (71%) of seven patients in the stavudine plus nelfinavir and saquinavir plus nelfinavir arms, respectively. Plasma concentrations of HIV-1 RNA are given in table 2. Treatment failure (>400 HIV-1 RNA copies/mL in plasma on two consecutive occasions) was found in two patients on stavudine plus nelfinavir, and in one patient on saquinavir plus nelfinavir compared to none of the patients continuing quadruple drug therapy.

Table 2 Characteristics of patients with at least 36 weeks follow-up

Characteristic	Prolonged Induction therapy			Maintenance therapy			p [a]
	All	Detect.	Undetect.	All	Detect.	Undetect.	
Patients, N	11	1	10	14	9	5	
Δ $CD4^+$	200	570	175	216	210	190	0.74
(cells/mm^3) [b]	(100-290)	(-)	(70-255)	(124)	(140-260)	(120-290)	
Mean, SD							
Δ $CD8^+$	20	360	-245	-30	-40	-20	0.69
(cells/mm^3) [b]	(-680-220)	(-)	(-695-195)	(-450 - 240)	(-450-240)	(-290-140)	
Mean, SD							
K (day^{-1}) [c]	0.24	-0.11	0.25	0.27	0.19	0.35	0.008
Median, range	(-0.11-0.52)	(-)	(0.12-0.52)	(0.0-0.58)	(0.0-0.32)	(0.29-0.58)	
HIV-1 RNA[d]	-	55	<28	-	289	< 22	0.003
(copies/ml)			(<15-<79)		(57-3200)	(<16-<28)	
Median,range							

[a] Comparison of characteristics between patients on maintenance therapy with an undetectable and detectable plasma HIV-1 RNA concentration at week 36 (Wilcoxon rank-sum test). [b]change from baseline at week 24. [c]k = virion clearance rate constant for HIV-1 RNA in plasma. [d] plasma HIV-1 RNA concentration at week 36

Patients on maintenance therapy, with or without a detectable plasma concentration of HIV-1 RNA at week 36, were similar in plasma concentration of HIV-1 RNA at baseline and change in CD4 and CD8 cell-counts during induction therapy (table 2). The time to a plasma concentration of HIV-1 RNA of less than 50 copies/mL differed between patients with or without a sustained viral suppression during maintenance therapy (figure 3, p=0.061). Subsequently, the initial virion-clearance rate (k) was calculated for each patient. The virion-clearance rate was similar for patients on maintenance therapy and on prolonged induction therapy (Wilcoxon's rank-sum test: p= 0.40, table 2). However, in the patients on maintenance therapy, this rate was significantly higher for patients with an undetectable plasma concentration of HIV-1 RNA than for patients with a detectable plasma concentration of HIV-1 RNA at week 36 (k=0.35 vs k=0.19, respectively (p=0.008), table 2).

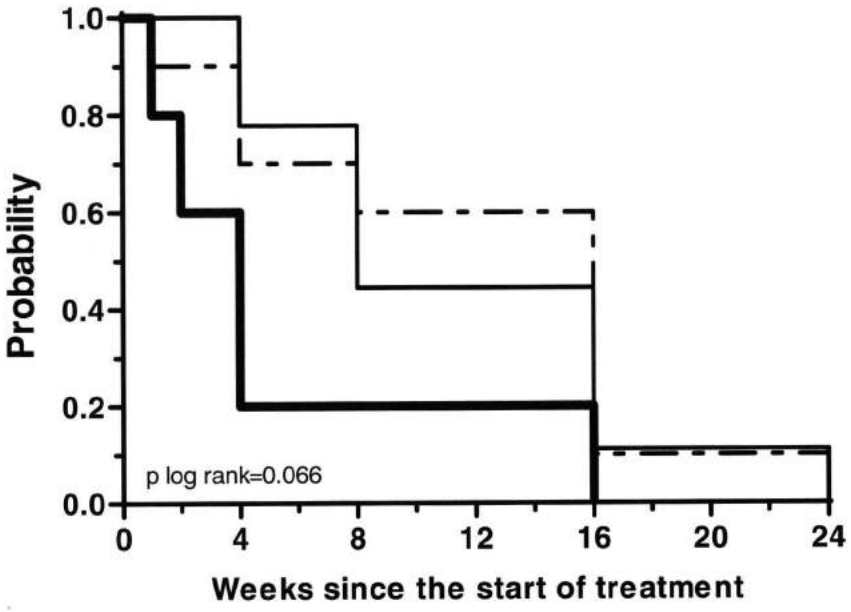

Figure 3 Kaplan-Meier curve for time to below 50 HIV-1 RNA copies/mL in plasma of patients on maintenance therapy. The thin line represents patients with a detectable plasma HIV-1 RNA concentration at week 36, the bold line represents patients with a undetectable plasma HIV-1 RNA concentration at week 36. Log rank statistic was performed for these two groups. The dashed line represents patients on prolonged induction therapy with an undetectable plasma HIV-1 RNA concentration at week 36.

Discussion

Despite rapid suppression of viral replication during the induction phase, maintenance therapy with two drugs proved to be inferior to prolonged induction therapy. Only 10 weeks after randomisation, plasma concentrations of HIV-1 had become detectable again in 64% of the patients switching to maintenance therapy, versus 9% of those receiving prolonged induction therapy. Our results are supported by those of the Trilège[16] and ACTG 343 trials.[17] In these two large randomised trials induction therapy (zidovudine, lamivudine, and indinavir) followed by maintenance therapy with two drugs, or even one drug, was investigated. A higher proportion of patients with measurable recurrence of viral replication was observed in the group of patients receiving maintenance therapy than in the group of patients remaining on the triple drug therapy. Our results and those of the Trilège and ACTG 343 trials led us to amend the protocol and discontinue randomisation at week 26.

Although the results of three randomised trials on induction-maintenance therapy regimens for HIV-1 infection have thus far been disappointing, it is intriguing that not all patients have immediate rebound of viral replication. Moreover, in the unrelated INCAS trial,[3] five antiretroviral-naïve patients who had begun treatment with zidovudine, didanosine, and nevirapine, violated the study protocol and discontinued didanosine for at least 6 weeks after plasma concentrations of HIV-1 RNA below the limit of detection (20 copies/mL) were achieved. They were all documented to have sustained viral suppression at this concentration during the period of ddI interruption.[22] Just like a small proportion of the patients in our study, these proof-of-concept cases seem to succeed in maintaining viral suppression after a reduction in the number of drugs taken.

Are there specific successful strategies of induction therapy followed by maintenance therapy or are there individual factors in patients that determine whether maintenance therapy is feasible? It has been shown that most cases of lasting viral suppression are only attained in patients whose plasma concentration of HIV-1 RNA can be maintained at very low concentrations (20-50 copies/mL).[18,23] In our study, randomisation to maintenance therapy was therefore restricted to patients with undetectable HIV-1 RNA plasma concentrations (<50 copies/mL). The ACTG 343 study showed that a rapid reduction of HIV-1 RNA in plasma after the start of treatment may be important as well. The time to an undetectable plasma concentration (<200 copies/mL) of HIV-1 RNA in the induction phase was a predictor of virological failure during maintenance therapy. Likewise, in our study, patients on maintenance therapy with a sustained undetectable plasma

concentration of HIV-1 RNA at week 36 had a faster reduction to a plasma concentration of HIV-1 RNA of less than 50 copies/mL during induction therapy than those with recurrence of a detectable plasma HIV-1 RNA concentration at week 36. When the initial virion-clearance rate was calculated, a significantly higher clearance rate was found in patients on maintenance therapy with an undetectable plasma concentration of HIV-1 RNA than in patients with a detectable plasma concentration of HIV-1 RNA at week 36. This implies that only patients with a relatively high initial virion-clearance rate succeeded to show a sustained undetectable plasma concentration of HIV-1 RNA during maintenance therapy. In addition, patients on prolonged induction therapy with a sustained viral suppression at week 36 had a virion-clearance rate comparable with the patients on maintenance therapy with a detectable plasma concentration of HIV-1 RNA. Apparently, a less rapid virological control in the first phase of the treatment may allow for viral escape during maintenance therapy and not during prolonged induction therapy. The approach of comparing patients who did or did not succeed to suppress virus replication during maintenance therapy, as regards viral-decay characteristics early in the induction phase, is a hypothesis-generating approach. This subgroup analysis is based on small numbers and should therefore be interpreted with caution. Future trials implementing a prospective approach in a different setting can estimate and therefore validate the prognostic values of these characteristics. The HIV-1 clearance rate might be influenced by the degree of exposure of the virus to antiretroviral agents for each patient. Hoetelmans and colleagues[24] showed that patients with a higher exposure to nelfinavir or saquinavir had a faster initial virion-clearance rate, while Weverling and colleagues[25] showed that five antiretroviral agents were superior to three agents in attaining an undetectable plasma concentration of HIV-1 RNA. The relevance of improving the potency of induction therapy for the feasibility of maintenance therapy may, however, eventually be limited. Tissue characteristics, such as the blood-brain barrier, and the obligate intracellular phosphorylation of the nucleoside reverse transcriptase inhibitors, may prove to be pharmacological limitations that impede the success of maintenance therapy to control replication of HIV-1 in body compartments other than the blood.[26,27]

If it is possible for the virus to replicate during maintenance therapy, the virus escape might be aided by the availability of a large pool of target cells. The influence of target cell availability on the rebound of wild-type virus after cessation of therapy and the emergence of resistant mutants during HAART has been described and mathematically modelled.[28,29] This predator-prey phenomenon seems

to be of relevance to induction therapy followed by maintenance therapy because the increase of CD4 cells was a predictor of virological failure of maintenance therapy in the ACTG 343 study. The induction therapy in our study induces a median rise in CD4 cell count of more than 200 cells/μL. Although the small numbers of patients in the different arms were comparable for the change in CD4 cell count, this increase may have adversely affected the suppression of viral replication during maintenance therapy.

We did not observe a difference in CD4 cell counts at week 36 between patients with sustained viral suppression and those with detectable plasma HIV-1RNA. Kaufmann and colleagues[30] have reported that even in individuals who remain viraemic while receiving HAART, a substantial rise in CD4 cells may be seen. The rise, however, appeared to be less than that seen in individuals with undetectable viraemia. Moreover, Li and colleagues[31] have reported that recovery of CD4 T-cell function is dependent on the amplitude and duration of viral-load reduction. This suggests that CD4 cell numbers and the recovery of CD4 T-cell function will eventually be less in the patients with inferior viral suppression during maintenance therapy. The 10-week period between randomisation and virological failure in our study would, however, appear to be too short to detect such differences.

The quadruple drug regimen of stavudine, lamivudine, saquinavir, and nelfinavir provided a potent induction regimen. Nevertheless, only patients with a relatively high initial virion-clearance rate had a sustained suppression of viral replication during maintenance therapy. The initial virion-clearance rate might be used as a parameter for the potency of the antiretroviral regimen used in an individual patient. Our findings, and those reported by others,[16,17] make it currently inadvisable to continue attempts at induction therapy followed by maintenance therapy in day-to-day practise.

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Chapter 3

The ADAM study continued: maintenance therapy after 50 weeks of induction therapy.

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-submitted for publication-

Abstract

Introduction

Several studies have shown an inferior suppression of viral replication during maintenance therapy compared to continued induction therapy in HIV-1 infected patients after three to six months of induction therapy. The ADAM study investigated whether the duration of induction therapy was of importance to the risk of virological failure during maintenance therapy.

Methods

Antiretroviral therapy naive HIV-1 infected subjects were treated with induction therapy (stavudine + lamivudine + saquinavir + nelfinavir) for either 26 or 50 weeks. Patients were randomised to maintenance therapy (either stavudine + nelfinavir or saquinavir + nelfinavir) or prolonged quadruple therapy at weeks 26 or 50. The patients randomised to maintenance or continued quadruple therapy were compared for the proportion of patients with a plasma HIV-1 RNA concentration >LLQ during follow-up. Subsequently, the time to a viral rebound during maintenance therapy was compared among the patients randomised at week 26 and at week 50.

Results

In total 65 patients were included, of whom 16 were randomised to maintenance therapy at week 26. An interim analysis demonstrated inferior suppression of viral replication during maintenance therapy compared to prolonged induction therapy. Randomisation at week 26 and further enrolment were discontinued. At week 50, 17 of the remaining 49 patients were randomised. Two out of seven patients randomised to continued quadruple therapy at week 50, decided to discontinue their study medication. Two of 10 patients randomised to maintenance therapy, refused maintenance therapy. Treatment failure was observed in 4/8 patients on maintenance therapy compared to only 1/5 patients on continued quadruple therapy (p-value is 0.56). The time to a plasma HIV-1 RNA concentration above the LLQ or above 400 copies/mL during maintenance therapy was comparable among patients randomised after 26 and 50 weeks of induction therapy.

Conclusion

Patients randomised to maintenance therapy after 26 weeks or 50 weeks of induction therapy seem to have an equally rapid rebound to a detectable HIV-1 RNA concentration in plasma. Apparently, a longer period of quadruple drug therapy does not postpone viral rebound.

Introduction

Induction-maintenance regimens in antiretroviral therapy might improve patient compliance by facilitating drug intake, reducing pill burden and reducing toxicity of antiretroviral drug regimens.[1] The induction-maintenance strategy was investigated in three studies: the ACTG 343, the Trilège, and the ADAM study. These three studies used different periods of induction therapy, patient populations, and antiretroviral agents. Nevertheless, each trial indicated that maintenance therapy with two or one antiretroviral drug could not sustain suppression of viral replication in a significant proportion of patients.[2-4] Although these results were disappointing, factors associated to treatment failure during maintenance therapy could be identified.

First of all, a lower potency of induction therapy by means of viral decay rate[4] or time to undetectability[2] was associated with virological failure during maintenance therapy. Secondly, the level of increase in $CD4^+$ T-cells was a predictor of virological failure during maintenance therapy in the ACTG 343 study, indicating a role for the 'predator-prey' phenomenon in viral rebound (e.g. a large pool of target cells facilitates the outgrowth of virus).[2] Finally, the presence of drug resistant mutants at the start of therapy increased the risk for virological failure during maintenance therapy.[2]

The duration of the induction therapy varied from three to six months in these three studies. Perhaps this was too short to make maintenance therapy possible. At the time of the design of the ADAM study, it was speculated that with the duration of therapy, the amount of HIV-1 RNA in cellular and anatomical reservoirs would further decrease,[5] reducing the risk of viral escape.[6,7]

The design of the ADAM study therefore provided the opportunity to investigate whether the duration of induction therapy was of significance for the efficacy of maintenance therapy. Albeit with small patient numbers, we here present the efficacy of maintenance therapy after induction therapy with a quadruple drug regimen for 50 weeks and discuss the effect of the duration of induction therapy on the efficacy of maintenance therapy.

Material and Methods

Patients

The enrolment of HIV-1-infected patients in this open-label randomised controlled study started in March 1997 and was ended prematurely on the 6th of April 1998.[4] Patients, aged 18 years or older, were eligible if they had at least 200 $CD4^+$ T-cells per mm^3 in their peripheral blood, 1000 or more HIV-1 RNA copies/mL in plasma and if they were naive for antiretroviral therapy. Further exclusion criteria have previously been described.[4,8] The study was approved by the institutional review boards of all participating institutions.

Study design

All patients started the induction phase with a quadruple drug regimen consisting of stavudine (d4T, 40 mg bid, or 30 mg bid if body weight <60 kg), lamivudine (3TC, 150 mg bid), saquinavir (SQV) hard-gelatin capsules (saquinavir-HGC, 600 mg tid) and nelfinavir (NFV, 750 mg tid). When saquinavir soft-gelatin capsules (saquinavir-SGC) became available (November 1st, 1997), all patients using saquinavir-HGC switched to saquinavir-SGC (800 mg tid). Patients were switched to saquinavir-SGC (1200 mg bid) and nelfinavir (1250 mg bid) on September 1st, 1998, in order to facilitate drug intake.[9,10] Patients were instructed to take their medication with food. One of the objectives of the trial was to compare patients randomised after 26 weeks of induction therapy with patients randomised after 50 weeks of induction therapy for the efficacy of maintenance therapy. However, further enrolment and the randomisation at week 26 were discontinued on the 6th of April 1998, since the interim analysis showed an inferior suppression of viral replication in the maintenance therapy arms.[4] The trial was continued to investigate the efficacy of maintenance therapy after 50 weeks of induction therapy.

At week 50, patients with a plasma HIV-1 RNA concentration below the lower limit of quantification (LLQ) of an ultrasensitive assay at both week 48 and 49 were randomised to continued quadruple drug therapy or to one of the following maintenance regimens: stavudine + nelfinavir or saquinavir + nelfinavir. Patients were allocated using a computerised minimisation program, weighting imbalance of allocations according to the $CD4^+$ T-cell count (more or less than 400 cells/mm^3) and HIV-1 RNA (more or less than 50,000 copies/mL) at baseline, and a plasma HIV-1 RNA concentration >LLQ at week 24/25. Treatment allocation was

performed in a 1:1:1 ratio (continued quadruple drug therapy, stavudine + nelfinavir or saquinavir + nelfinavir, respectively).

Follow-up

Patients not yet randomised at week 26 were scheduled to visit the outpatient clinic for clinical assessment and routine laboratory monitoring at the start of treatment and at weeks 1, 2, 4, 8, 16, 24, 25, 26, 36, 48, 49, 50, 51, 52, and every following month. Laboratory monitoring included plasma HIV-1 RNA concentration and $CD4^+$ and $CD8^+$ T-cell count, except for weeks 25, 49, 51, and 52 when only a plasma HIV-1 RNA concentration assessment was done. If patients were not randomised or had discontinued their medication for other reasons, follow-up was continued every three months. Assessment of saquinavir and nelfinavir drug concentrations was done batchwise on stored plasma.

HIV-1 RNA Quantification

During the first 26 weeks, HIV-1 RNA concentrations in plasma were measured using commercially available assays: NASBA and NucliSens HIV-1 RNA QT (Organon Teknika, Boxtel, the Netherlands) with a fixed limit of quantification of 1000 and 400 HIV-1 RNA copies per mL, respectively, or the Amplicor assay (Roche Diagnostic Systems, Inc., Branchburg, NJ, USA) with a variable limit of quantification (median quantification limit: 248 copies/mL, range: 125-755 copies/mL (n=53)). If a plasma HIV-1 RNA concentration below the limit of quantification was reached, a more sensitive assay was used: either the NucliSens HIV-1 RNA QT assay with a fixed limit of quantification of 50 copies/mL or the ultrasensitive procedure of the Roche Amplicor assay with a variable lower limit of quantification (LLQ). At week 26 and during further follow-up, only the ultrasensitive procedure of the Roche Amplicor assay was used with a median LLQ of 22 copies/mL (range: 9-107 copies/mL (n=384)). After July 1999, the COBAS Amplicor assay was used with a fixed quantification limit at 50 copies/mL.

Endpoint definition and discontinuations

During the induction period, treatment failure was defined as two consecutive assessments with a plasma HIV-1 RNA concentration above 400 copies per mL. As outlined before, the primary endpoint after randomisation was a plasma HIV-1 RNA concentration above the LLQ of the ultrasensitive assay at a time beyond week 50. Patients with a detectable HIV-1 RNA concentration of more than 100 copies/mL or

more than 400 copies/mL during maintenance therapy on two consecutive occasions were advised to continue with the original quadruple regimen or to change their therapy to three completely different antiretroviral agents, respectively.

In case of grade 4 toxicity, or grade 3 toxicity[11] without improvement after temporary discontinuation (max. 2 weeks), or recurrence of grade 3 toxicity after re-challenge, permanent discontinuation of the study medication was obligatory. After treatment failure or discontinuation of the study medication, further therapy was at the discretion of the investigator.

Assessment of drug exposure

The quantification of nelfinavir and saquinavir plasma concentrations was performed using a validated and sensitive reverse-phase high-performance liquid chromatography (RP-HPLC) assay.[12] To adjust for the time interval between drug ingestion and the drawing of the sample, the drug plasma concentration ratio was calculated for each sample, as described elsewhere.[8,13] These plasma concentrations ratios were used as a measure for exposure to saquinavir and nelfinavir.

Analysis

Induction phase

The plasma HIV-1 RNA concentrations and median $CD4^+$ and $CD8^+$ T-cell counts over time were described for patients on induction therapy. The proportion of patients on quadruple drug treatment, with a plasma HIV-1 RNA concentration below 400 copies/mL and below the LLQ of the used assay was calculated at each time point. The proportion of patients experiencing a certain side effect (severity: ≥ grade 2) was assessed. In addition, the duration of the side effect within 50 weeks of quadruple drug induction therapy was calculated.

Drug exposure

The median drug concentration ratio was calculated for each patient. Median drug concentration ratios of saquinavir and nelfinavir during the use of saquinavir-HGC and saquinavir-SGC were compared. To evaluate the effect of time and the effect of the introduction of saquinavir-SGC on the plasma concentration ratios of saquinavir and nelfinavir, an analysis of repeated measures with mixed effects was performed (Mixed Models procedure of the statistical package SAS 6.12 for Windows).

Maintenance phase

To evaluate the efficacy of maintenance therapy after 50 weeks of induction therapy, the proportion of patients with a plasma HIV-1 RNA concentration above the LLQ of the ultrasensitive assay at week 60 was compared between patients randomised to continued quadruple therapy and to maintenance therapy. In addition, the occurrence of treatment failure (a plasma HIV-1 RNA concentration above 400 copies/mL) during the available follow-up was compared among these same patients (Wilcoxon test).

Subsequently, patients randomised to maintenance therapy at week 26 and week 50 were compared for baseline characteristics, change in $CD4^+$ T-cell count at time of randomisation, initial virion-clearance rate, and median concentration ratio of saquinavir and nelfinavir per patient. Finally, to compare the efficacy of maintenance therapy after 26 and 50 weeks of induction, the time to a plasma HIV-1 RNA concentration above the LLQ of the ultrasensitive assay and above 400 copies/mL during maintenance therapy was compared for patients randomised at week 26 and at week 50. Statistical comparisons were based on the Wilcoxon test.

Results

Patients

A total of 65 patients (61 males, 94%) was enrolled (Figure 1). Baseline characteristics have already been reported.[8] The median age at baseline was 39 years. The $CD4^+$ T-cell count at baseline was 410 cells/μL and the median plasma HIV-1 RNA concentration 4.51 log_{10} copies/mL. Three patients were lost to follow-up (see also Figure 1).

Induction phase

Efficacy

The response of the $CD4^+$ T-cell count during 50 weeks of quadruple drug therapy is shown in Figure 2A. The median $CD4^+$ T-cell count rose from 410 cells/μL at baseline to 560 cells/μL at week 24 and to 680 cells/μL at week 48. The median $CD4^+$ T-cell count as a percentage of the total lymphocyte count rose from 21% at baseline to 30% at week 24 and 32% at week 48. The mean $CD8^+$ T-cell count was 1050 cells/μL at baseline and 950 cells/μL, and 940 cells/μL at weeks 24 and 48, respectively. The median $CD8^+$ T-cell count as a percentage of the total lymphocyte count showed a decrease from 61% at baseline to 49% and 46% at weeks 24 and 48, respectively. The decline in plasma HIV-1 RNA concentration during treatment with the quadruple drug regimen is depicted in Figure 2B. Two patients had treatment failure on quadruple drug therapy before randomisation at week 50. The percentage of patients with a plasma HIV-1 RNA concentration below the LLQ of the ultrasensitive assay during quadruple drug therapy was 69% at week 24 and 71% at week 48. The proportion of patients with a plasma HIV-1 RNA concentration below 400 copies/mL was higher (96% and 97%, respectively).

Toxicity and discontinuations

Side effects with a severity grade 2 or more were reported in 44 out of 65 patients. Of the ten patients who discontinued their study medication for reasons of toxicity, seven patients stopped the medication within the first 26 weeks as described previously (Figure 1).[8] The remaining three patients complained about general malaise, depression or lipodystrophy as the reason for discontinuation. Toxicity during 50 weeks of induction therapy mainly concerned gastro-intestinal complaints and fatigue. Twenty-seven of the 65 patients had diarrhoea (median duration 56 days (interquartile range (IQR): 18-153 days), and eight patients had complaints of abdominal pain (median duration 50 days (IQR 7-100 days)).

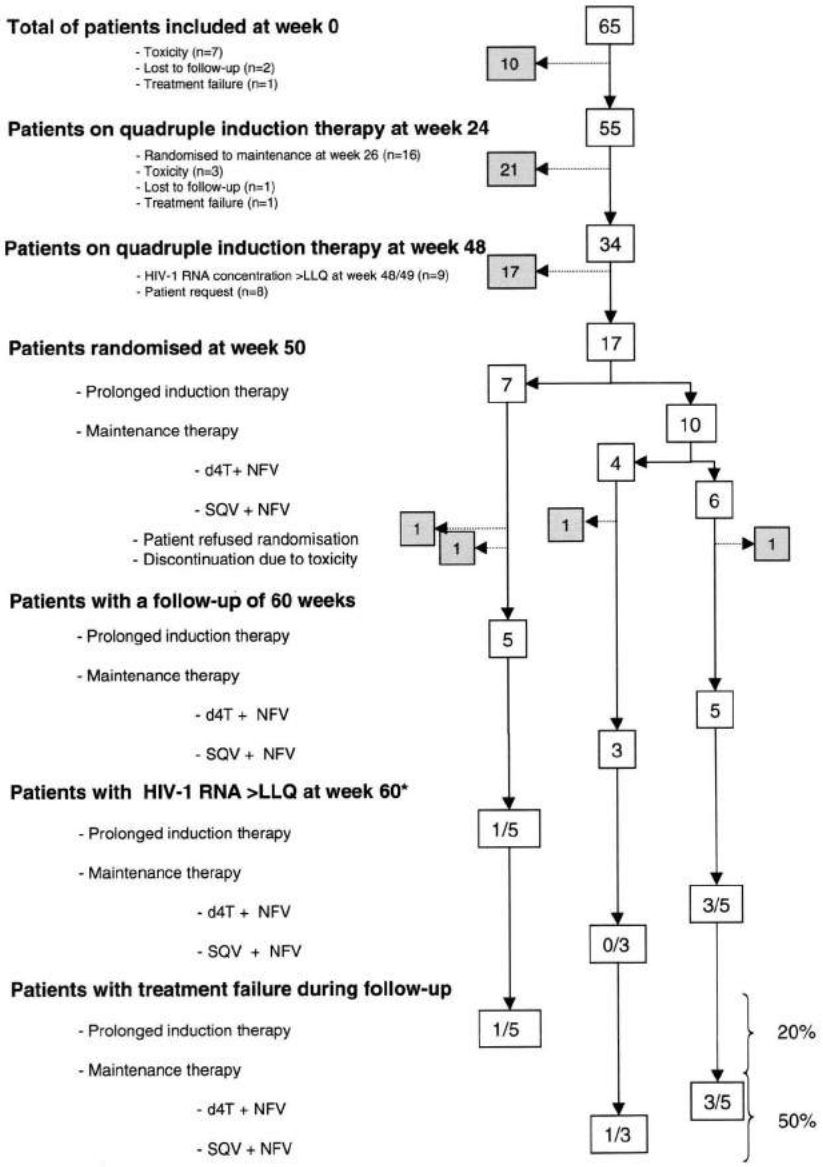

Figure 1 Trial profile. The numbers in shaded boxes indicate patients who discontinued the study medication.

Fatigue occurred in eight patients and was usually quite persistent (median duration 83 days (IQR 41-224)). Laboratory monitoring revealed raised liver enzymes in 8/65 patients. Cholesterol levels in plasma were elevated in 28/65 patients. However, in only 6/65 patients cholesterol temporarily rose above 7.7 mmol/L. Triglyceride elevations in plasma were observed in 13/65 patients, of whom only two were found to have triglyceride levels above 8.4 mmol/L in plasma.

Drug exposure

The median plasma drug concentration ratio per patient (n=56) was 0.78 for saquinavir (IQR: 0.42-1.25) and 0.61 for nelfinavir (IQR: 0.45-0.83). Median plasma concentration ratios of saquinavir were lower during the use of saquinavir-HGC than during the use of saquinavir-SGC (0.56 (IQR 0.28-1.28) and 0.81 (IQR 0.41-1.67) respectively, p=0.002). Median nelfinavir ratios were comparable among the two treatment periods (0.61 (IQR 0.41-0.89) and 0.60 (IQR 0.37-0.85), respectively; p=0.46). These findings were confirmed, using repeated measures analysis with mixed effects to investigate the effect of time and the use of saquinavir-SGC. High plasma concentrations of saquinavir were associated with the use of saquinavir-SGC (test of fixed effects, p=0.03 and p=0.27 for saquinavir and nelfinavir, respectively). No change in plasma drug concentration ratios could be found over time (test of fixed effects; p=0.53 and p=0.09 for saquinavir and nelfinavir, respectively).

For the evaluation of the period that saquinavir-SGC and nelfinavir were used in a bid regimen, not enough data were available at the time of analysis.

Maintenance phase

Patients

At week 50, 17 of the 65 patients were randomised (Figure 1). Reasons for not randomising at week 50 were: lost to follow-up (3), prior randomisation to maintenance therapy (16), treatment failure (2), toxicity (10), a plasma HIV-1 RNA concentration above the LLQ at week 48 and 49 (9) or at the request of the patient (8). One of the seven patients randomised to continued quadruple therapy decided to take a 'drug holiday' and another patient experienced lipodystrophy, leading to a switch of therapy. Ten patients were randomised to maintenance therapy with either stavudine + nelfinavir (n=6) or saquinavir + nelfinavir (n=4). One patient in each maintenance arm refused maintenance therapy (Figure 1).

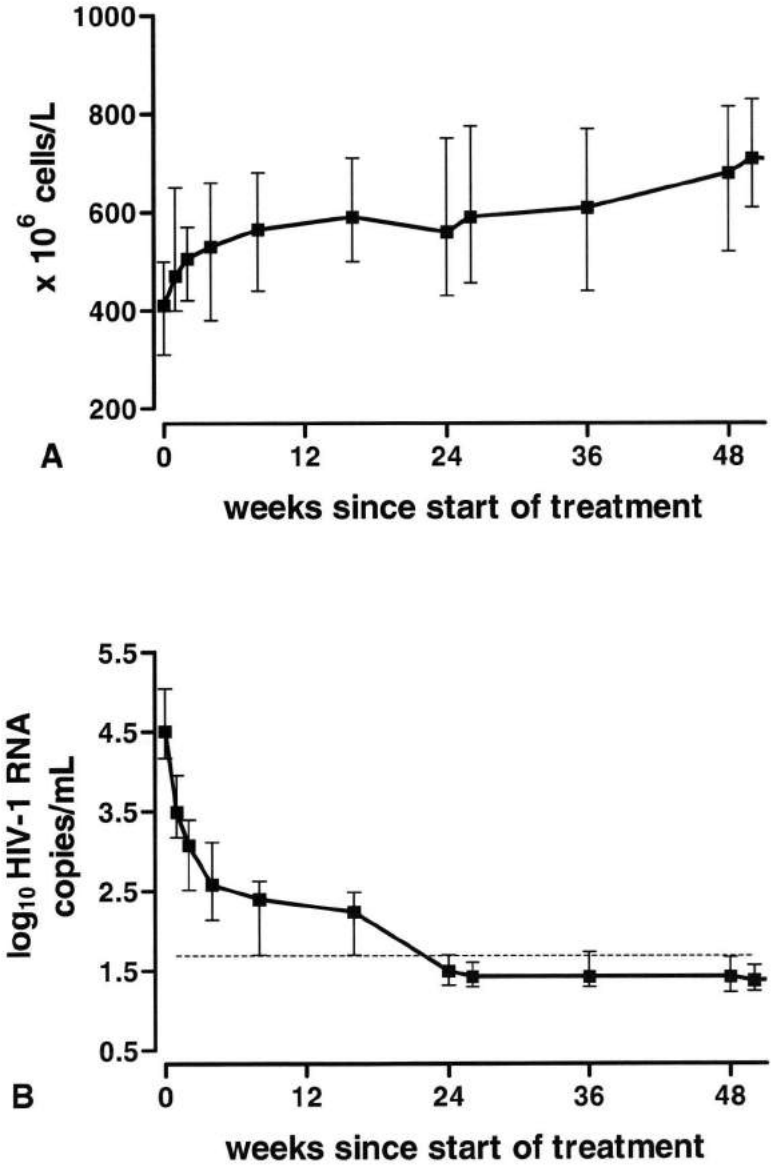

Figure 2 Median $CD4^+$ T-cell count and plasma HIV-1 RNA concentration during the induction therapy. The dashed line represents 50 copies/mL or 1.69 $\log_{10}$ copies/mL; Bars represent interquartile ranges.
Panel A: Median $CD4^+$ T-cell count;
Panel B: Median $\log_{10}$ HIV-1 RNA copies/mL in plasma.

Efficacy

At week 60, no difference was observed between the randomisation arms regarding the proportion of patients with a quantifiable plasma HIV-1 RNA concentration (Figure 1). One out of five patients in the continued quadruple drug therapy arm had a plasma HIV-1 RNA concentration above the LLQ compared to 3 out of 8 patients on maintenance therapy. After a median follow-up of 34 weeks in the quadruple therapy arm and 46 and 40 weeks in the two maintenance therapy arms (stavudine + nelfinavir and saquinavir + nelfinavir, respectively), treatment failure had occurred in one out of five patients (20%) using quadruple drug therapy, and in four out of eight patients (50%) using maintenance therapy (Fisher exact: p=0.56; Figure 1).

Table 1 The characteristics of patients randomised to maintenance therapy after 26 or 50 weeks of induction therapy

Characteristic		Week 26 (n=16)	Week 50 (n=10)	p-value
At baseline				
$CD4^+$ T-cell count	($x10^6$cells/L)[a]	360 (320-570)	370 (320-440)	0.71
$CD8^+$ T-cell count	($x\ 10^6$cells/L)[a]	950 (750-1490)	103 (970-1760)	0.16
Log_{10} HIV-1 RNA	(copies/mL)[a]	4.69 (4.09-5.04)	4.32 (3.99-4.57)	0.15
At randomisation				
$\Delta CD4^+$ T-cell count	($x\ 10^6$ cells/L)[a]	200 (120-310)	280 (100-470)	0.33
$\Delta CD8^+$ T-cell count	($x\ 10^6$ cells/L)[a]	-30 (-290-250)	40 (-290-320)	0.86
Virological decline during induction therapy				
initial virion-clearance rate	($days^{-1}$)[a]	0.31 (0.25-0.34)	0.30 (0.28-0.34)	0.81
Median drug concentration ratio in plasma within the first 26 weeks of induction therapy[b]				
saquinavir[a]		0. 0.18-0.74	0.86 0.62-1.51	0.03
nelfinavir[a]		0. 0.41-0.75	0.62 0.46-0.74	0.88

Values are [a] median (interquartile range); [b] For the patients randomised at week 50: n=8.

Subsequently, we compared patients randomised to maintenance therapy at week 26 (n=16) and week 50 (n=10, Table 1). Patients were comparable for their baseline characteristics and change in $CD4^+$ and $CD8^+$ T-cell counts at time of randomisation. Compared to patients randomised at week 26, patients randomised at week 50 had significantly higher plasma concentration ratios of saquinavir and were more often treated with saquinavir-SGC during the first 26 weeks of induction therapy (p=0.03 and p<0.001, respectively; Table 1).

The time to a plasma HIV-1 RNA concentration above the LLQ of the ultrasensitive assay or above 400 copies/mL during maintenance therapy was comparable among patients randomised to maintenance therapy after 26 or 50 weeks of induction therapy, as is illustrated in the Kaplan Meier plots (Figure 3A and 3B, respectively).

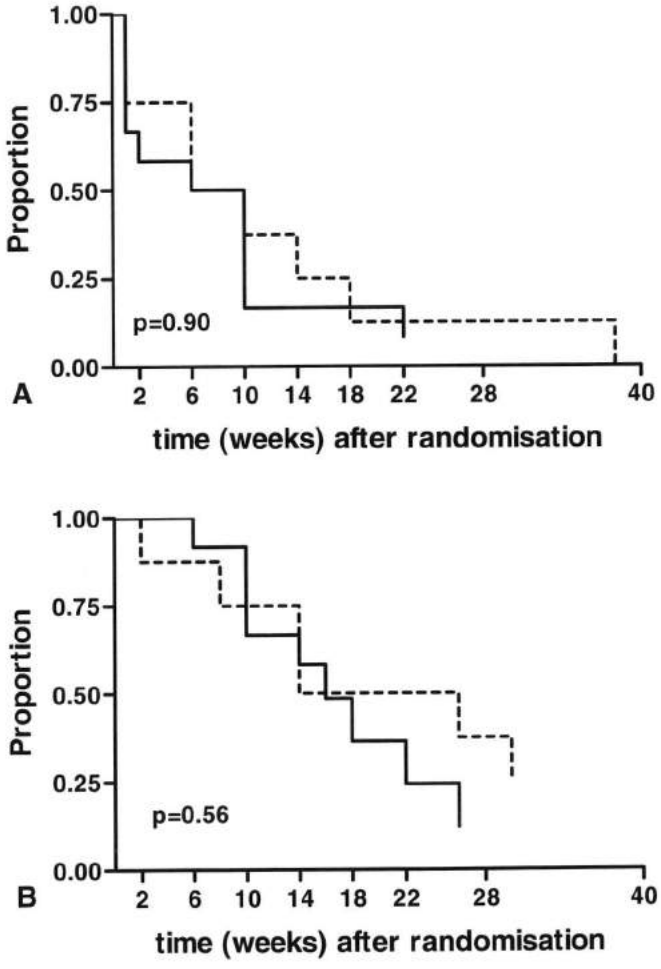

Figure 3 Kaplan Meier curve for the time to a plasma HIV-1 RNA concentration above the variable lower limit of quantification of the ultrasensitive assay (median quantification limit: 22 copies/mL; panel A) or above 400 copies/mL (panel B) during maintenance therapy. The solid line represents the patients (n=14) randomised to maintenance therapy at week 26, the dashed line represents the patients (n=10) randomised to maintenance therapy at week 50. The P-value was calculated using Log-Rank testing.

Discussion

Maintenance therapy in HIV-1 infected patients, even after an induction therapy for 50 weeks, seemed not efficacious. Four out of eight patients randomised to maintenance therapy were found to have a rebound of viral replication in contrast to one out of five patients randomised to continued quadruple drug therapy. Although the difference was not statistically significant, prolonging the induction therapy from 26 to 50 weeks does not seem to contribute to the efficacy of the maintenance regimen, since patients randomised after 50 weeks of induction therapy had an equally rapid rebound of the plasma HIV-1 RNA concentration to >400 copies/mL than patients randomised after 26 weeks of induction therapy.

The switch from saquinavir-HGC (600 mg tid) to saquinavir-SGC (800 mg tid) did result in a higher drug exposure during induction therapy in patients randomised to maintenance therapy at week 50. However, this did not result in differences in the viral decline of these patients compared to patients randomised to maintenance therapy at week 26 (Table 1), nor did it result in improved suppression of viral replication during maintenance therapy.

Although only two patients discontinued the study medication for reason of virological failure during the induction therapy, it is remarkable that a considerable proportion of the patients who discontinued medication was not available for randomisation at week 50. Besides a drop-out of patients due to toxicity (mostly within the first 26 weeks), a considerable amount of patients refused study medication for personal reasons, both before (n=8) and after (n=2) randomisation. It is likely that the negative results of the interim analysis have influenced patients to abandon randomisation at week 50. This is in line with results of the quality of life study indicating that the knowledge of an incomplete suppression of the viral load was of significance to the quality of life of the patients randomised to maintenance therapy.[14] Finally, the lower limit of detection was set to 50 copies/mL at week 24/25 but was lower and variable at week 48/49. We therefore may have been more stringent in selecting patients for randomisation at week 50.

With the low number of patients randomised at week 50, it is difficult to demonstrate a statistically significant difference in virological failure between patients randomised to maintenance therapy and to continued quadruple drug therapy. A population of 80 patients would be needed to show statistical significance for the difference observed (50% vs. 20%), with 80% power. This,

however, seems not realistic, since patients already tended to discontinue the study at the knowledge of results from earlier publications, as discussed above.

Since the design of the study, several studies have indicated that even after long-term suppression of viral replication below 50 copies/mL, low level replication remains present.[15-18] It is therefore not surprising, that viral replication can expand when the antiviral potency is reduced during maintenance therapy. In the ACTG 343 and Trilège studies, failure during maintenance therapy indeed appeared to be highly attributable to insufficient potency of the maintenance regimen.[19,20]

Target cell availability was already suggested to be of significance to failure of induction-maintenance regimens.[2] In our study, no difference in the increase of $CD4^+$ T-cell count was observed among the patients randomised at week 26 or at week 50 (Table 1). Fleury et al. recently showed, that the pool of proliferating $CD4^+$ T-cells was found to be enlarged for at least 48 weeks after the start of antiretroviral treatment, suggesting that randomisation after 50 weeks of induction therapy was still accompanied by increased target cell availability.[21] Maybe future induction-maintenance strategies could decrease target cell availability by the use of agents that selectively suppress the activation of $CD4^+$ T-cell counts.[22]

With the increasing awareness of the disadvantages of antiretroviral therapy, strategies reducing pill burden, toxicity and complexity of regimens are needed more than ever. Unfortunately, we here again show that induction-maintenance strategies with the currently available classes of agents are not successful.[2-4] In future studies targeting induction- maintenance therapy in HIV-1 infected patients, more benefit may be expected from an increase in the antiretroviral efficacy of the maintenance regimen, or a reduction of the required antiretroviral efficacy during maintenance therapy than from a prolongation of the induction phase beyond 26 weeks.

Appendix

In the ADAM Study Team participated:
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Chapter 4

The steady-state pharmacokinetics of nelfinavir (Viracept®) and saquinavir (Invirase®) during a quadruple antiretroviral drug regimen in HIV-1-infected individuals.

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-submitted for publication-

Summary

Aims

Objective of this multicentre, observational study was to assess the steady-state plasma pharmacokinetics of nelfinavir (Viracept®) and saquinavir (Invirase®) during a quadruple antiretroviral drug regimen consisting of nelfinavir (750 mg tid), saquinavir (600 mg tid), stavudine (40 mg bid), and lamivudine (150 mg bid).

Methods

Eighteen antiretroviral naive HIV-1-infected male patients who have been treated with the quadruple antiretroviral drug regimen for at least 4 weeks were included in this study. For each individual, plasma concentrations of nelfinavir and saquinavir were quantified during a full (8-hour) dosing interval. Plasma pharmacokinetics of both protease inhibitors were calculated using noncompartmental methods. The positive pharmacokinetic interaction between nelfinavir and saquinavir was further explored by comparing saquinavir pharmacokinetics to those observed in historical controls treated with a saquinavir dosage of 1,200 mg tid (Invirase®), without the use of nelfinavir.

Results

The average values for the area under the plasma concentration versus time curve (AUC) were 18.1 h*mg/L and 2.37 h*mg/L for nelfinavir and saquinavir, respectively. The average maximum and trough plasma concentrations were 5.2 and 0.62 mg/L for nelfinavir, and 0.57 and 0.10 mg/L for saquinavir. The average elimination half-lives were 2.0 h and 2.3 h for nelfinavir and saquinavir, respectively. Observed interindividual variation in pharmacokinetic parameters was approximately 4-fold for nelfinavir, and approximately 6-fold for saquinavir. Significant positive correlation was observed between the values for nelfinavir and saquinavir pharmacokinetics. Nelfinavir increases saquinavir plasma concentrations at least 2-fold, and reduces intrapatient fluctuation of saquinavir plasma concentrations. Interpatient variability in saquinavir pharmacokinetics was not reduced by concomitant administration of nelfinavir.

Conclusions

The results of this pharmacokinetic study provide a pharmacologic rationale for the combined use of nelfinavir and saquinavir.

Introduction

The "*Amsterdam Duration of Antiretroviral Medication*" study (ADAM) investigates the feasibility of a quadruple drug induction therapy followed by a double drug maintenance therapy in HIV-1 infected antiretroviral naive individuals.[1] Induction therapy consisted of a quadruple regimen containing two protease inhibitors (saquinavir, Invirase®, and nelfinavir, Viracept®) plus two reverse transcriptase inhibitors (stavudine, d4T, Zerit®, and lamivudine, 3TC, Epivir®).

Saquinavir and nelfinavir were chosen as the two protease inhibitors for the induction phase of the study since they show a positive pharmacokinetic interaction (leading to an increased exposure of saquinavir),[2] the primary drug resistance mutations differ,[3] they can be concomitantly ingested, and are relatively well tolerated drugs.

Since data regarding the steady-state pharmacokinetics of nelfinavir and saquinavir when used in a quadruple combination regimen are lacking, a pharmacokinetic substudy was conducted during the quadruple drug induction phase of the ADAM study. We feel that a detailed understanding of the pharmacokinetics of both protease inhibitors is essential, as exposure to these drugs was shown to be significantly and positively related with the initial rate of decline of plasma HIV-1 RNA in this study.[4] Consecutively, it was demonstrated that patients with a relatively low initial rate of decline of plasma HIV-1 RNA had a higher risk of virological failure during maintenance therapy in the ADAM study.[1]

A positive effect on saquinavir plasma concentrations by the addition of nelfinavir was reported by Merry et al.[2] They found an approximately 5-fold increase in saquinavir AUC after addition of nelfinavir in 6 HIV-1 infected patients. The pharmacokinetic profile of nelfinavir, however, was not reported, nor were steady-state pharmacokinetics investigated. We here report the steady-state pharmacokinetics of saquinavir and nelfinavir when used in combination with two nucleoside reverse transcriptase inhibitors, and define further the pharmacokinetic interaction between them.

Methods

Patients were recruited from the outpatient clinics of the *Onze Lieve Vrouwe Gasthuis*, Amsterdam, the *Kennemer Gasthuis*, Haarlem, the *Academic Medical Centre*, Amsterdam, and the *Slotervaart Hospital*, Amsterdam, all in the Netherlands, from April 1997 to October 1997.

Patients participating in the ADAM study of 18 years and older with confirmed HIV-1 infection could be included in this sub-study. Alanine (ALAT) and aspartate aminotransferase (ASAT), and alkaline phosphatase had to be less than three times the upper limit of the normal ranges; the creatinine concentration had to be less than 130 μmol/L. On the pharmacokinetic study day, patients had to use the quadruple drug regimen for at least 4 weeks. Exclusion criteria consisted of a haemoglobin less than 6.0 mmol/L, and known allergy to study medication. Approval was obtained from the local review boards of the participating institutions and the patients gave written informed consent. Patients who concomitantly used drugs that might influence nelfinavir or saquinavir pharmacokinetics by inhibition or induction of the cytochrome P450 enzymes were excluded from participation in this sub-study.[5] Other concurrent medication was allowed.

After an overnight fast, patients ingested 600 mg saquinavir (Invirase®), 750 mg nelfinavir, 40 mg stavudine, and 150 mg lamivudine after a breakfast. Ten-mL venous blood samples were collected in heparinised tubes just before, and 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, and 8 hours after ingestion of the drugs. Blood samples were kept on melting ice and after plasma was isolated by centrifugation (10 min at 3,000 G) on the same day, the plasma samples were stored at -30° C until analysis.

Nelfinavir and saquinavir plasma concentrations were quantified using a sensitive and validated isocratic, reversed-phase ion-pair high-performance liquid chromatographic assay.[6] Briefly, sample preparation consists of a solid-phase extraction using C_2 columns. The analytical column is a Zorbax® SB-C_{18} column (75 x 4.6 mm I.D./particle size 3.5 μm; Rockland Technologies, Newport, DE, USA) and the mobile phase is composed of acetonitrile plus destilled water containing 25 mM sodium acetate and 25 mM hexane-1-sulfonic acid, adjusted to pH 4.0. Between-day and within-day variation of quality control samples of nelfinavir and saquinavir in plasma range from 3.4 to 5.2%. The lower limit of quantification of the assay is 25 μg/L for nelfinavir and 2.5 μg/L for saquinavir. The assay is linear up to concentrations of at least 20.0 mg/L for nelfinavir and 4.0 mg/L for saquinavir.

Plasma concentration (C) - time (T) data for nelfinavir and saquinavir were analysed by

non-compartmental methods.[7] The highest observed plasma concentration was defined as C_{max} with the corresponding sampling time as T_{max}. The plasma concentration observed at 8 h after ingestion of the drugs was defined as C_{min}. To provide a measure for the fluctuation of concentrations during the dosing interval, τ, the percentage peak-trough fluctuation (PTF) was calculated as $100 * (C_{max} - C_{min})/C_{min}$. The terminal, log-linear period ($\log_{10}$ C versus T) was defined by the last data points (N ≥ 4) by visual inspection. The absolute value of the slope (ß/ln 10) was calculated by least squares analysis. The elimination half-life ($t_{½}$) was estimated by the equation ln 2/ß. The area under the plasma concentration versus time curve ($AUC_{[0-8]}$) was obtained using the trapezoidal rule from zero (T_0) to eight hours (T_8). The average steady-state concentration (C_{av}) was calculated by dividing $AUC_{[0-8]}$ by τ. The apparent clearance (CL/F) was calculated by dividing the dose by the $AUC_{[0-8]}$, and the apparent volume of distribution (V/F) by dividing CL/F by ß (where F represents the oral bioavailability). The accumulation index (R_{AC}) was calculated using the equation: $R_{AC} = 1/(1-e^{-ß*\tau})$.

Statistical calculations were performed with the Statistical Product and Service Solutions (SPSS) for Windows, version 6.1, SPSS Inc., Chicago, IL, USA. A significance level of 0.05 was used for all tests.

Results

Patients

Eighteen male patients participating in the ADAM study were enrolled for the pharmacokinetic sub-study. Patient characteristics are described in Table 1. Co-medication (other than the study medication) on the pharmacokinetic sampling day consisted of loperamide in 3 patients.

Table 1 Patient characteristics.

	mean	range
age (years)	41	23 – 56
weight (kg)	75	61 – 90
weeks on therapy	7	4 – 15
ALAT (U/L)	17	8 – 33
ASAT (U/L)	16	9 – 27
alkaline phosphatase (U/L)	47	33 – 58
creatinine (µmol/L)	84	52 – 99
bilirubin (µmol/L)	7.9	4.0 - 11.0
haemoglobin (mmol/L)	9.0	7.7 - 10.4
leucocytes (x 10^9/L)	7.1	4.1 - 12.5
platelets (x 10^9/L)	280	160 – 363
$\log_{10}$ **HIV RNA load** (copies/mL)	4.47	2.36 - 6.58
CD4$^+$ T-lymphocyte count (cells/mm^3)	429	150 –770

Abbreviations: ALAT = alanine aminotransferase, ASAT = aspartate aminotransferase

Pharmacokinetics

Nelfinavir and saquinavir were detected and quantified in all plasma samples. Table 2 summarises the values for the plasma pharmacokinetic parameters of nelfinavir and saquinavir, respectively, which were calculated from the individual concentration-time profiles in 18 patients.

The average nelfinavir AUC was 18.1 h*mg/L and showed an approximately 4-fold variability between patients (ranging from 8.6 to 34.8 h*mg/L). The average C_{max} and C_{min} were 5.2 and 0.62 mg/L, respectively. Nelfinavir was eliminated from plasma with an average half-life of 2.0 h. The mean accumulation index was 1.07. Figure 1 represents the mean (+ standard error) plasma concentration-time profile of nelfinavir in this population (N=18) during the 8-hour dosing interval.

The average saquinavir AUC was 2.37 h*mg/L and showed an approximately 6-fold interpatient variation (ranging from 0.96 to 5.96 h*mg/L). The average C_{max} and

C_{min} were 0.57 and 0.10 mg/L, respectively. Saquinavir was eliminated from plasma with a mean half-life of 2.3 h. The mean accumulation index was 1.10. Figure 2 represents the mean (+ standard error) plasma concentration-time profile of saquinavir in this population (N=18) during the 8-hour dosing interval.

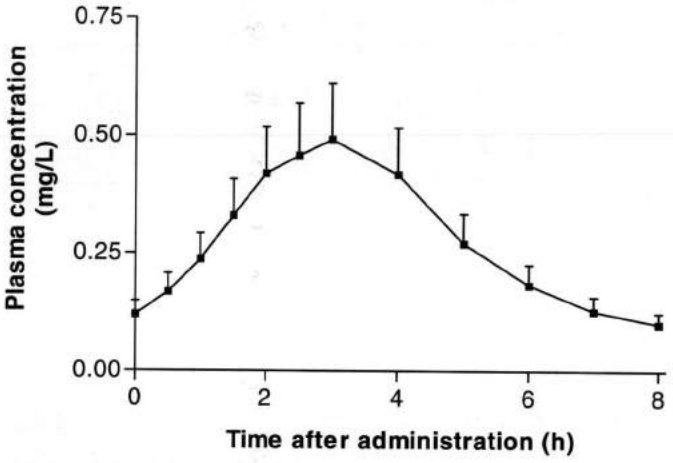

Figure 1 Mean plasma concentration-time profile (+standard error) of nelfinavir in steady-state concentrations in a dosing regimen of 750 mg *tid* in combination with saquinavir 600 mg *tid* (N=18).

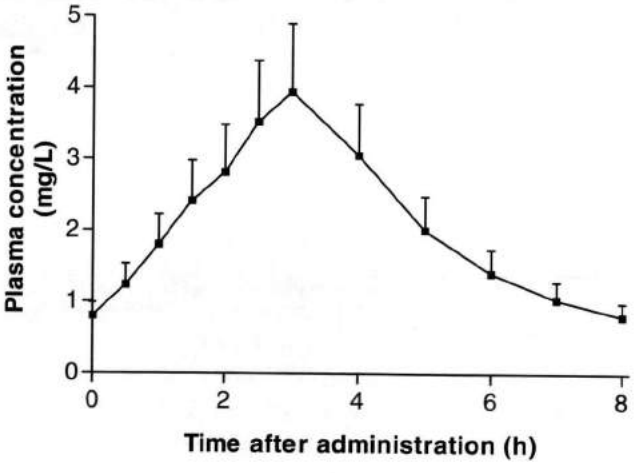

Figure 2 Mean plasma concentration-time profile (+standard error) of saquinavir (Invirase®) in steady-state concentrations in a dosing regimen of 600 mg *tid* in combination with nelfinavir 750 mg *tid* (N=18).

Table 2 The steady-state plasma pharmacokinetics of nelfinavir and saquinavir

	nelfinavir mean	nelfinavir range	saquinavir mean	saquinavir range
$AUC_{[0-8]}$ (h*mg/L)	18.1	8.6 - 34.8	2.37	0.96 - 5.96
C_{max} (mg/L)	5.2	2.8 - 10.2	0.57	0.26 - 1.49
C_{min} (mg/L)	0.62	0.25 - 1.4	0.10	0.04 - 0.34
C_{av} (mg/L)	2.26	1.1- 4.4	0.30	0.12 - 0.74
%PTF	845	445 - 1,475	560	143 - 1,488
T_{max} (h)	2.7	1.5 - 4.1	2.9	0.7 - 4.3
$t_{½}$ (h)	2.0	1.1- 3.2	2.3	1.1 - 3.8
CL/F (L/h)	46	20 72	272	92 – 526
CL/F (L/h/kg)	0.61	0.26- 1.11	3.6	1.3 - 8.1
V/F (L)	141	35- 330	865	156 - 2,196
V/F (L/kg)	1.91	0.50 - 4.40	11.7	2.1 - 33.8

Abbreviations: $AUC_{[0-8]}$ = area under the plasma concentration versus time curve from 0 to 8 hours, CL/F = apparent clearance with F = availability, C_{av} = average steady-state concentration, C_{max} = maximum plasma concentration, C_{min} – plasma trough concentration, %PTF = percentage peak-trough fluctuation, T_{max} = time to maximum plasma concentration, $t_{½}$ – plasma elimination half-life, V/F = apparent volume of distribution.

Using univariate regression analysis, significant positive relationships were observed between all pharmacokinetic parameters of nelfinavir and saquinavir, except for the elimination half-life (Table 3).

Table 3 Correlation between saquinavir and nelfinavir pharmacokinetics

	p-value	r
$AUC_{[0-8]}$	0.012	0.60
C_{max}	0.004	0.67
C_{min}	0.002	0.70
%PTF	0.026	0.54
T_{max}	0.002	0.69
$t_{½}$	0.18	0.34

Abbreviations: $AUC_{[0-8]}$ = area under the plasma concentration versus time curve from 0 to 8 hours, C_{max} = maximum plasma concentration, C_{min} = plasma trough concentration, %PTF = percentage peak-trough fluctuation, T_{max} = time to maximum plasma concentration, $t_{½}$ = plasma elimination half-life.

Using the Mann-Whitney test, the steady-state pharmacokinetics of saquinavir (Invirase®) 600 mg *tid* in combination with nelfinavir 750 mg *tid* as observed in the current study were compared with the steady-state pharmacokinetics obtained in 20 HIV-1 infected patients who were treated with saquinavir (Invirase®) 1,200 mg *tid* in combination with 2 reverse transcriptase inhibitors (without nelfinavir).[8] The results are presented in Table 4. Statistical significant differences were only observed for the percentage peak-trough fluctuation (p=0.017).

Table 4 **Comparison of the steady-state pharmacokinetics of saquinavir 600 mg *tid* with nelfinavir 750 mg *tid* (n=18), and the steady-state plasma pharmacokinetics of saquinavir observed in a 1,200 mg *tid* regimen without nelfinavir (n=20).**

	600 mg *tid* plus nelfinavir	1,200 mg *tid* without nelfinavir	p-value
$AUC_{(0-8)}$ (h*mg/L)	2.37	2.32	0.86
C_{max} (mg/L)	0.57	0.72	0.29
C_{min} (mg/L)	0.10	0.08	0.66
%PTF	560	956	0.017
T_{max} (h)	2.9	2.9	0.97
$t_{½}$ (h)	2.3	1.7	0.16
time >0.10 mg/L (h)	7.0	5.7	0.040

Abbreviations $AUC_{(0-8)}$ = area under the plasma concentration versus time curve from 0 to 8 hours, CL/F = apparent clearance, with F = bioavailability, C_{max} = maximum plasma concentration, C_{min} = plasma trough concentration, %PTF = percentage peak-trough fluctuation, T_{max} = time to maximum plasma concentration, $t_{½}$ = plasma elimination half-life, V/F = apparent volume of distribution.

Discussion

The concept of a quadruple drug induction therapy followed by a double drug maintenance therapy for treatment of HIV-1 infected individuals has been investigated in the ADAM study. At this moment, the strategy of induction-maintenance therapy cannot be advocated for routine practice.[1]

In this study, the importance of pharmacologic exposure to antiretroviral drugs for antiretroviral therapy became evident, as the initial rate of decline of plasma HIV-1 RNA in patients participating in the ADAM study was significantly and positively related with the exposure to nelfinavir and saquinavir in plasma.[4] The aim of the currently described pharmacokinetic substudy was to gain more insight into the steady-state pharmacokinetics and interpatient variability of nelfinavir and saquinavir when used in combination, and to further understand the pharmacokinetic interaction between them.

In 1995, saquinavir (Ro 31-8959) became the first member of the class of protease inhibitors to be licensed in the USA by the FDA under its accelerated approval regulations for use in combination with approved nucleoside reverse transcriptase inhibitors in patients with advanced HIV infection. The saquinavir hard gelatin capsule formulation (saquinavir HGC, Invirase®) was subsequently approved in the European Community in October 1996.

The pharmacokinetics of saquinavir are complex. The drug exhibits nonlinear pharmacokinetics (with increasing doses leading to a more than proportional increase in AUC and maximum plasma concentration), the bioavailability is low and the drug should always be ingested within 2 hours after a meal. Saquinavir is highly bound to plasma proteins (>98%) and is rapidly (and mainly) metabolised by the cytochrome P450 3A4 isoenzyme to a number of inactive mono- and dihydroxylated metabolites.[9] Elimination of saquinavir is predominantly non-renal: after an oral dose of 600 mg, only 1% is excreted in the urine.[9] Furthermore, the pharmacokinetics of saquinavir are subject to large interindividual variability, and, interestingly, plasma concentrations of saquinavir are higher in patients infected with HIV as compared with healthy volunteers.[5] Finally, due to the drug's affinity for cytochrome P450 enzymes, various drug-drug interactions have been identified.[5]

Nelfinavir is a more recently licensed protease inhibitor with an average oral availability in various animal species ranging from 17 to 47%.[10] The nelfinavir AUC in 6 fasted volunteers was 27 to 50% of the AUC achieved in fed volunteers after administration of single doses of 400 and 800 mg and the drug should therefore

always be ingested with food.[11] *In vitro* studies with human microsomes revealed that nelfinavir is primarily metabolised by the CYP3A isoenzyme, but CYP2C19, CYP2D6, and possibly CYP2C9 are also involved in the metabolism of the drug.[12] Nelfinavir is a less potent inhibitor of CYP3A than either indinavir or ritonavir. The currently observed average nelfinavir $AUC_{(0-8)}$, C_{max}, T_{max}, and $t_{½}$ are in agreement with those previously reported.[11,13,14] We found an approximately 4-fold interindividual variation in exposure to nelfinavir (as measured by plasma AUC) in our population (values for the AUC range from 8.6 to 34.8 h*mg/L). Comparable variations were observed for maximum and trough plasma concentrations. Exposure to nelfinavir as achieved in our patients is expected to result in a substantial antiretroviral response.[15]

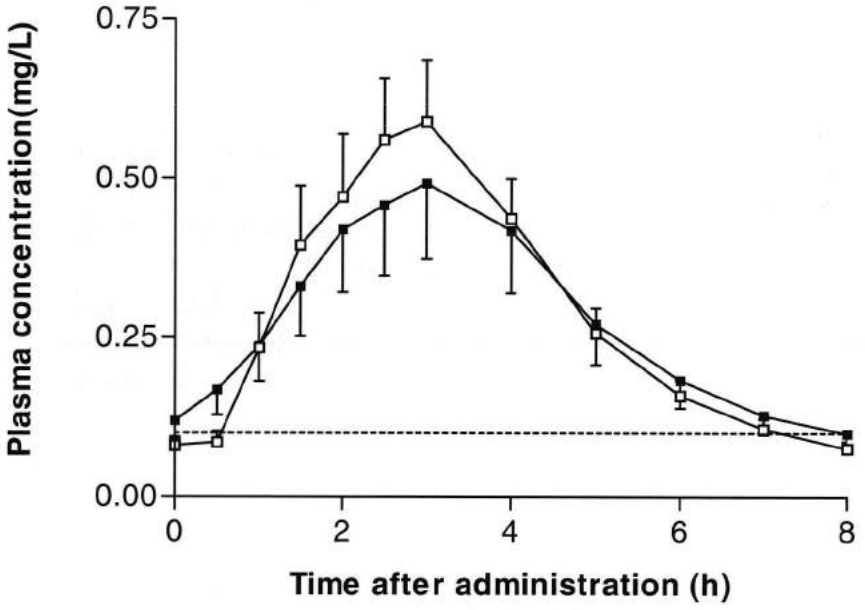

Figure 3 Mean steady state plasma concentration – time profiles (+ standard error) of saquinavir 600 mg *tid* in combination with nelfinavir and 3 reverse transcriptase inhibitors (N=18), and of saquinavir 1,200mg tid in combination with 2 reverse transcriptase inhibitors (N=20)[8].

For the saquinavir AUC we found an approximately 6-fold interindividual variation with values ranging from 0.96 to 5.96 h*mg/L. Comparable variations were observed for maximum and trough plasma concentrations.

To further define the effect of nelfinavir on the exposure to saquinavir, the saquinavir pharmacokinetics in this study were compared to those obtained earlier by us when saquinavir (Invirase®) is administered in a dosage of 1,200 mg *tid* combined with 2 reverse transcriptase inhibitors and without nelfinavir.[8] Exposure to saquinavir (as measured by plasma AUC) in the currently described 600 mg *tid* regimen with concomitant use of nelfinavir 750 mg *tid* and two reverse transcriptase inhibitors was not significantly different from exposure to saquinavir in a 1,200 mg *tid* regimen with two reverse transcriptase inhibitors and without the use of nelfinavir (p=0.86, Mann-Whitney test). This observation confirms the positive interaction between nelfinavir and saquinavir. Since the pharmacokinetics of saquinavir are non-linear (with increasing doses leading to a more than proportional increase in plasma AUC) this indicates that addition of nelfinavir to saquinavir-containing regimens increases saquinavir exposure by more than 2-fold. No significant differences were observed for C_{min} (p=0.66), C_{max} (p=0.29), $t_{\frac{1}{2}}$ (p=0.16), and T_{max} (p=0.97) using the Mann-Whitney test for independent groups. A significant (p=0.017), approximately two-fold reduction in saquinavir %PTF was observed in patients from the currently reported study as compared to patients who used saquinavir in a dosage of 1,200 mg *tid* without nelfinavir. This decrease in intrapatient fluctuation of saquinavir concentrations resulted from a lower C_{max} and a higher C_{min}.

Data from the group of 20 patients who used saquinavir in a 1,200 mg *tid* regimen suggest that saquinavir trough concentrations of at least 0.10 mg/L should be reached for adequate antiretroviral response.[8] Therefore, the time that saquinavir plasma concentrations were > 0.10 mg/L during the dosing interval was calculated for each patient. In patients from the ADAM study, saquinavir plasma concentration were > 0.10 mg/L during 88% of the dosing interval (50% of the patients had plasma concentrations > 0.10 mg/L during the whole dosing interval). For patients who used saquinavir 1,200 mg *tid* with 2 reverse transcriptase inhibitors, the average time > 0.10 mg/L was 71% (p=0.04) and only 5% of the patients had plasma concentrations > 0.10 mg/L during the whole interval.

Thus, the improved pharmacokinetic profile of saquinavir 600 mg *tid* in combination with nelfinavir 750 mg *tid* might lead to an improved virological response as compared to the dosing of saquinavir in a 1,200 mg *tid* regimen without the use of nelfinavir. This concept of an improved pharmacokinetic profile of saquinavir is illustrated in Figure 3.

When the interpatient variability of saquinavir pharmacokinetics was investigated, addition of nelfinavir had no effect on the variability of saquinavir pharmacokinetics ($p=0.88$, F-test). We conclude that concomitant administration of nelfinavir and saquinavir leads to a more than 2-fold increase in saquinavir plasma concentrations, and that observed exposure to nelfinavir and saquinavir is expected to result in substantial antiretroviral response. Furthermore, the pharmacokinetic profile of saquinavir is more favourable compared to the profile obtained when saquinavir is used in a 1,200 mg *tid* regimen without the addition of nelfinavir, since the average time that plasma concentrations are above 0.10 mg/L, a recently suggested target trough concentration, is significantly increased in the protease inhibitor combination regimen. The results of this pharmacokinetic study provide a rational basis for the combined use of nelfinavir and saquinavir in HIV-1 infected patients.

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Chapter 5

The effect of plasma drug concentrations on HIV-1 clearance rate during quadruple drug therapy.

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AIDS 1998, **12**:F111-F115.

Abstract

Objective

To investigate the relationship between exposure to antiretroviral drugs and the initial decline of plasma HIV-1 RNA.

Design

Open-label study in antiretroviral naive HIV-1 infected patients using a quadruple drug regimen (nelfinavir (NFV), saquinavir (SQV), stavudine, and lamivudine).

Methods

The elimination rate constant (k) for HIV-1 clearance was calculated during the first 2 weeks of treatment in 29 patients. Exposure to NFV and SQV was quantified on each study visit. Observed NFV and SQV concentrations were related to those expected in a reference population and a concentration ratio was calculated. The median concentration ratios for NFV and SQV, the baseline CD4+ lymphocyte count and baseline $\log_{10}$ HIV-1 RNA were correlated with k.

Results

A significant positive correlation was observed between k and the median NFV ($p=0.001$) or SQV concentration ratio ($p=0.016$) in a univariate analysis. In multivariate analyses, the median NFV concentration ratio remained significantly correlated with k.

Conclusions

The variation in the rate of decline of plasma HIV-1 RNA between patients after the initiation of a quadruple drug regimen could be explained by differences in exposure to NFV or SQV. Determination of k could be used to optimize further antiretroviral drug therapy and may be a first tool to assess antiretroviral activities of new or increasing doses of drugs administered in combination regimens. Furthermore, our data suggest that exposure to antiretroviral drugs should be incorporated in mathematical models to describe HIV-1 dynamics in more detail.

Introduction

The finding that replication of HIV-1 *in vivo* occurs continuously at high rates and the increased availability of antiretroviral drugs has led to fundamental changes in treatment strategies.[1,2] They have given rise to the widespread opinion that the minimum goal of antiretroviral drug therapy should be to suppress HIV replication as much as possible for as long as possible.[3] Several mathematical models have been used to describe the dynamics of HIV-1 replication *in vivo*.[1,2,4,5] These models assume full (or at least constant) inhibition of viral replication by antiretroviral drugs in each patient. However, interindividual variability in exposure to antiretroviral drugs is large.[6] This could result in different degrees of inhibition of viral replication between patients treated with the same drug regimen. We have investigated the relationship of exposure to antiretroviral drugs and HIV-1 clearance rates in the Amsterdam Duration of Antiretroviral Medication (ADAM) study.

Material and methods

ADAM is an ongoing, multicenter, open-label, randomised study to investigate the feasibility of long-term suppression of viral replication by a quadruple induction therapy followed by a double drug maintenance therapy after 6 or 12 months in antiretroviral-naive HIV-1-infected patients. Induction therapy consists of a regimen containing 750 mg thrice daily nelfinavir (NFV), 600 mg thrice daily saquinavir (SQV), 40 mg twice daily stavudine (d4T), and 150 mg twice daily lamivudine (3TC). Eligible patients have CD4+ lymphocyte counts $\geq 200 \times 10^6$cells/L and $\geq$1,000 copies HIV-1 RNA/ml.

Patients

Patients included in the ADAM study with at least two evaluable NFV and SQV concentrations during the first 8 weeks of therapy, and with viral load data available until 15 November 1997, were included in this substudy. Patients were instructed to ingest NFV and SQV simultaneously during a meal. The study protocol was approved by local reviewing boards and all patients gave written informed consent.

HIV-1 RNA dynamics

HIV-1 RNA levels in plasma were measured using commercially available assays (NASBA HIV-1 RNA QT and NucliSens, Organon Teknika, Boxtel, the Netherlands) according to the instructions of the manufacturer. Initially the NASBA HIV-1 RNA QT assay was used. When HIV-1 RNA levels declined to < 1,000 copies/ml (the lower limit of detection of the assay), levels were quantified using the NucliSens assay. Subsequently, when levels declined to < 400 copies/ml, an ultrasensitive protocol was used. Briefly, the RNA purified according to the NucliSens protocol was eluted in a two-step procedure from the silica particles. Subsequently, RNA was concentrated by precipitation with ethanol and sodium acetate in the presence of a pellet dye (pellet paint co-precipitate; Novagen, Madison, Wisconsin, USA). The complete RNA pellet was then used in the NucliSens RNA amplification and detection procedure according to the instruction of the manufacturer. In case of the ultrasensitive protocol, the lower limit of detection of the assay was 50 copies HIV-1 RNA/ml.

HIV-1 RNA levels in plasma were determined at baseline, and at days 7, 14, 28, and 56. For calculating the HIV-1 RNA clearance rate in plasma an exponential function was used to describe the rate of HIV-1 RNA decline during the first two

weeks for each patient. The following function was used to describe the decline of viral load (first-order elimination):

$$V_{(t)} = V_{(0)} \times e^{(-k*t)}$$

where $V_{(t)}$ represents plasma HIV-1 RNA in copies/ml at time t, $V_{(0)}$ represents the baseline viral load, k is the elimination rate constant (day^{-1}), and t is the time after start of treatment (days). All HIV-1 RNA measurements ≥50 copies/ml were used for each patient from the start of treatment until a value below 50 copies/ml was reached (including this measurement) or until day 14. For each patient the value for k was estimated using least squares regression analysis (ln $V_{(t)}$ versus t plot). The half-life of clearance of HIV-1 RNA in plasma was calculated with the equation $t_{1/2} = \ln 2/k$.

NFV and SQV exposure

On each study visit a blood sample was drawn for the quantitation of plasma concentrations of NFV and SQV using a validated and sensitive reverse-phase high-performance liquid chromatography (RP-HPLC) assay.[7] The time between drug ingestion and the drawing of the sample was noted. NFV and SQV plasma concentrations were divided by the expected concentrations of NFV and SQV, respectively, at the same time after drug administration in a reference population of 18 patients in whom full (8-h) pharmacokinetic profiles of both drugs were assessed. By this means, concentration ratios for NFV and SQV were calculated at days 7, 14, 28, and 56 to estimate exposure to both protease inhibitors adequately. For each patient, at least two NFV and SQV concentration ratios had to be evaluable (e.g. the time interval between drug ingestion and drawing of the sample was known). Individual median values for NFV and SQV concentration ratios were used as a measure of exposure to these drugs in each patient.

Statistical analysis

Univariate and multivariate linear regression analysis was performed with k as the dependent variable. Median NFV and SQV concentration ratios, baseline CD4+ lymphocyte count, and baseline $\log_{10}$ HIV-1 RNA were used as independent variables. Statistical calculations were performed with the Statistical Product and Service Solutions (SPSS) for Windows, version 6.1 (SPSS Inc., Chicago, Illinois, USA). A significance level of $P=0.05$ was used throughout.

Results

HIV-1 RNA measurements in plasma were available from 34 patients in the ADAM study during the first 56 days after start of treatment at the time of analysis. All patients had HIV-1 RNA levels < 50 copies/mL at day 56. For 5 patients, less than two evaluable NFV and SQV concentration ratios were available. Thus, it was possible to include a total of 29 patients in this substudy.

The median values and interquartile ranges for k, baseline viral load, baseline CD4+ lymphocyte count, and median NFV and SQV concentration ratios in this population of 29 patients are presented in Table 1. In our reference population of 18 patients the average values for area under the plasma concentration versus time curve (during 8 hours), maximum plasma concentration, and plasma elimination half-life were 18.1 h*mg/l, 5.2 mg/l, and 2.0 h for NFV, and 2.37 h*mg/l, 0.57 mg/l, and 2.3 h for SQV, respectively.

Table 1 Summary of baseline characteristics, HIV-1 RNA clearance rates, and exposure to protease inhibitors

	median	interquartile range	range
baseline characteristics			
HIV-1 RNA (copies/ml)	58,000	23,400 - 139,600	10,000 - 690,000
$\log_{10}$ HIV-1 RNA (copies/ml)	4.76	4.37 - 5.14	4.00 - 5.84
CD4+ lymphocytes (x 10^6 cells/l)	410	303 - 508	150 - 770
HIV clearance rates			
k (day^{-1})	0.29	0.23 - 0.32	0.13 - 0.49
$t_{1/2}$ (days)	2.42	2.19 - 3.07	1.41 - 5.33
median NFV concentration ratio	0.83	0.60 - 1.01	0.25 - 1.60
median SQV concentration ratio	0.93	0.66 - 1.51	0.11 - 2.56

k = elimination rate constant for HIV-1 RNA in plasma, NFV = nelfinavir, SQV = saquinavir

Univariate linear regression analysis was performed using k as the dependent variable, and median NFV and SQV concentration ratios, baseline CD4+ lymphocyte count and baseline $\log_{10}$ HIV-1 RNA as independent variables. Results of the univariate linear regression analysis are shown in Table 2. NFV and SQV concentration ratios were significantly and positively correlated with k (Figure 1).

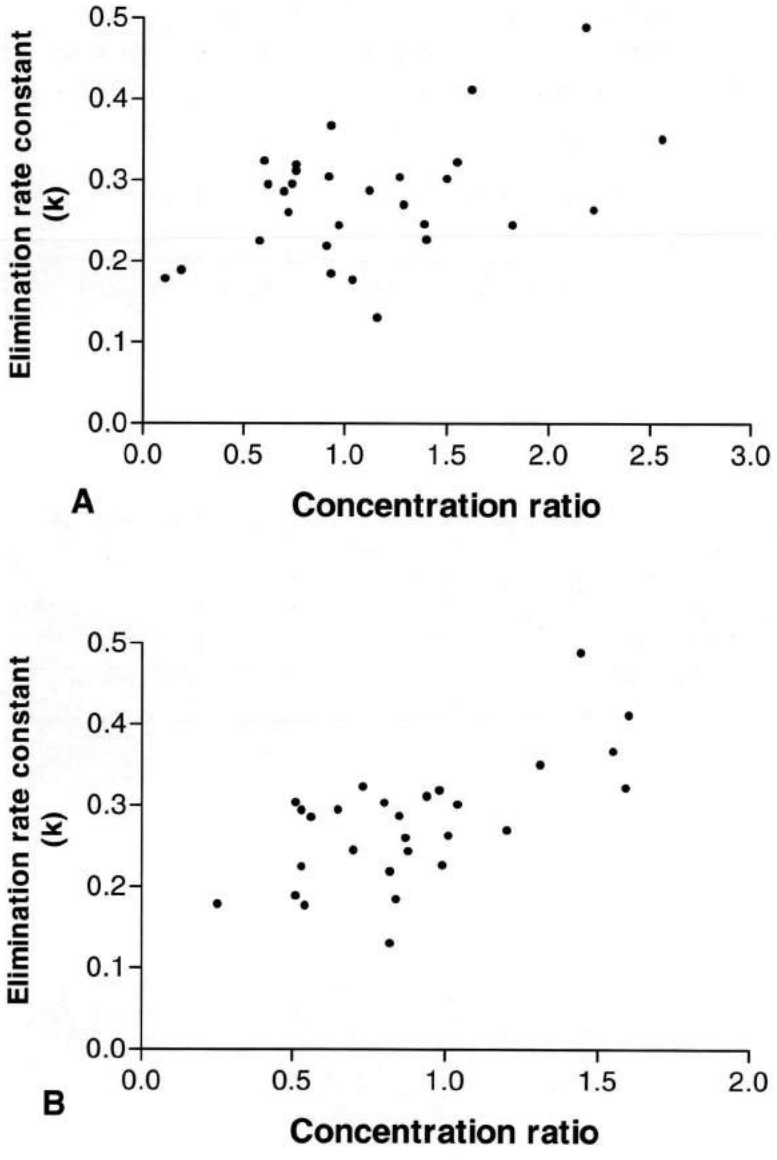

Figure 1 Relationship between (A) saquinavir concentration ratio and (B) nelfinavir concentration ratio and the elimination rate constant k for the clearance of HIV-1 RNA from plasma.

Median concentration ratios of NFV and SQV were significantly correlated (p=0.030). If NFV and SQV concentration ratios were simultaneously analysed in a multivariate linear regression model, the median NFV concentration ratio remained significantly related with k in contrast to the median SQV concentration ratio (Table 2). If baseline CD4+ lymphocyte count was added to this model, the NFV concentration ratio was still significantly correlated with k.

Table 2 Univariate and multivariate linear regression analysis using k as the dependent variable.

	Univariate linear regression analysis		Multivariate linear regression analysis			
independent variable	coefficients	p	coefficients	p	coefficients	p
median NFV concentration ratio	0.108 (0.031)	0.001	0.082 (0.035)	0.026	0.088 (0.032)	0.011
median SQV concentration ratio	0.055 (0.022)	0.016	0.030 (0.022)	0.193	0.033 (0.021)	0.128
baseline $CD4^+$ lymphocyte count*	- (0.0084) 0.016	0.064	-0.0054 (0.0081)	0.515	-	-
baseline $\log_{10}$ HIV-1 RNA	0.008 (0.030)	0.800	-	-	-	-

k = elimination rate constant for HIV-1 RNA in plasma, NFV = nelfinavir, SQV = saquinavir, *per 100 CD4 cells.
Figures are regression coefficients (with SE = standard error), and p-values.

Discussion

In this study an approximately four-fold variation in the elimination rate constant for HIV-1 RNA clearance in plasma was observed (range: 0.13 - 0.49). We have made an attempt to explain this variation, at least in part, by the differences in exposure to antiretroviral drugs between patients.

The patients in the current study used two protease inhibitors (NFV and SQV) and two nucleoside reverse transcriptase inhibitors (d4T and 3TC). Since the nucleoside analogues require intracellular phosphorylation to their active triphosphates, and plasma concentrations do not necessarily reflect the intracellular amount of pharmacological active triphosphates, these drugs were not taken into account. Protease inhibitors, on the other hand, do not require an activation step, and relationships between plasma concentrations and antiretroviral effect have been reported.[6] Thus, we decided to investigate the relationship between exposure to both protease inhibitors and HIV-1 RNA clearance rates.

Univariate linear regression analysis revealed that baseline CD4+ lymphocyte count and baseline $\log_{10}$ HIV-1 RNA did not correlate with k. However, significant positive relationships were found between the median NFV or SQV concentration ratio and k (Fig. 1). Since median SQV and NFV concentration ratios were positively correlated, and baseline CD4+ lymphocyte count almost reached significance in the univariate analysis, these parameters were analysed in multivariate linear regression analyses. The baseline CD4+ lymphocyte count was not correlated with k in a multivariate analysis. The NFV concentration ratio remained significantly related with k in contrast to the SQV concentration ratio. This observation can be interpreted in several ways. It could mean that NFV has a stronger effect than SQV on the initial elimination rate of HIV-1 RNA in plasma when used in this drug regimen. On the other hand, the effect of SQV on viral replication could be maximal at observed plasma concentrations (as opposed to NFV), and a relationship between exposure and k could therefore not be found at currently observed concentrations. Furthermore, SQV and NFV concentrations are significantly correlated due to the fact that NFV increases SQV concentrations in plasma.[6] This correlation makes it difficult to separate the effects of both protease inhibitors in this study.

A more rapid decline of HIV-1 RNA in plasma after start of treatment may be important to prevent the emergence of drug-resistant virus. Recently, the results of AIDS Clinical Trials Group 343 pointed out that the time to HIV-1 RNA load below 200 copies/mL was a risk factor for treatment failure.[8] This observation justifies that a

fast decline of HIV-1 RNA after start of therapy should be investigated as a potential objective of antiretroviral therapy.

The decrease of HIV-1 RNA in plasma was described during the first two weeks of therapy by measuring the HIV-1 RNA load on three occasions. A more frequent sampling strategy would have resulted in a more accurate estimate of k. The currently observed values for k are somewhat lower than reported by Ho *et al.*[1] and Perelson *et al.*[5] This can be explained by the less frequent sampling schedule in our study and the gradual decrease of the HIV-1 RNA decline after start of treatment. The possibility of an underestimation of k does not affect the primary outcome of this study (a relationship exists between drug exposure and the elimination rate constant). In this study we have used single timepoint measurements to estimate the exposure to drugs in the patients. Determination of the area under the concentration versus time curve might have been more accurate to estimate exposure of these drugs, but is also less practical.

The main outcome of this study is that higher exposure to NFV or SQV resulted in a higher clearance rate of HIV-1 RNA in this population. This observation could be used for further improvement of the initial response to antiretroviral drug therapy in individual patients by optimising plasma drug concentrations (through dose adjustment). Assessing the value of k could also be used as a first tool to measure antiretroviral activity of new or increasing doses of drugs in combination therapy regimens. Furthermore, up to now, mathematical models to describe HIV-1 dynamics *in vivo* have assumed full (or at least constant) inhibition of viral replication in all patients treated with the same drug regimen.[5] The currently reported results justify, however, that exposure to antiretroviral drugs should be taken into account in these models to describe HIV-1 dynamics *in vivo* more accurately.

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Chapter 6

Toxicity and drug exposure in a quadruple drug regimen in HIV–1 infected patients participating the ADAM study.

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Abstract

Objective

To study the relationship between toxicity and the exposure to nelfinavir and saquinavir as part of a quadruple drug regimen.

Design

The ADAM study is a randomized study to investigate the feasibility of induction-maintenance therapy in HIV-1 infection.

Methods

HIV-1-infected patients with no prior use of antiretroviral treatment started induction therapy consisting of stavudine + lamivudine + nelfinavir + saquinavir for a period of 26 weeks. Data regarding toxicity of the quadruple regimen and exposure to the protease inhibitors were collected.

Results

Seven of the 65 patients enrolled had to switch therapy for reasons of toxicity within the first 26 weeks. Diarrhoea was frequently reported (49 of 65, one discontinuation), but could be relieved by using antidiarrhoeal agents. Laboratory monitoring revealed elevated liver enzymes (leading to 4 discontinuations) and mild to moderate elevations of triglycerides and cholesterol (nine and 23 of 65, respectively). The exposure to saquinavir and nelfinavir was lower than expected. Abdominal pain was associated with a higher exposure to nelfinavir or saquinavir. The association of nausea and abdominal distension with drug exposure appeared to vary over time.

Conclusions

The quadruple drug regimen was quite well tolerated. Diarrhoea was frequently reported but could be relieved by the use of antidiarrhoeal agents. In comparison to other protease inhibitor combinations, lipid abnormalities in plasma were infrequent and mild. With the exception of diarrhoea, all gastrointestinal complaints observed were found to be associated with the level of exposure to nelfinavir or saquinavir. The exposure to the protease inhibitors was relatively low, although the virologic efficacy of the regimen used was satisfactory.

Introduction

A durable suppression of viral replication in HIV-1 infection can be achieved by giving a combination of three antiretroviral agents.[1-3] Highly active antiretroviral therapy (HAART) can result in clinical benefit in terms of a prolonged (disease-free) survival.[4,5] The use of more than three agents or a higher exposure to a specific antiretroviral agent might even increase the potency of an antiretroviral regimen.[6-9] However, the occurrence of side effects may hamper the use of intensified treatment regimens .[10,11]

Protease inhibitor combinations are pharmacokinetically favourable in antiretroviral therapy.[12,13] All protease inhibitors show mild to moderate gastrointestinal side effects in addition to a rather unique safety and tolerability profile. Recently, the association between the use of protease inhibitors and the occurrence of a new onset of diabetes mellitus, hyperlipidaemia or peripheral lipodystrophy has been described.[14] However, there is only limited data regarding the safety profiles of PI combinations, such as ritonavir or nelfinavir plus saquinavir.[15-19]

A quadruple induction regimen, consisting of stavudine, lamivudine, nelfinavir, and saquinavir, was used for at least 26 weeks in antiretroviral therapy-naïve patients in the Amsterdam Duration of Antiretroviral Medication (ADAM) study.[20] In this study, the feasibility of an induction-maintenance strategy in HAART was investigated. The preliminary results of the efficacy of this induction-maintenance regimen have already been described.[20] In this paper, we focus on the toxicity of the quadruple induction regimen used in the ADAM study and its relationship with the exposure to nelfinavir and saquinavir.

Materials and methods

Patients

The enrolment of HIV-1-infected patients in this open-label randomised-controlled study started in March 1997 and was prematurely discontinued on April 6, 1998, as the interim analysis demonstrated increased viral replication in patients on maintenance therapy.[20] Patients, aged 18 years or more, were eligible for participation if they had a CD4+ cell count of at least 200 x 10^6/l in their peripheral blood, 1000 or more HIV-1 RNA copies/mL in plasma, and if they were antiretroviral therapy-naïve. Exclusion criteria were the existence of an active opportunistic infection, active hepatitis C or presence of the hepatitis B surface antigen, women who were breast-feeding or pregnant, the use of immunomodulatory drugs or investigational drugs up to 1 month prior to the start of the study medication, and certain laboratory parameters (Hb <7 mmol/l (male) or <6.5 mmol/l (female), neutropenia <0.75x10^9/l, aspertate-amino transferase (ASAT)/alanine-amino transrefase (ALAT) >5 x upper limit of normal (ULN), serum creatinine >1.5 x ULN). Drugs such as rifampin or ketoconazole, which have a strong pharmacokinetic interaction with protease inhibitors, were not allowed. Written informed consent was obtained from all patients. The Institutional Review Boards of all participating centres approved the study protocol.

Study design

All patients started therapy with a quadruple drug regimen consisting of stavudine (40 mg twice daily, or 30 mg twice daily for those with body weight < 60 kg), lamivudine (150 mg twice daily), nelfinavir (750 mg three times a day) and saquinavir hard-gelatin-capsules (saquinavir-HGC, 600 mg three times a day). When saquinavir soft-gelatin-capsules (saquinavir-SGC) became available (1 November 1997), all patients using saquinavir-HGC switched to saquinavir-SGC (800 mg three times a day). Roche (Roche NL, Mijdrecht, the Netherlands) provided both nelfinavir and saquinavir-SGC. Patients were instructed to take their medication with food.

Follow-up

During the induction phase, patients were scheduled to visit the outpatient clinic for clinical assessment and routine laboratory monitoring at the start of treatment and at weeks 1, 2, 4, 8, 16, 24, 25 (for plasma HIV-1 RNA concentration

assessment only) and 26. Laboratory monitoring included plasma HIV-1 RNA concentration (Nuclisens HIV-1 RNA QT assays (Organon Teknika, Boxtel, Netherlands)), CD4+ and CD8+ cell count and plasma concentrations of nelfinavir and saquinavir. At baseline no plasma concentrations of nelfinavir and saquinavir were assessed.

Toxicity grading

The occurrence of side effects (signs, symptoms, or laboratory abnormalities) was assessed during each study visit. The severity of each event was graded according to the World Health Organization (WHO) classification (grades 1 to 4; Appendix 1) and the probability of a relationship with the study medication used was indicated (see also Appendix 1).[21] As the abnormalities in plasma cholesterol were not graded in the WHO classification, a more recently defined grading system by the AIDS Clinical Trial Group (ACTG) was used for the analysis of this parameter.[22] The probability of a relationship with the study medication used was indicated and the use of concomitant medication to relieve side effects was recorded.

Assessment of drug exposure

The quantification of plasma concentrations of nelfinavir and saquinavir was performed using a validated and sensitive reverse-phase high-performance liquid chromatography (RP-HPLC) assay.[23] For each sample the drug plasma concentration was divided by the expected drug concentration at the corresponding time-point, to adjust for the time interval between drug ingestion and the drawing of the sample. The expected concentrations of nelfinavir or saquinavir at different time points were obtained from full (8h) pharmacokinetic profiles of both drugs assessed in 18 patients participating in the ADAM study.[24] These plasma concentration ratios were used as a measure of exposure to both protease inhibitors.

Treatment failure and discontinuations

After attaining a plasma HIV-1 RNA concentration below the quantification limit of the ultrasense assay (<50 copies/ml), a plasma HIV-1 RNA concentration above 400 copies/ml at two consecutive time points was considered as a treatment failure.

In case of a grade 4 toxicity (WHO classification) or grade 3 toxicity with no improvement after temporary discontinuation (maximum 2 weeks), or the recurrence of a grade 3 toxicity after re-challenge, permanent discontinuation of the

study medication was obligatory.[21] Further therapy was at the discretion of the investigator after treatment failure or discontinuation of the study medication.

Analysis

The baseline values and the changes from baseline to week 4, 8, 16 and 26 for plasma HIV-1 RNA concentration, and CD4+ and CD8+ cell counts of all patients were assessed. Patients with and without a specific side effect were compared for baseline values of these parameters and the change from baseline to week 26 (t-test).

The proportion of patients experiencing a specific side effect was assessed. In addition, the duration of the side effect within the first 6 months was calculated. Only side effects with an incidence of >10% and a possible relationship with the study medication used were taken into account for analysis.

The correlation coefficient between nelfinavir and saquinavir per patient was assessed. For each patient, the median plasma concentration ratios of nelfinavir and saquinavir over time were calculated. To evaluate whether the plasma concentration ratio of nelfinavir and saquinavir varied over time, a generalized linear model with mixed effects was estimated (Mixed Models procedure of the statistical package SAS 6.12 for Windows, SAS institute, Cary, North Caroline, USA).

The median plasma concentration ratios of nelfinavir and saquinavir of patients on treatment with or without a specific side effect were compared (Wilcoxon test). In addition to this comparison between groups of patients, the odds ratio for experiencing a specific side effect at a specific time point for the corresponding drug exposure was estimated by using the generalized estimating equations (GEE) method, taking into account the 'within patient' correlation between time points.[25] Time (as a factor) and plasma concentration ratio (as a covariate) were included in the model as main effects, as well as the interaction between these two variables. The analysis was based on the logistic link function and the exchangeable working correlation matrix. Calculations were performed by using the GENMOD procedure of the statistical package SAS 6.12 for Windows. To calculate *P*-values from the results of the GENMOD procedure, a SAS macro was written using the Wald statistic.

Results

Patients and discontinuations

In total, 65 patients (61 males, 94%) were enrolled. The baseline characteristics of these patients are summarized in Table 1. Two patients were lost to follow-up (one for logistic reasons, one withdrew from further medical follow-up after moving to another city). One patient discontinued his study medication due to virologic failure at week 24. Of the remaining 62 patients, seven patients discontinued the study medication due to side effects within the first 26 weeks (see '*Toxicity*').

Table 1 Baseline characteristics

Characteristic		All patients (n=65)
Male[a]		61 (94)
Age (years)[b]		39 (±8)
CDC*classification[26]		
A[a]		47 (72)
B[a]		16 (25)
C[a]		2 (3)
CD4+ cell count	(x 10^6 cells/l)[c]	410 (320-510)
CD8+ cell count	(x 10^6 cells/l)[c]	1050 (910-1460)
$\log_{10}$ HIV-1 RNA	(copies/mL)	4.51 (4.18-5.04)

Values are [a] number (%); [b] mean (± SD); [c] median (interquartile range).

CD4+ and CD8+ cell count and plasma HIV-1 RNA concentrations during therapy

The median $CD4^+$ cell count rose from 410 x 10^6 at baseline to 560 x 10^6 cells/l at week 26. The median $CD8^+$ cell count decreased from 1050 x 10^6 at baseline to 970 x 10^6 cells/l at week 26. The median baseline plasma HIV-1 RNA concentration was 4.51 $\log_{10}$ copies/ml. At weeks 4, 8, 16 and 26, the median plasma HIV-1 RNA concentration declined to 2.58, 2.42, 2.25 and 1.54 $\log_{10}$ copies/ml, respectively.

Toxicity

The frequency of the occurrence of side effects, the median of the total duration of the side effect per patient in this population and the percentage with a grade 1, 2, 3, or 4 severity are summarized in Table 2. If the use of loperamide was longer than the duration of diarrhoea, the duration of diarrhoea was extended for the period during which loperamide was used and the severity was graded as 1.

Clinical side effects

Forty-nine of the 65 patients suffered from diarrhoea, mostly consisting of loose or watery stools, two or three times daily. Loperamide was used in 30 patients for a mean duration of 128 days (SD: 61) to relieve the complaint. Diarrhoea led to discontinuation of the study medication in one patient, after 8 weeks. Other side effects leading to discontinuation of the study medication were headache (one patient, week 22), and peripheral neuropathy, probably related to the use of stavudine (one patient, week 24). In addition to gastrointestinal side effects such as abdominal pain, nausea and abdominal distension, fatigue and headache were frequently reported. No peripheral lipodystrophy was observed within the first 26 weeks of treatment.

Table 2 Toxicity

Side effect	Frequency		Duration (days)	Severity (%)[a]			
	N	(%)	Median (IQR)	1	2	3	4
Clinical							
Diarrhoea[b]	49	(75)	98 (42-164)	51	43	6	-
Nausea	13	(20)	12 (4-162)	77	23	-	-
Abdominal pain	9	(14)	40 (13-122)	57	29	14	-
Abdominal distension	8	(12)	63 (19-107)	75	25	-	-
Fatigue	18	(27)	55 (21-167)	72	28	-	-
Headache	11	(17)	44 (30-127)	73	18	-	-
Laboratory							
Elevated liver enzymes	12	(18)	27 (14-56)	50	17	17	16
Elevated cholesterol	23	(35)	14 (14-67)	-	88	12	-
Elevated triglycerides	9	(14)	70 (14-168)	-	100	-	-

[a] See also Appendix 1-1, 2; [b] Corrected for the use of loperamide. IQR, interquartile range.

Abnormalities of laboratory values

Mild to severe elevations of the liver enzymes occurred in 12 patients. In four of these patients the elevated liver enzymes resulted in a discontinuation of the study medication (at week 4, 8, 16, and 20, respectively). The occurrence of elevated cholesterol in plasma was frequent; 23 out of 65 patients (35%). However the cholesterol level rose above 7.7 mmol/l in only six of these 23 patients. Hypertriglyceridaemia was less frequent and did not exceed grade 2 severity. No hyperglycaemia or glycosuria was observed within the first 26 weeks of treatment.

Toxicity and patient characteristics

Patients with or without a specific complaint did not differ with respect to their baseline characteristics. Only patients complaining about fatigue had a lower

baseline CD4+ cell count and a higher plasma HIV-1 RNA concentration compared to patients without this complaint (t-test $P = 0.02$ and $P = 0.03$, respectively). The patients complaining of abdominal pain had a greater decrease in their plasma HIV-1 RNA concentration at week 26 than patients without abdominal pain (t-test $P = 0.02$; data not shown).

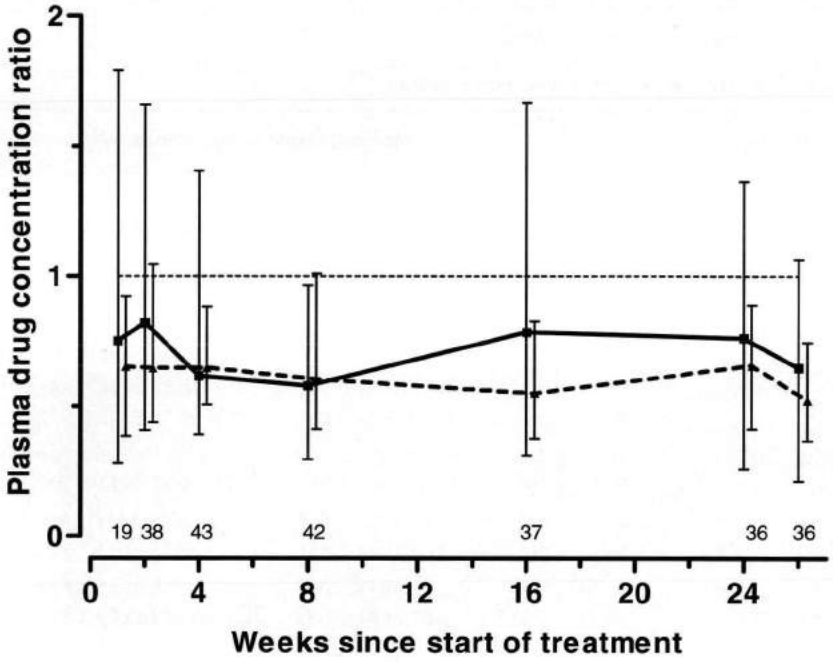

Figure 1 Median plasma concentration ratios of nelfinavir (dashed line) and saquinavir (solid line) over time. The numbers at the bottom of the figure represent the number of samples available for the different time points. Bars represent interquartile ranges.

Drug exposure

The correlation coefficient between the nelfinavir and saquinavir plasma concentrations per patient was calculated. The mean correlation coefficient in this group of patients was 0.55 (95% confidence interval (CI) 0.41 - 0.67); calculation of the confidence intervals was based on the Fisher transformation of the mean. As a result of unknown time intervals (e.g. either an unknown time of medication intake and/or the drawing of the sample), plasma drug concentration ratios could only be calculated at two or more time points in 56 patients. The median plasma concentration ratio over time per patient for nelfinavir and saquinavir varied between 0.11 and 1.31 for nelfinavir (median 0.60; inter-quartile range (IQR) 0.44-

0.86) and between 0.11 and 6.50 for saquinavir (median 0.75; IQR 0.42-1.37). For both nelfinavir and saquinavir, the median plasma concentration ratios were significantly lower than 1 (95% CI 0.59 - 0.93 and 0.51 - 0.68, respectively); calculation of the confidence intervals was based on the geometric mean. The plasma concentration ratios of nelfinavir and saquinavir in these patients did not change over time (Fig. 1; analysis of repeated measures $P = 0.76$ and $P = 0.68$ for nelfinavir and saquinavir, respectively).

Table 3 Toxicity and plasma concentration ratios

Side effect		N	Median plasma concentration ratio NFV median (IQR)	SQV median (IQR)
Diarrhoea	Yes	41	0.59 (0.44-0.83)	0.61 (0.34-1.02)*
	No	15	0.62 (0.51-1.05)	1.19 (0.65-1.66)*
Nausea	Yes	11	0.59 (0.41-0.77)	0.78 (0.27-1.52)
	No	45	0.60 (0.45-0.87)	0.70 (0.43-1.36)
Abdominal distension	Yes	8	0.57 (0.43-0.81)	0.38 (0.29-0.85)
	No	48	0.60 (0.44-0.88)	0.78 (0.45-1.57)
Abdominal pain	Yes	8	0.95 (0.61-1.23)**	1.73 (0.83-2.70)**
	No	48	0.59 (0.44-0.83)**	0.68 (0.42-1.10)**
Headache	Yes	10	0.59 (0.44-0.90)	0.70 (0.45-1.36)
	No	46	0.60 (0.45-0.85)	0.75 (0.42-1.39)
Fatigue	Yes	17	0.58 (0.51-0.81)	0.78 (0.43-1.52)
	No	39	0.61 (0.41-0.89)	0.72 (0.89-1.36)
Elevated liver enzymes	Yes	9	0.60 (0.44-0.87)	0.78 (0.45-1.34)
	No	47	0.60 (0.45-0.85)	0.72 (0.42-1.39)
Elevated cholesterol	Yes	19	0.61 (0.41-0.95)	0.67 (0.28-1.39)
	No	36	0.60 (0.46-0.83)	0.75 (0.44-1.23)
Elevated triglycerides	Yes	9	0.94 (0.51-1.01)	0.67 (0.28-1.82)
	No	46	0.60 (0.44-0.83)	0.75 (0.42-1.34)

IQR, inter quartile range * $P = 0.04$ (Wilcoxon Test); ** $P = 0.02$ for nelfinavir and $P = 0.05$ for saquinavir (Wilcoxon Test).

Toxicity and drug exposure

The median plasma drug concentration ratios over time of patients with and without a specific complaint, are tabulated in Table 3. Patients with abdominal pain appeared to have higher plasma concentration ratios for nelfinavir and saquinavir than patients without this complaint (Wilcoxon test: $P = 0.02$ and $P = 0.05$, respectively).

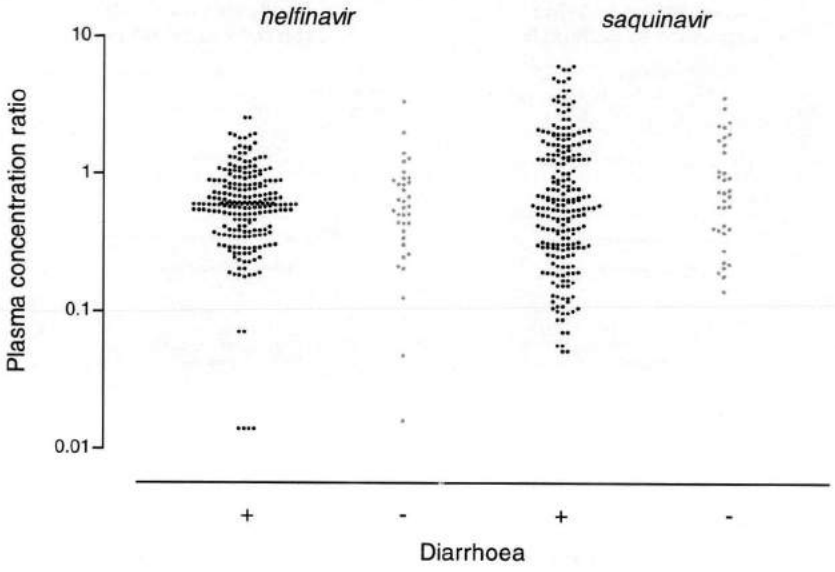

Figure 2 Plasma concentration ratios of nelfinavir and saquinavir in patients with and without diarrhoea. Left side, nelfinavir; right side, saquinavir. Dots, patients with diarrhoea; circles, patients without diarrhoea.

The patients with diarrhoea had lower saquinavir plasma concentration ratios than patients without diarrhoea (Fig. 2; Wilcoxon test: $P = 0.04$). No differences in drug exposure were found for any of the other side effects.

Using the GEE method, the occurrence of diarrhoea was found to be associated with the exposure to nelfinavir and not with the exposure to saquinavir (Fig. 3A), even if the diarrhoea was not corrected for the use of loperamide (data not shown). For the other gastrointestinal complaints, evidence was found for a time-dependent relationship with the plasma drug concentration ratios (Figures 3B-D). Both abdominal distension and nausea became associated with a low exposure to nelfinavir and saquinavir after the first weeks of therapy and for nausea, this association seemed to increase over time ($P<0.001$). The association between abdominal pain and a high drug exposure remained present during the entire treatment period ($P<0.001$). For all other complaints, no evidence was found for a relationship with plasma drug exposure (data not shown).

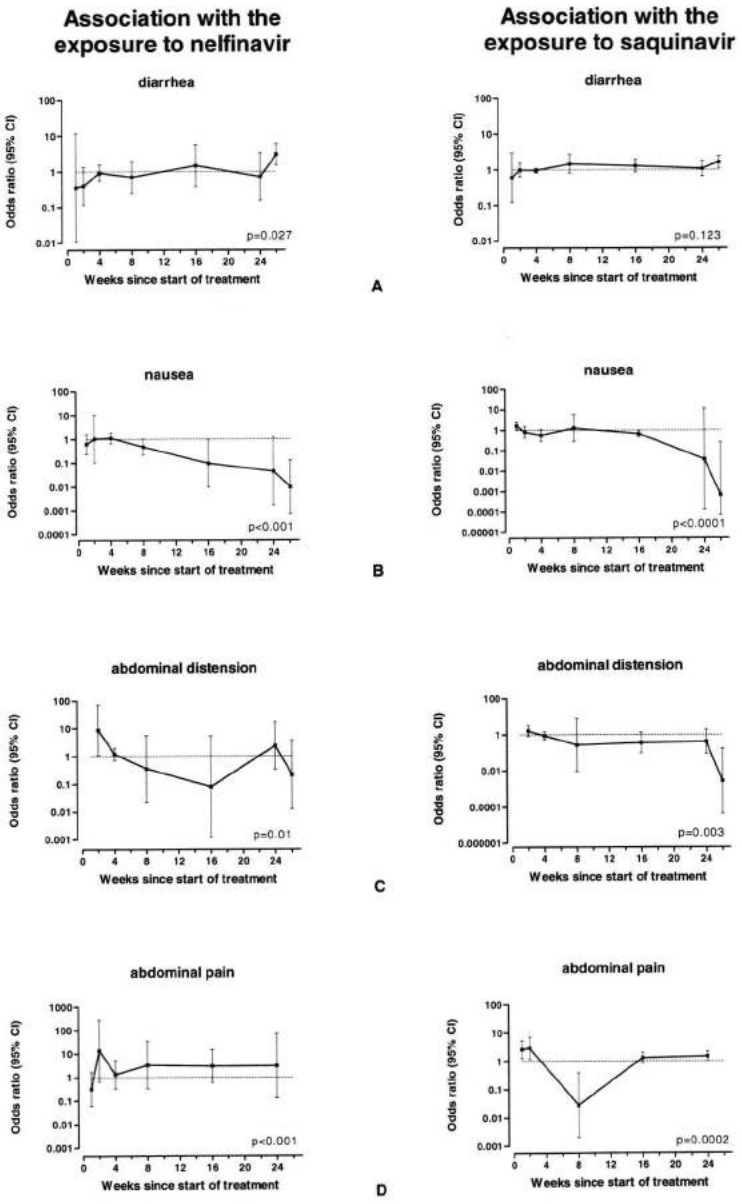

Figure 3 The association between a certain side effect and exposure to nelfinavir (left column of figures) or saquinavir (right column of figures). In each figure, the dotted line represents an odds ratio of one, e.g. no association, whereas the solid line represents the odds ratio between a certain side effect and exposure to nelfinavir or saquinavir over time. Bars represent the 95% confidence interval.

Discussion

The quadruple induction therapy in the ADAM study was quite well tolerated. Seven out of 65 patients had to discontinue their study medication within the first 26 weeks of therapy. The incidence and severity of the observed side effects were comparable with those reported in other studies.[15,16,26,27] The most frequently reported side effect, diarrhoea, led to discontinuation of the antiretroviral drug combination in only one patient. Although tolerable with the use of loperamide, diarrhoea was persistent in almost all patients. Furthermore, the occurrence of diarrhoea was not consistently associated with the exposure to nelfinavir or saquinavir, in contrast to other gastrointestinal complaints.

Recently, many studies have focussed on the association between protease inhibitor use and the occurrence of a new onset of diabetes mellitus, lipodystrophy or hyperlipidaemia.[14] The median time to the development of peripheral lipodystrophy was reported to be ten months which might explain why this side effect was not yet observed within our population.[14] Hyperlipidaemia was present within the observed treatment period, although the elevations of triglycerides and cholesterol were usually mild and infrequent in comparison to those reported in studies using a combination of ritonavir and saquinavir.[17-19]

Surprisingly, the plasma concentrations of nelfinavir and saquinavir obtained were relatively low compared with the expected levels obtained under standardized conditions. These expected plasma drug concentrations obtained in a clinical setting and after the use of a standard meal, probably resulted in higher plasma drug levels than those obtained in daily practice. This was found even though all patients in this study were instructed to take their medication with food. Despite this low exposure, virologic efficacy of the quadruple regimen was found to be satisfactory in the first 26 weeks. Only one patient failed to attain plasma HIV-1 RNA concentrations below 400 copies per ml. However, it remains to be seen whether these plasma drug concentrations are sufficient for durable suppression of the viral replication.

In contrast to the findings of Khaliq *et al.* the plasma concentration ratios of the protease inhibitors in the antiretroviral combination used in the ADAM study did not decline during the first 26 weeks.[29] However, by replacing saquinavir HCC with saquinavir SGC, the exposure to saquinavir within this population may have been maintained over time.[30,31]

Figure 1 illustrates the variation of plasma concentration ratios of nelfinavir and saquinavir in patients over time. Considering this wide variation, the odds ratio for the occurrence of a specific side effect at a specific time point as a result of drug exposure was estimated. Most gastrointestinal complaints were found to have a time-dependent relationship with drug exposure. For both nausea and abdominal distension, the association with drug exposure varied within the first weeks of treatment. This change of association within the first weeks of treatment could indicate that the pharmacokinetics of the drugs and the tolerance of the patient had not yet reached an equilibrium. In addition, the use of stavudine and lamivudine might have concealed a possible relationship of nausea with the exposure to protease inhibitors. After the first weeks of therapy, however, abdominal distension and nausea were both associated with a low exposure to the protease inhibitors used. For nausea this association became even stronger over time. This could indicate a reduced uptake of the protease inhibitors in these patients, but could also illustrate that especially patients with nausea developed a less compliant behaviour, resulting in low plasma drug concentrations. Plasma drug monitoring in these patients could be useful when virologic efficacy appears insufficient.

The patients with abdominal pain were found to have both a high drug exposure as well as a greater decrease in HIV-1 RNA within the first 26 weeks. Hoetelmans *et al.* found that a higher clearance rate of HIV-1 RNA was found in patients with a higher exposure to nelfinavir and saquinavir.[9] It may therefore be concluded that the elevation of drug exposure to nelfinavir and saquinavir in order to increase virologic effectiveness might be hampered by the occurrence of abdominal pain.

In dose-finding studies, diarrhoea appeared to be the dose-limiting side effect of nelfinavir,[32,33] while a higher bio-availability of saquinavir was more likely to cause diarrhoea, suggesting a relationship between the exposure to a PI and the occurrence of this side effect.[31,34] The patients with diarrhoea in our study were found to have lower levels of saquinavir than patients without diarrhoea (Wilcoxon test). This was however not confirmed using the GEE method. In fact, using this more sensitive method, the occurrence of diarrhoea was not related to the exposure to saquinavir, but had an inconsistent association with the exposure to nelfinavir. It is possible that the occurrence of diarrhoea has been both a cause and an outcome of drug exposure, which might have concealed a significant association.

None of the other clinical or laboratory abnormalities could be related to the observed drug exposure. The occurrence of fatigue and headache might be explained by the (continuous) use of nucleoside reverse transcriptase inhibitors as

well. In addition, fatigue is a side effect that is more likely to occur in patients with a more advanced disease stage. This was confirmed in our study since patients experiencing fatigue had a significantly lower CD4+ cell count and a higher HIV-1 RNA concentration in plasma at baseline than patients without this complaint.

In conclusion, the quadruple induction therapy was quite well tolerated. Diarrhoea was the most frequently reported side effect. During the first 26 weeks of therapy, the elevations of plasma triglycerides and cholesterol were mild and infrequent in comparison to those observed in other protease inhibitor combinations. Clear and time-dependent relationships with the amount of drug exposure were only found for nausea, abdominal distension and abdominal pain. The exposure to the protease inhibitors in this population was lower than expected, suggesting that the compliance to timing, dosing and food intake required may not be achieved in daily practice. Nevertheless, the virologic efficacy of the quadruple drug regimen was quite satisfactory.

Acknowledgements

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Side effect	Grade I	Grade II	Grade III	Grade IV
Nausea	Mild or transient: reasonable intake maintained	Moderate discomfort or intake decreased for <3 days	Severe discomfort or minimal intake for ≥3 days	Hospitalization required
Abdominal pain	Transient or mild discomfort: no limitation in activity; no medical intervention /therapy required	Mild to moderate limitation in activity; some assistance may be needed: no or minimal medical intervention/therapy required	Marked limitation in activity; some assistance usually required: medical intervention/therapy required, hospitalizations possible	Extreme limitation in activity; significant assistance required; significant medical inter-vention/ therapy required, hospitalization or hospice care probable
Abdominal distension	Transient or mild discomfort: no limitation in activity; no medical intervention /therapy required	Mild to moderate limitation in activity; some assistance may be needed: no or minimal medical intervention/therapy required	Marked limitation in activity; some assistance usually required: medical intervention/therapy required, hospitalizations possible	Extreme limitation in activity; significant assistance required; significant medical inter-vention/ therapy required, hospitalization or hospice care probable
Diarrhoea	Mild or transient: 2-3 episodes/day or mild diarrhoea lasting <1 week	Moderate or persistent: 5-7 loose stools/day or diarrhoea lasting ≥1 week	Bloody diarrhoea or orthostatic hypotension or >7 loose stools/day or IV therapy required	Hypotensive shock or hospitalization required
Fatigue	Normal activity reduced <25%	Normal activity reduced 25%-50%	Normal activity reduced >50% cannot work	Unable to care for himself/herself
Headache	Mild: no medication required	Moderate: non-narcotic analgesia therapy required	Severe: responds to initial narcotic therapy	Intractable: requiring repeated narcotic therapy
Elevated liver enzymes	1.25 - 2.5 x ULN of AST, ALT, AF or γ- GT	>2.5 - 5.0 x ULN of AST, ALT, AF or γ- GT	>5.0 - 10.0 x ULN of AST, ALT, AF or γ- GT	>10.0 x ULN of AST, ALT, AF or γ- GT
Elevated triglycerides	-	4.5 - 8.4 mmol/L or 400 - 750 mg/dL	8.5 - 13.5 mmol/L or 751 - 1200 mg/dL	>13.5 mmol/L or > 1200 mg/dL
Elevated cholesterol*	-	6.2 - 7.7 mmol/L or 240 - 300 mg/dL	7.8 - 10.3 mmol/L or 301- 400 mg/dL	>10.3 mmol/L or > 400 mg/dL

*ACTG - guidelines for toxicity 1997 ULN: Upper limit of normal.

Chapter 7

Quality of life in maintenance- vs prolonged induction therapy for HIV.

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To the Editor:

The feasibility of induction-maintenance therapy for human immunodeficiency virus type 1 (HIV) infection has been studied as a strategy to simplify antiretroviral regimens.[1-3] In the Amsterdam Duration of Antiretroviral Medication study, maintenance dual therapy after 26 weeks of quadruple induction therapy resulted in less viral suppression than prolonged induction therapy.[3] However, a prolonged quadruple regimen may have a negative impact on patients' quality of life (QOL) because of pill burden and adverse effects. We compared QOL in maintenance vs prolonged induction therapy.

Methods

Antiretroviral-naive HIV-infected patients with a CD4 cell count of at least 200 x 10^6/L (200/μL) and 1000 HIV RNA copies/mL received 26 weeks of induction therapy comprising stavudine, lamivudine, saquinavir, and nelfinavir. If the plasma HIV RNA concentration at weeks 24 and 25 was less than 50 copies/mL, patients were randomly assigned to receive prolonged 4-drug induction or maintenance therapy (either stavudine and nelfinavir or saquinavir and nelfinavir). From week 26, plasma HIV RNA concentrations were assessed by an ultrasensitive assay procedure (Amplicor HIV-1 Monitor Ultrasensitive; Roche Diagnostics, Branchburg, NJ) with a variable quantification limit. Clinical results have been reported elsewhere.[3]

In a subsample, QOL was assessed at weeks 24 and 48 by the Medical Outcome Study (MOS) HIV Health Survey, comprising 10 subscales.[4] We calculated changes in QOL scores from week 24 to week 48. Effect sizes for between-group differences were calculated by dividing mean differences by pooled SD.[5] Effect sizes equaling 0.20, 0.50 and 0.80 are considered to indicate small, moderate and large effects, respectively.[5] We calculated correlation coefficients between the plasma HIV RNA concentration at week 48 and changes in QOL scores. Analysis was by intention to treat.

Results

Ten out of 16 patients assigned to receive maintenance therapy and 9 of 15 patients assigned to receive prolonged induction therapy participated in the QOL study. Both groups were comparable ($p > .20$) in terms of age (39 vs 44 years), sex (91% vs 100% men), Centers for Disease Control and Prevention HIV classification A (73% vs 67%), median baseline CD4 cell count (370 x 10^6/L vs 420 x 10^6/L), and median baseline HIV RNA $\log_{10}$ copies/mL (4.50 vs 4.58).[6]

Participants were similarly comparable to those who did not participate. Patients assigned to receive maintenance therapy showed more decline in QOL scores than patients assigned to receive prolonged induction therapy on the following MOS-HIV subscales: physical function (-11 points; effect size, 0.4), role function (-18 points; effect size, 0.4), social function (-17 points; effect size, 0.5), overall QOL (-19 points; effect size, 0.7), health distress (-17 points; effect size, 0.7), health perceptions (-13 points; effect size, 0.5) and energy/fatigue (-8 points; effect size, 0.3). At week 48, plasma HIV RNA was higher in the maintenance group than in the prolonged induction group (2.3 $\log_{10}$ copies/mL vs 1.6 $\log_{10}$ copies/mL; p=.05), although concentrations in both groups were quite low. A higher plasma HIV RNA concentration was correlated with more decline in QOL scores for energy/fatigue (r= -0.51; p=.03), social function (r= -0.66; p=.003), health distress (r= -0.64; p=.005), health perceptions (r= -0.55; p=.02) and overall QOL (r= -0.58; p=.009) (Figure).

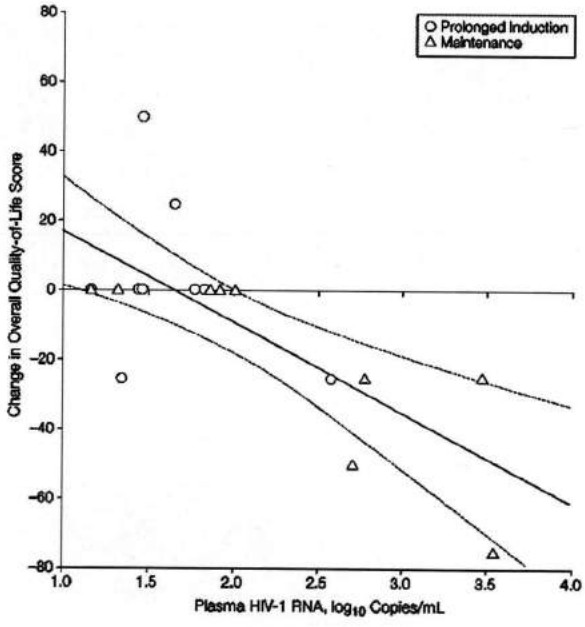

Figure 1 HIV indicates human immunodeficiency virus type 1. Values on the y-axis that are less than 0 indicate decline in quality of life, whereas values greater than 0 indicate improvement in quality of life. Solid line is regression and regression prediction line of the mean; dashed lines, 95% confidence interval.Horizontal line indicates no change in quality of life. There were 10 patients allocated to maintenance therapy and 9 to prolonged induction therapy.

Comment

Quality-of-life scores declined more during maintenance therapy than during prolonged induction therapy. The data from this small unblinded study raise the interesting possibility that the negative effects of inferior viral suppression on QOL were greater than the added burden of a 4-drug regimen.

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Chapter 8

Concentrations of stavudine, lamivudine, nelfinavir, and saquinavir in plasma, cerebrospinal fluid, and seminal plasma of HIV-1-infected individuals.

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-submitted for publication-

Abstract

In the ADAM study, a study investigating the feasibility of induction maintenance therapy, a combination of four antiretroviral agents was used as an induction therapy for 26 or 50 weeks. In 12/65 HIV-1-infected patients participating this study, concentrations of stavudine, lamivudine, nelfinavir, and saquinavir, were assessed in plasma, cerebrospinal fluid (CSF) and semen after 26 or 50 weeks of continuous treatment. Five of them could not obtain semen on the requested day. Two patients refused a lumbar puncture. In all but one patient, the HIV-1 RNA concentration in both plasma and CSF had declined below 400 copies/mL at the time of sampling. The median plasma concentration of stavudine, lamivudine, saquinavir, and nelfinavir was 84 ng/mL, 359 ng/mL, 387 ng/mL, and 1190 ng/mL, respectively. Of the protease inhibitors, only saquinavir was detected at a low concentration in seminal plasma. Neither nelfinavir nor saquinavir was detected in the CSF. Concentrations of stavudine and lamivudine in seminal plasma were higher than in CSF: 104 vs. 49 ng/mL for stavudine (NS) and 1329 vs. 100 ng/mL for lamivudine ($p=0.006$). For lamivudine, seminal plasma/blood plasma ratios were significantly higher than CSF/blood plasma ratios. These data support the hypothesis that poor penetration of the central nervous system and the male genital tract by antiretroviral drugs can contribute to differences in viral dynamics in these compartments.

Introduction

Antiretroviral therapy decreases the levels of human immunodeficiency virus type 1 (HIV-1) in plasma, cerebrospinal fluid (CSF) and semen.[1-3] However, the decline of HIV-1 RNA concentrations and the evolution of virus in the CSF or semen during therapy has not always been comparable to those in plasma, indicating viral compartmentalisation.[4,5] A poor penetration of the male genital tract and the central nervous system by antiretroviral drugs is suggested to contribute to the differences in the viral dynamics in these compartments.

Compared to other antiretroviral drugs, the penetration of the protease inhibitors (PIs) into the CSF is relatively poor. In general, PIs are highly bound to plasma proteins and are not very lipid-soluble, impeding the entry of these drugs into the CSF.[6] Reported concentrations of ritonavir, saquinavir and nelfinavir in CSF are low or below the lower limit of quantification.[7,8] As an exception (possibly due to limited binding to plasma proteins), indinavir has been reported to enter the CSF in concentrations above the *in vitro* 95% inhibitory concentration.[9] NRTIs penetrate the CSF, although the reported concentrations in CSF are usually lower than in plasma.[10]

The penetration into the genital tract seems to vary between the different PIs. Taylor et al. showed good penetration of indinavir in contrast to poor penetration of ritonavir and saquinavir into the seminal plasma.[11] Recently, Pereira et al. reported the concentrations of zidovudine and lamivudine in the semen and plasma of nine patients. Zidovudine concentrations and especially lamivudine concentrations in semen were higher than in plasma and were associated with a reduction of HIV-1 RNA concentrations in both seminal and blood plasma.[12]

In the ADAM study, a study investigating the feasibility of induction maintenance therapy, a combination of four antiretroviral drugs was used as an induction therapy for 26 or 50 weeks.[13] In this study, plasma, CSF, and semen were obtained in a subset of patients and the concentrations of stavudine, lamivudine, nelfinavir, and saquinavir were investigated.

Methods

The ADAM study

In the ADAM study, an open-label randomised-controlled study, the feasibility of induction maintenance therapy in antiretroviral treatment was investigated. A quadruple induction regimen, consisting of stavudine, lamivudine, saquinavir, and nelfinavir, was used for 26 weeks in antiretroviral therapy-naive patients. At week 26, patients were randomised to either maintenance therapy or the prolongation of the quadruple drug regimen. In the latter group, a second randomisation was performed after 50 weeks of treatment. Patients, methods and the efficacy results of the induction-maintenance regimen in the ADAM study have been described elsewhere.[13]

As part of the ADAM study, patients were asked to participate in a sub-study to assess the concentrations of the antiretroviral medication used in plasma, CSF and semen. The institutional review boards of all participating institutions approved this sub-study and written informed consent was obtained.

Patients

At the time of assessment, patients had to be using a quadruple drug regimen consisting of stavudine, (40 mg BID, or 30 mg BID if body weight < 60 kg), lamivudine (150 mg BID), saquinavir hard-gelatin-capsules (600 mg TID) and nelfinavir (750 mg TID). When saquinavir soft-gelatin-capsules became available (November 1st 1997), all patients using saquinavir hard-gelatin-capsules (600 mg TID) switched to saquinavir soft-gelatin capsules (800 mg TID). Patients were instructed to take their medication with food.

Samples

On the day of sampling, patients were instructed to collect semen in a 50 mL sterile collection tube. Within 6 h of collection, semen was frozen at -70°C until testing. On the same day, a lumbar puncture was performed at a random time point. Part of the obtained CSF was used for an immediate assessment of the number of cells and the concentration of proteins and glucose. The other part was stored at -70°C until analysis. Immediately after the lumbar puncture, heparinized blood was obtained by venipuncture for an assessment of stavudine, lamivudine, saquinavir, and nelfinavir concentrations. The time of last medication intake and the time of sample withdrawal were recorded.

The concentrations of stavudine and lamivudine in plasma, CSF, and seminal plasma were quantified simultaneously using an HPLC-assay.[14] Stavudine and lamivudine were extracted from plasma, CSF, and seminal plasma using silica extraction columns prior to isocratic, reversed-phase HPLC with ultraviolet detection at 270 nm. The method has been validated over the range of 10-5,000 ng/mL using a 500 µL sample volume. Simultaneous quantification of saquinavir and nelfinavir in plasma, CSF, and seminal plasma was performed using an HPLC-assay, as previously published.[15] Briefly, sample pre-treatment consisted of solid-phase extraction. Seminal plasma and CSF samples were diluted with blank human plasma (1:1 v/v) prior to isocratic ion-pair, reversed-phase HPLC and were detected at 239 and 210 nm, respectively. The lower limit of quantification for saquinavir and nelfinavir was 25 and 50 ng/mL, respectively, using a 600 µL sample volume. The assay was linear up to concentrations of at least 25 µg/mL.
The HIV-1 RNA concentration in plasma, CSF, and seminal plasma was measured using a commercially available PCR assay with a variable lower limit of detection (Amplicor HIV Monitor Test, Roche Diagnostic Systems Inc., Branchburg, NJ, USA).

Statistical analysis

All data were tabulated. Median and interquartile ranges were calculated. If the value of the HIV-1 RNA concentration or the drug concentration was below the lower limit of quantification, the value of the cut-off point was used for all calculations. Seminal plasma/blood plasma concentration ratios and CSF/blood plasma concentration ratios were used as a measure for drug penetration into these tissues.

Results

Patients

Of the 65 patients in the ADAM study, 12 patients were willing to participate in the sub-study. In one patient the time of assessment was not according to protocol, he had a lumbar puncture at week 12 for a medical reason and did not supply any semen. Four patients did not succeed in obtaining semen on the requested day. Two patients refused the lumbar puncture for logistic or personal reasons, but were able to collect semen.

Table 1 **Patient characteristics**

Patient	*age*	*heigth cm*	*weight kg*	*CDC*	*HIV-1 RNA copies/mL*	*CD4⁺ cells/mm³*	*CD8⁺ cells/mm³*
1	49	180	76	A	200000	310	2090
2	48	174	62	A	110000	420	530
3	43	183	86	A	46000	500	1800
4	35	200	91	A	4200	490	500
5	30	190	92	B	180000	440	2710
6	31	170	78	A	29000	300	1510
7	42	176	66	A	120000	230	710
8	41	187	83	A	270000	410	1820
9	46	168	70	B	17000	180	780
10	44	191	84	A	16163	300	320
11	50	175	74	A	112758	340	2840
12	39	182	90	A	10464	560	1000
Median	43	181	81		78000	380	1260
Range	30-50	168-200	62-92		4200-270000	180-560	320-2840

Patient characteristics

Characteristics of all participating patients at baseline and at time of sampling are tabulated in Tables 1 and 2. None of the patients was diagnosed as having AIDS. The median $CD4^+$- and $CD8^+$ cell count at baseline was 380 and 1260 cells/mm^3, respectively. All but one patient (12) were using saquinavir – SGC at time of sampling. In all patients the $CD4^+$ cell count increased during therapy. The median plasma HIV-1 RNA concentration at baseline was 4.89 $\log_{10}$ copies/mL. In one patient the plasma HIV-1 RNA concentration was >400 copies/mL at the time of sampling. In all other patients, the plasma HIV-1 RNA concentration had declined below 400 copies per mL, although in four of these patients the plasma HIV-1 RNA was detectable (Table 1).

None of the patients had a neurologic disease or genital infection at the time of sampling. The median total protein concentration in CSF was 0.45 g/L (IQR 0.28-0.47 g/L), and the total cell count in CSF did not exceed 14 cells/µL (data not shown).

Table 2 Parameters at the time of sampling

Patient	week	*CD4*⁺ cells/mm³	*CD8*⁺ cells/mm³	*HIV-1 RNA* copies/mL plasma	*HIV-1 RNA* copies/mL *CSF*
1	50	490	930	<18	<27
2	50	630	630	64	<23
3	50	810	1650	<16	<28
4	26	750	790	<20	23
5	26	660	1950	186	-
6	26	340	1040	<20	<27
7	26	430	720	34	<29
8	26	690	1980	26	NA*
9	26	240	790	<39	<4
10	12	320	230	<27	<21
11	26	390	1180	13890	1514
12	26	670	860	<18	-
Median		560	900	<27	<27
Range		240-810	230-1980	<16-13890	<4-1514

*NA: not assessed; - : not sampled

HIV-1 RNA

The HIV-1 RNA concentrations in plasma and CSF were assessed in 12 and 10 samples respectively (Table 2). HIV-1 RNA concentrations in CSF were in the same order of magnitude than those in plasma. Two patients had a detectable HIV-1 RNA concentration in CSF. One of these patients also had a high HIV-1 RNA in plasma. This patient might not have been compliant with the study medication, since drug levels of this patient were in the lower range (See Table 3). Unfortunately, HIV-1 RNA assessment in semen failed due to processing errors.

Drug concentrations of the NRTIs and PIs in plasma, CSF and seminal plasma are listed in Table 3. The median plasma concentration of stavudine and lamivudine was 84 ng/mL (range <10-321 ng/mL) and 359 ng/mL (range 135-1187 ng/mL), respectively. Nelfinavir and saquinavir were present in plasma at a median concentration of 1190 ng/mL (range 870-2860 ng/mL) and 387 ng/mL (range 57-1062 ng/mL), respectively.

Table 3 **Drug concentrations in plasma, CSF, and semen**

Patient	stavudine (ng/mL) plasma	CSF	semen	lamivudine (ng/mL) plasma	CSF	semen
1	173	41	-	1016	110	-
2	77	65	-	1173	409	-
3	33	74	103	329	120	1329
4	78	33	-	231	38	-
5	210	-	183	1187	-	1349
6	157	32	33	736	90	1894
7	321	58	30	789	65	454
8	36	61	104	244	110	794
9	91	80	127	388	119	664
10	39	41	-	166	63	-
11	<10	34	-	135	67	-
12	194	-	1273	319	-	6642
Median	84	49	104	359	100	1329
Range	10-321	34-80	30-1273	135-1187	38-409	454-6642

Drug concentrations

The penetration of the CSF by NRTIs was limited. The median concentration of stavudine and lamivudine in CSF was 49 ng/mL (range 32-80) and 100 ng/mL (range 38-409) respectively. These concentrations of stavudine and lamivudine in CSF were usually above the *in vitro* IC_{50} concentrations for most reported wild-type HIV-1 strains.[16,17] Neither saquinavir nor nelfinavir was detected in the CSF.

Both stavudine and lamivudine were present in semen (median 104 ng/mL (range 30-1273), and median 1329 ng/mL (range 454-6642 ng/mL), respectively). Of the PIs only saquinavir was present at a low concentration in three of eight semen samples.

In some patients the time interval between drug intake and time of sampling was not known. CSF and plasma were drawn as a paired sample at a median time interval of 3.5h (range 0.33-7h, n=9)). Semen was usually sampled close to the time-point of medication intake (median 0.1h (range -0.5-1.75h, n=5)). Figure 1 illustrates the CSF, semen and plasma concentrations of stavudine and lamivudine in relation to the time interval between drug intake and time of sampling.

The CSF/blood plasma and seminal plasma/blood plasma concentration ratios were used as a measure for penetration of the NRTI into the CSF and semen. Using these ratios, the penetration of stavudine into the CSF and semen appeared comparable (median CSF/blood plasma ratio 0.87, IQR 0.23-1.69; median seminal plasma/blood plasma ratio 1.41, IQR 0.06-3.18).

Table 3 continued

	saquinavir (ng/mL)			nelfinavir (ng/mL)		
Patient	*plasma*	*CSF*	*semen*	*plasma*	*CSF*	*semen*
1	821	<25	-	1270	<50	-
2	1062	<25	-	2860	<50	-
3	84	<25	45	870	<50	<50
4	57	<25	-	910	<50	-
5	619	-	<25	1530	-	<50
6	220	<25	<25	1110	<50	<50
7	323	<25	<25	910	<50	<50
8	65	<25	<25	920	<50	<50
9	450	<25	46	2055	<50	<50
10	497	<25	-	1490	<50	-
11	110	<25	-	980	<50	-
12	549	-	62	2300	-	<50
Median	387	-	<25	1190	-	-
Range	57-1062	-	<25-62	870-2860	-	-

- : not sampled

The penetration of lamivudine was, however, significantly higher in semen than in CSF (Figure 2; median CSF/blood plasma ratio 0.33, IQR 0.12-0.38; median seminal plasma/blood plasma ratio 2.57, IQR 1.14-4.04).

Upon comparing absolute concentrations of plasma, CSF and semen, the lamivudine concentrations in semen were higher than the concentrations in both CSF and plasma (Figure 3; $p=0.006$ and $p=0.02$, respectively).

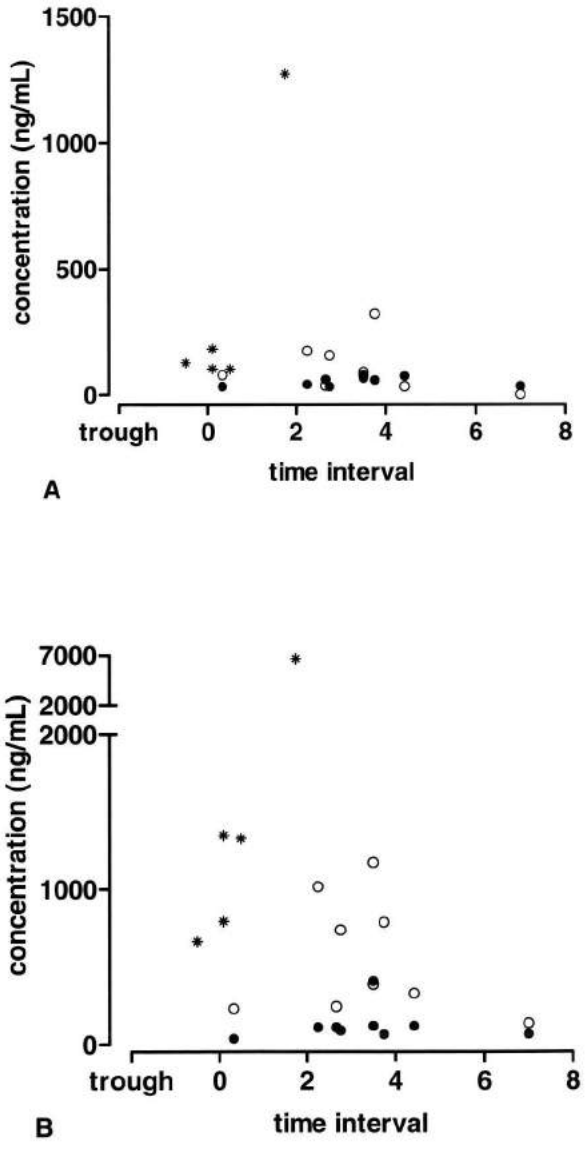

Figure 1 The concentrations of NRTIs and the time interval between medication intake and sampling. The white dots represent blood plasma concentrations and the black dots and stars represent CSF and seminal plasma concentrations, respectively.
Panel A: Stavudine.
Panel B: Lamivudine.

Discussion

To our knowledge, these are the first data regarding the concentrations of stavudine, lamivudine, nelfinavir, and saquinavir in both CSF and semen of HIV-1-infected patients. In most patients, the concentration of stavudine and lamivudine that entered the semen was higher than the CSF. In most cases, nelfinavir and saquinavir were not detectable in CSF and semen. These findings confirm that the male genital tract and the central nervous system should be considered as different biological compartments (as compared to plasma) from a pharmacokinetic point of view. Different penetration of antiretroviral agents may therefore attribute to distinct viral dynamics in the different body compartments.

Although data are limited, the blood tissue barrier of the male genital tract is assumed to behave like a lipid barrier.[18] Passive diffusion from blood to semen is therefore hypothesised to become facilitated if an agent has high lipid solubility, a low plasma protein-binding, and a favourable dissociation constant.[18] This dissociation constant indicates the pH at which equimolar concentrations of non-ionised and ionised forms of the drug exist. Non-ionised compounds diffuse more easily across lipid barriers. In addition, ion-trapping mechanisms can attribute to the accumulation of acid compounds in alkaline compartments and of basic compounds in acidic compartments. In our study, the concentration of stavudine and lamivudine that entered the genital tract was higher than the concentration present in plasma. The seminal plasma/blood plasma concentration ratios of the NRTIs in our study were not as high as the seminal plasma/blood plasma concentration ratios of zidovudine (6) and lamivudine (13) as recently reported by Pereira et al.[12] However, the comparison is difficult to make, since both studies have low numbers of patients and the two studies differ for their design, especially regarding the time interval between sampling of semen and blood. These studies indicate that, in addition to passive diffusion, active transportation or accumulation might contribute to the relatively high concentrations of NRTIs in semen. Stavudine and lamivudine differ for their proteinbinding (negligible vs. 10-50%) and dissociation constant (pKa: 10 vs. 4.3), thus providing a possible explanation for the observed differences in the seminal plasma/blood plasma concentration ratios of stavudine and lamivudine. In addition to differences in passive diffusion, the physico-chemical characteristics of lamivudine may be more favourable for entrapment in the protein-loaded, alkaline semen.[18]

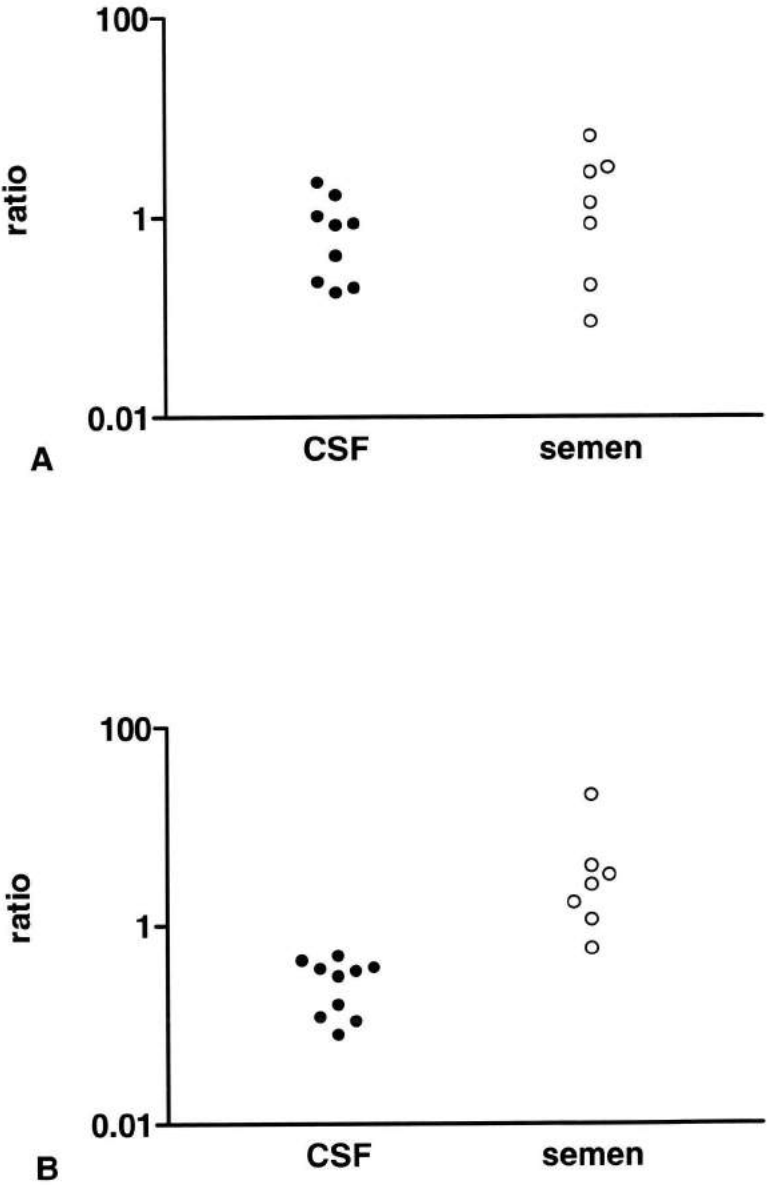

Figure 2 The seminal plasma/blood plasma ratios and CSF/blood plasma ratios of stavudine and lamivudine. The black dots represent the CSF/blood plasma ratios. The white dots represent the seminal plasma/blood plasma ratios. Panel A: Stavudine. Panel B: Lamivudine.

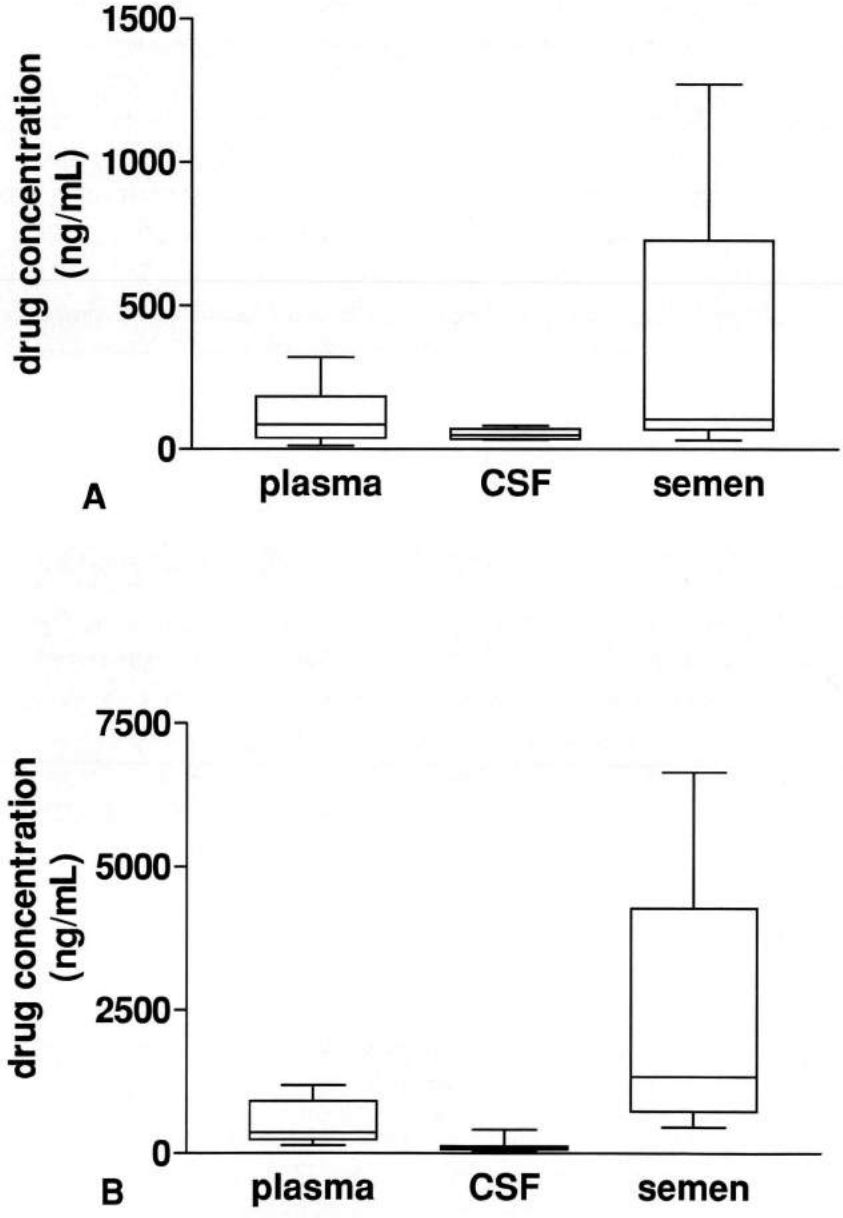

Figure 3 The drug concentrations of stavudine and lamivudine in plasma, CSF, and semen. Data are median and interquartile range; the bars represent the complete range.
Panel A: Stavudine.
Panel B: Lamivudine.

A more trivial explanation for the observed differences between the semen/plasma ratios is the difference in time to C_{max} of stavudine (3.8 h) and lamivudine (1.0-1.5 h).

Since the sampling time of plasma was often closer to the time to C_{max} of stavudine than of lamivudine, and semen concentrations were suggested to be rather stable over time [13,20], seminal plasma/blood plasma concentration ratios of stavudine may have been underestimated as compared to lamivudine.[18]

The concentrations of stavudine and lamivudine in CSF were low compared to the concentrations in semen. Differences in time of sampling are probably not significant for this comparison, since both the concentrations in semen and in CSF are considered to be rather stable over time.[6,19] There are however several differences in the physiology of CSF and semen that might influence the penetration of these antiretroviral drugs. The blood tissue barrier of the central nervous system is probably more impermeable and more extensive than that of the male genital tract.[20] The choroid plexuses actively produce CSF. The renewal of CSF occurs 4-5 times daily.[6] Semen, however, consists of a composition of secretions from the testes, the seminal vesicles and the prostate, all of which have their own physiological characteristics.[18] The renewal of semen is partly dependent on the frequency of ejaculation. The impact of these fluid characteristics on antiretroviral drug concentrations in both CSF and semen needs further exploration.

In contrast to the substantial penetration of the NRTIs into CSF and semen, neither nelfinavir nor saquinavir was present in the semen or the CSF at significant concentrations. The lack of penetration of both nelfinavir and saquinavir into the CSF confirms earlier observations of poor penetration of highly protein-bound PIs with a high molecular weight into the CSF.[6] Even if protein-binding capacity, lipophilicity and molecular weight would not prevent the influx into these compartments, it is possible that an active efflux by, for example, P-glycoprotein interfered with the achievement of detectable levels of these agents in the central nervous system and the male genital tract.[20-22]

Our data support the hypothesis that poor penetration of the central nervous system and the male genital tract by antiretroviral drugs can partly explain the distinct viral dynamics in these tissues as compared to plasma.[4,5] We were unable to assess the efficacy of the quadruple regimen in these compartments, since no samples for the assessment of baseline HIV-1 RNA concentrations in CSF or semen were obtained. The HIV-1 RNA concentrations in plasma and CSF were however in the same order of magnitude after 26 or 50 weeks of quadruple drug therapy.

Future studies might shed more light on the consequences of varying levels of antiretrovirals in CSF and semen, not only for the durability of viral suppression, but also on HIV-1 transmission within the population.

Appendix

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Chapter 9

Stavudine concentrations in the plasma and cerebrospinal fluid: A possible interaction with ritonavir and/or indinavir?

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Abstract

The metabolism of stavudine, a frequently used nucleoside analogue reverse transcriptase inhibitor (NRTI), is partly unknown. Except for the interaction of protease inhibitors (PIs) with zidovudine on the level of glucuronidation, no clinically important in vivo pharmacokinetic interactions between NRTIs and PIs have been reported. In order to investigate the pharmacokinetics of stavudine, stavudine concentrations were assessed in paired samples of plasma and cerebrospinal fluid (CSF) of patients using four different treatment regimens. All of the 39 patients available for this analysis used 40 mg stavudine bid for at least 12 weeks. Eleven patients did not use a PI. Nelfinavir and saquinavir were used in ten patients, while eight patients used ritonavir and saquinavir. The remaining ten patients used a five-drug regimen containing indinavir, indinavir/ritonavir or nelfinavir.

Baseline characteristics of the patients were comparable. Patients using stavudine without the addition of a PI or in combination with nelfinavir and saquinavir were found to have significantly lower stavudine concentrations in plasma and CSF than patients using ritonavir and/or indinavir ($p<0.001$). CSF/plasma concentration ratios of stavudine were comparable ($p=0.6$). In a multivariate linear regression analysis, the use of ritonavir and/or indinavir and the baseline $CD4^+$ T-cell count were significantly associated with the stavudine concentrations in plasma ($p=0.0007$ and $p=0.03$, respectively). The concentration of stavudine in CSF was associated with the use of ritonavir ($p=0.0001$). This unexpected finding suggests that stavudine metabolism may, at least in part, be inhibited by ritonavir and/or indinavir.

Introduction

Stavudine is a thymidine nucleoside analogue, used as an antiretroviral agent in the treatment of HIV-1 infection.[1-4] Stavudine is phosphorylated intracellularly into the active triphosphate anabolite that inhibits HIV-1 reverse transcripts.[2] Stavudine entry into for example lymphocytes appears to occur by non-facilitated diffusion.[5] Stavudine has a high absolute oral bioavailability and has a plasma elimination half-life of about 1 to 1.67 hours.[3,6] C_{max} is achieved after 0.5-0.75 hours and varies between 600 and 890 ng/mL (40 mg stavudine twice daily (bid)) in phase I and II studies.[2,6,7] Although the reported concentrations in cerebrospinal fluid (CSF) are usually lower than in plasma, stavudine penetrates the CSF, reaching concentrations above the IC_{50} of wild type HIV-1 strains *in vitro*.[2,8] About 40% of the stavudine dose is excreted unchanged in urine;[7,9] the remaining is metabolized to unknown derivatives, or excreted unchanged in the feces.

Combination of antiretroviral agents has shown to be necessary to maintain a sustained suppression of HIV-1 replication.[10,11] Stavudine is one of the main NRTIs used in triple combination regimens. To our knowledge, no clinically relevant pharmacokinetic interactions have been reported with respect to the combination of NRTIs and PIs. Didanosine elimination was unchanged with concurrent use of ritonavir.[12] In addition, no interactions were found between PIs and the intracellular metabolism of the NRTIs *in vitro*.[13] Zidovudine concentrations showed a not clinically relevant decrease when ritonavir was added. It has been suggested that ritonavir induces the glucuronidation of zidovudine.[14] It is, however, unclear whether glucuronidation is of importance for the elimination of stavudine.[15]

The level of exposure to PIs and non-nucleoside RT inhibitors was found to be associated with the antiretroviral efficacy of combination regimens.[16,17] For the NRTIs a clear relationship between the observed drug concentrations and the observed antiviral efficacy may not be found, since they need to be metabolized intracellularly before they exert an antiretroviral effect.[18] The assessment of intracellular concentrations is difficult and time-consuming, leaving plasma concentrations of the non-metabolized compound as a more accessible parameter for drug exposure.

In this report the concentrations of stavudine in both plasma and CSF were compared between the different regimens of four clinical studies.

Material and Methods

050 study

The 050-study was an open label, randomized trial in HIV-1-infected, antiretroviral therapy- naive patients comparing the efficacy of two NRTI combinations (zidovudine 200 mg three times daily (tid) plus lamivudine 150 mg bid or stavudine 40 mg bid plus lamivudine 150 mg bid). All patients were asked to participate in a neurological sub-study to assess HIV-1 RNA and drug concentrations in plasma and CSF. All patients had a baseline $CD4^+$ T-cell count above 200 cells/mm^3 and an HIV-1 RNA concentration in plasma above 10,000 copies/mL. CSF and plasma samples were obtained after 12 weeks of therapy.[8]

ADAM study

The ADAM study was an open label, randomized study in HIV-1-infected, antiretroviral therapy-naive patients to investigate the feasibility of an induction-maintenance regimen. Patients started a quadruple drug induction regimen consisting of stavudine (40 mg bid), lamivudine (150 mg bid), nelfinavir (750 mg tid or 1,250 mg bid), and saquinavir soft gelatin formula (800 mg tid or 1,200 mg bid) for at least 26 or 50 weeks. Only patients with more than 200 $CD4^+$ T-cells/mm^3 and more than 1,000 HIV-1 RNA copies/mL in plasma were included. In a sub-study, CSF and plasma samples were obtained at week 26 or week 50.[19]

The occurrence of side effects (signs, symptoms, or laboratory abnormalities) was assessed at each study visit.[20]

Prometheus study

The Prometheus study was an open label, randomized study in HIV-1-infected patients, to compare the efficacy and toxicity of a combination of ritonavir and saquinavir (each 400 mg bid) with a combination of stavudine (40 mg bid), ritonavir and saquinavir (each 400 mg bid). Patients could not enter the study if they had previously used PIs or stavudine. CSF and serum samples were collected in a subgroup of the patients after 12 weeks of therapy.[21]

The occurrence of side effects (signs, symptoms, or laboratory abnormalities) was assessed at each study visit.[22]

ERA study

In this open label study, patients with either a chronic or primary HIV-1 infection were treated with five agents. All patients selected for this analysis used a quintuple drug regimen containing stavudine. Patients could be pretreated or naive for antiretroviral agents. Stavudine was combined with lamivudine 150 mg bid or didanosine (400 mg once daily (qd)), nevirapine (200 mg bid or 400 mg qd), abacavir (300 mg bid), and indinavir (1,000 mg tid) or indinavir/ritonavir (800/100 mg bid) or nelfinavir (1,250 mg bid). A serum pharmacokinetic profile was obtained at weeks 8, 24, 48, 72 and 96. Blood was collected prior to drug administration and at 1, 2, 4, 6, 8 and 10 hours thereafter. At weeks 8, 24, 48 and 72, a CSF sample was collected one hour after taking the drugs.[23]

Within every study, written informed consent was obtained from all patients. The institutional review boards of all participating centers approved with the four study protocols.

HIV-1 RNA

In the four studies, the HIV-1 RNA concentrations in CSF and plasma were measured using a commercially available PCR assay with a variable lower limit of quantification (Amplicor HIV Monitor Test, Roche Diagnostic Systems Inc., Branchburg, NJ, USA for the ADAM, Prometheus, and 050 studies) or a fixed lower limit of quantification (NASBA- and NucliSens HIV-1 RNA QT assay, Organon Teknika, Boxtel, the Netherlands, for the baseline data of the ADAM study and the complete ERA study).

Stavudine concentrations

The concentrations of stavudine in plasma and CSF were quantified simultaneously using an HPLC-assay.[24,25] Stavudine was extracted from plasma (ADAM and 050) or serum (Prometheus and ERA) and CSF with silica extraction columns prior to isocratic, reversed-phase HPLC with ultraviolet detection at 270 nm. The method has been validated over the range of 10-5,000 ng/mL using a 0.5 mL sample volume.

Statistical analysis

The HIV-1 RNA concentration and $CD4^+$ and $CD8^+$ T-cell counts at baseline and at time of sampling were compared between the four study populations (Kruskal-Wallis test). The four groups of patients were also compared for the time interval between drug intake and sampling, for treatment duration and for protein and cell concentration in CSF. The concentrations of stavudine in plasma and CSF and the CSF/plasma concentration ratio of stavudine were compared between the four study populations (Kruskal-Wallis test).

Univariate and multivariate linear regression analyses were performed using the stavudine concentration in plasma or CSF as the dependent variable, to distinguish variables that contributed to the stavudine concentrations. Variables with a p-value < 0.1 in the univariate model were entered in the multivariate model. Subsequently, the multivariate linear regression model was constructed using both forward and backward selection.

Patients in the Prometheus and ADAM studies were compared for the incidence of peripheral neuropathy during the time stavudine was used, using person-years analysis. Between these studies, the time to peripheral neuropathy was compared. Differences between groups were considered significant at a $p < 0.05$ level. All reported p-values were two sided. Analyses were performed using SAS, version 6.12 (SAS Institute, Cary, North Carolina, USA).

Results

Patient characteristics

Baseline characteristics of the patients in the four studies are listed in Table 1. Eleven patients in the 050 study were using stavudine and lamivudine at week 12. Of the ten patients in the ADAM study, one patient was not sampled according to the protocol. This patient had a diagnostic lumbar puncture at week 12. The other patients were sampled at week 26 (n=6) or at week 50 (n=3). In the Prometheus study, eight patients could be evaluated at week 12. Ten patients in the ERA study could be evaluated, three of whom started treatment at the time of primary infection. Baseline characteristics were comparable between the four studies, although the baseline $CD4^+$ T-cell count in the Prometheus study was low compared to the other studies.

Table 1 **Baseline characteristics and parameters at time of sampling**

Baseline characteristics	050 study (n=11) median (IQR)	ADAM study (n=10) median (IQR)	Prometheus study (n=8) median (IQR)	ERA study (n=10) median (IQR)
HIV-1 RNA in plasma	4.85	4.85	4.70	4.99
[$\log_{10}$ copies/mL]	(4.32-5.12)	(4.23-5.08)	(4.40-4.89)	(4.88-5.18)
$CD4^+$ cells	0.34	0.33	0.09	0.31
[$*10^9$ cells/L]	(0.25-0.42)	(0.30-0.42)	(0.02-0.34)	(0.11-0.36)
$CD8^+$ cells	0.94	1.15	0.78	1.36
[$*10^9$ cells/L]	(0.82-1.27)	(0.53-1.82)	(0.62-1.04)	(0.66-1.85)
At sample withdrawal				
HIV-1 RNA in plasma	1144	<27	<197	390
[copies/mL]	(<283-3281)	(<20-<39)	(<154-<272)	(263-490)
$CD4^+$ cells	0.47	0.46	0.25	0.54
[$*10^9$cells/L]	(0.34-0.57)	(0.34-0.75)	(0.10-0.51)	(0.28-0.64)
$CD8^+$ cells	0.96	0.86	1.15	1.05
[$*10^9$cells/L]	(0.76-1.33)	(0.77-1.18)	(0.81-1.25)	(0.85-1.20)
HIV-1 RNA in CSF	<281	<27	<336	<40
[copies/mL]	(<211-<334)	(<23-<28)	(<208-<602)	(<40-<40)[a]
Proteins in CSF	0.39	0.46	0.35	0.40
[g/L], mean (SD)	(0.27-0.56)	(0.28-0.53)	(0.29-0.42)	(0.30-0.69)[a]
Cell count in CSF	10	4	8	7
[$*10^9$cells/L], mean (SD)	(7-13)	(2-7)	(5-17)	(4-7)[a]

IQR : interquartile range; [a] n=5;

Patient characteristics at time of sampling

Median HIV-1 RNA concentration in plasma and CSF and median $CD4^+$- and $CD8^+$ T-cell counts are tabulated in Table 1. Since HIV-1 RNA concentrations were assessed with different assays and at different time points in the four studies, a comparison between the four studies for virological efficacy regarding differences in drug exposure was not feasible. No clear neurological disease was present in any of the patients.

The median time interval between medication intake and sampling was 5 hours in the 050 study, 3.5 hours in the ADAM study, and unknown in the Prometheus study (Table 2). For the comparison of stavudine concentrations in plasma of the patients participating the ERA study, the values at a time interval of four hours were selected. For the comparison of the CSF/plasma concentration ratio of stavudine, plasma concentrations corresponding with a time interval of one hour were used (time of lumbar puncture).

Table 2 Concentrations of stavudine in plasma and CSF, and CSF/plasma ratio

	050 study n=11, median (IQR)	ADAM study n=10, median (IQR)	Prometheus study n=8, median (IQR)	ERA study n=10, median (IQR)	p-value
Plasma [ng/mL]	142 (99-233)	77 (36-157)	488 (257-746)	424 (316-533)	0.0001
CSF [ng/mL]	47 (41-61)	49 (34-65)	213 (125-281)	132 (80-146)	0.0001
CSF/plasma	0.28 (0.18-0.68)	0.87 (0.23-1.69)	0.45 (0.29-0.67)	0.25 (0.23-0.26)	0.11
Time interval[a] Median [hrs]	5 (3-6)	3.5 (2.7- 3.8)	-	4[b] (4-4)	0.11

IQR: interquartile range; [a] Time interval: time between time of medication intake and time of sampling;
[b] For CSF/plasma ratio: 1(1-1)

Drug concentrations in the plasma

The stavudine concentrations in the plasma differed significantly among the four studies: median stavudine concentration: 050 study: 142 ng/mL, ADAM study: 77 ng/mL, Prometheus study: 488 ng/mL and the ERA study: 424 ng/mL (Table 2, p=0.0001). The Prometheus and ERA studies were comparable for stavudine concentrations in plasma (p=0.9), but differed significantly from the 050 and ADAM studies (050: p=0.02 and p=0.003, ADAM: p=0.005 and p=0.002, respectively).

Drug concentrations in the CSF

The stavudine concentrations in the CSF were significantly different among the four studies: median CSF stavudine concentration in the 050 study: 47 ng/mL, in the ADAM study: 49 ng/mL, in the Prometheus study: 213 ng/mL, and in the ERA study 132 ng/mL (Table 2, p=0.0001). The Prometheus and ERA studies were comparable for stavudine concentrations in CSF (p=0.09) but differed significantly from the 050 and ADAM studies (050: p=0.0004 and p=0.006, ADAM: p=0.0004 and p=0.01, respectively). Median stavudine CSF/plasma concentration ratios were comparable among the four studies (Table 2).

Univariate and multivariate linear regression analysis

Univariate linear regression analysis identified the $CD4^+$ T-cell count at baseline, the use of ritonavir, indinavir, or nelfinavir, and the treatment duration as contributing to the stavudine concentrations in plasma (Table 3). In a multivariate linear regression analysis including these variables, the use of ritonavir and indinavir and the baseline $CD4^+$ T-cell count remained significant. Since some patients used ritonavir plus indinavir, we introduced 'the use of ritonavir plus indinavir' into the model. However, the estimate of this parameter did not exceed the estimates of the two separate protease inhibitors, even if the model included the baseline $CD4^+$ T-cell count. Therefore, in the final model, we included 'the use of ritonavir and/or indinavir'. In this model the baseline $CD4^+$ T-cell count and the use of ritonavir and/or indinavir were significantly associated to stavudine concentrations (p=0.03 and p=0.0007, respectively). This is illustrated by Figure 1A in which the stavudine concentrations of patients with the use of ritonavir and/or indinavir and the stavudine concentrations of patients without the use of these PIs are plotted.

In the analysis of the CSF concentrations, less samples were available for the univariate linear regression model using the stavudine concentrations in CSF as the dependent factor. Potential factors were the $CD4^+$ T-cell count at baseline, plasma creatinine concentration, the protein concentration in CSF, and the use of ritonavir or nelfinavir. Here, the use of indinavir did not contribute to the stavudine concentrations, probably because less CSF samples of patients using indinavir were available (Figure 1B). Only the baseline $CD4^+$ T-cell count and the use of ritonavir remained of significance in the multivariate linear regression analysis (p=0.007 and p=0.0001, respectively).

Table 3 Linear regression analysis using the stavudine concentration in plasma as the dependent variable

Independent variable	Univariate		Multivariate			
	Coefficients	p	Coefficients	p	Coefficients	p
Baseline HIV-1 RNA (per $\log_{10}$ copies)	15 (99)	0.88				
Baseline CD4$^+$ T-cell count (per 100 cells)	-80 (24)	0.002	-65 (23)	0.009	-52 (22)	0.03
Baseline CD8$^+$ T-cell count (per 100 cells)	-11 (7)	0.12				
Age (per year)	-6 (5)	0.28				
Body mass index (kg/m^2)	-30 (20)	0.15				
Creatinine[a] (µmol/L)	-5 (3)	0.13				
Proteins in CSF[a] (g/L)						
Cell count in CSF[a] (cells/µL)						
Drugs						
Indinavir (yes)	244 (104)	0.03	174 (95)	0.07		
Ritonavir (yes)	259 (86)	0.005	144 (85)	0.10		
Saquinavir (yes)	-18 (90)	0.84				
Nelfinavir (yes)	-241 (91)	0.01	- -	-		
Ritonavir/indinavir[b] (yes)	334 (73)	0.0001			275 (74)	0.0007
Time interval[c] (hrs)	-34 (32)	0.28				
Treatment duration (weeks)	-8 (4)	0.06	- -	-		

For the independent variables in the models regression coefficients (SE), and p-values are shown. [a] at time of sampling; [b] the use of ritonavir plus indinavir, ritonavir or indinavir; [c] the time between medication intake and time of sampling

In the Prometheus study (n=136), ten events of peripheral neuropathy were observed during a total of 101 person years, compared to seven events in 103.7 person years in the ADAM study (n=65) (Incidence density: 9.9 (95%CI: 4.75-18.21), and 6.8 (95%CI: 2.71-13.91), respectively). Median time to peripheral neuropathy was 0.4 and 0.5 years in the two studies, respectively (NS).

Discussion

The concentrations of stavudine in both the plasma and the CSF of patients using this drug in combination with ritonavir and/or indinavir were significantly higher than those found in patients not using these specific PIs. The stavudine concentrations in plasma and CSF were retrieved from four differently designed studies, all with limited patient numbers, which may have biased our results. However, the differences among the studies may help to explain the different stavudine concentrations found in the four studies.

First of all the 050 and ADAM studies used plasma for measuring the stavudine concentrations while both the Prometheus and ERA studies used serum samples. HPLC assessments reportedly performed equally in plasma and serum.[25] However, even if the HPLC results would have differed for serum and plasma, the stavudine concentrations in CSF would not have been expected to differ among the four studies.

Secondly, the time interval between drug intake and sampling is of significance for the stavudine concentrations in plasma.[8] Although the time interval in the Prometheus study was unknown, the other three studies were comparable for the median time interval of the drawing of the samples. For CSF, the time interval in the ERA study was different from the 050 and ADAM studies. However, a correlation between stavudine concentration and time of sampling is expected to be less important, since the concentrations of stavudine in CSF appeared to be rather constant over time.[8]

The treatment duration before sampling was 12 weeks for the 050 and Prometheus studies; this differed significantly from the ADAM study (26 or 50 weeks). Apparently, an induction of metabolism of stavudine over time – as for saquinavir – is unlikely and can not explain the differences in stavudine concentrations among these studies.[26]

Except for the $CD4^+$ T- cell count, baseline characteristics were comparable among the four studies. Patients with a low $CD4^+$ T-cell count at baseline were found to have high stavudine concentrations in plasma. It is likely that patients with lower $CD4^+$ T-cell counts also had a lower body mass index (BMI) and subsequently a lower volume of distribution, which explains the higher stavudine concentrations in these patients.[27] Indeed, the baseline $CD4^+$ T-cell count was significantly and positively correlated with BMI (Pearson correlation coefficient: 0.36 ($p=0.03$)). Multivariate linear regression analysis with the use of ritonavir and/or indinavir, and

BMI as independent factors had almost a similar fit to a model with baseline $CD4^+$ T-cell count instead of BMI (data not shown).

The role of non-nucleoside reverse transcriptase inhibitors could not be evaluated in this study; however, in a triple drug regimen consisting of stavudine, lamivudine and nevirapine, Taylor et al. reported plasma stavudine concentrations comparable to the concentrations found in the 050 and ADAM studies.[28]

Table 3 Linear regression analysis using stavudine concentration in CSF as the dependent variable

	Univariate		Multivariate			
Independent variable	Coefficients	p	Coefficients	p	Coefficients	p
Baseline HIV-1 RNA (per $\log_{10}$ copies)	-7 (32)	0.83				
Baseline $CD4^+$ T-cell count (per 100 cells)	-26 (8)	0.003	-15 (5)	0.007	-16 (5)	0.004
Baseline $CD8^+$ T-cell count (per 100 cells)	-16 (26)	0.56				
Age (per year)	0.3 (1.8)	0.86				
Body mass index (kg/m^2)	-6 (7)	0.4				
Creatinine[a] (µmol/L)	-2 (1)	0.09	- -	-		
Proteins in CSF[a] (g/L)	-173 (99)	0.09	- -	-		
Cell count in CSF[a] (cells/µL)	-0.2 0.5	0.65				
Drugs						
Indinavir (yes)	30 (42)	0.47				
Ritonavir (yes)	143 (19)	0.0001	132 (19)	0.0001	128 (18)	0.0001
Saquinavir (yes)	47 (29)	0.11				
Nelfinavir (yes)	-69 (30)	0.03	- -	-		
Ritonavir/indinavir[b] (yes)	125 (21)	0.0001				
Time interval[c] (hrs)	0.4 (11)	0.97				
Treatment duration (weeks)	-1.5 (1.3)	0.23				

For the independent variables in the models regression coefficients (SE), and p-values are shown.
[a] at time of sampling; [b] the use of ritonavir plus indinavir, ritonavir or indinavir; [c] the time between medication intake and time of sampling

Forty percent of stavudine is excreted unchanged in urine. The remaining is excreted unchanged into the feces or metabolized, although pathways are still unknown.[15] Ritonavir and indinavir are both strong inhibitors of the cytochrome P450 enzyme system influencing the pharmacokinetics of several agents.

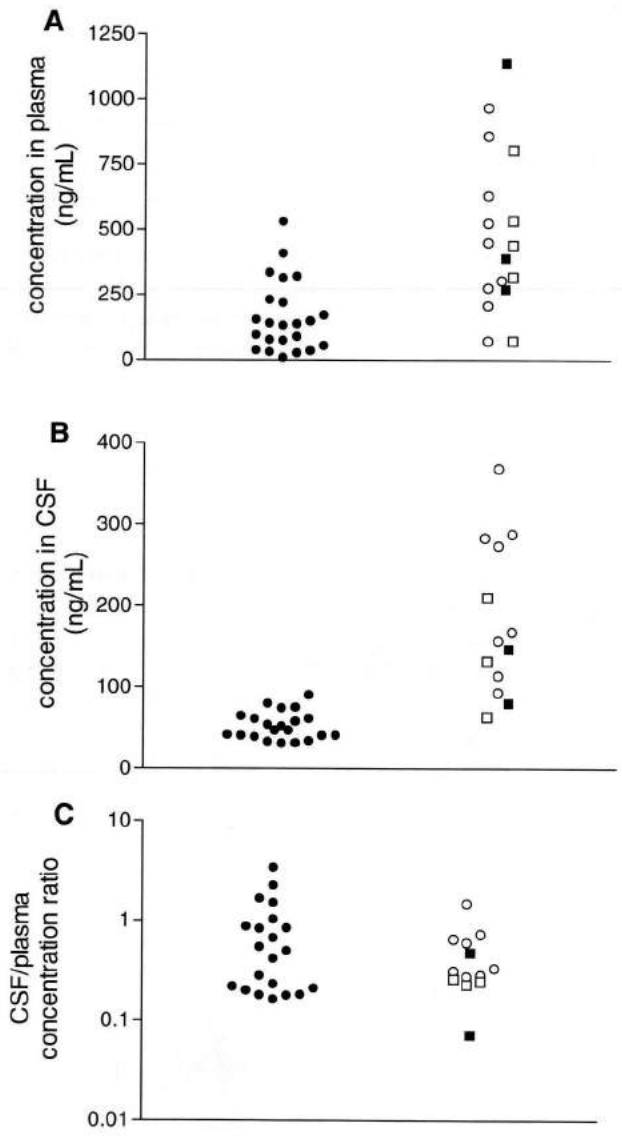

Figure 1 Stavudine concentrations in plasma or CSF and the CSF/plasma concentration ratio of stavudine in patients with an antiretroviral therapy combination not containing ritonavir or indinavir (left column), or containing ritonavir (right column, white dots), indinavir (right column, black squares) or ritonavir plus indinavir (right column, white squares).
Panel A: Stavudine concentrations in plasma or serum.
Panel B: Stavudine concentrations in CSF.
Panel C: CSF/plasma or serum concentration ratios of stavudine.

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Chapter 10

General discussion.

With the introduction of potent antiretroviral therapy, the aim of the treatment of HIV-1 infection has focussed on preventing instead of delaying disease progression. The suppression of viral replication as strongly as possible for as long as possible, has resulted in prevention or (partial) restoration of the damage to the immune system.[1-4] Subsequently, a significant decrease of the incidence of AIDS related morbidity and mortality has been observed in the Western world.[5-8] However, since viral eradication was not expected to be feasible within years ,[9-11] the use of antiretroviral agents was anticipated to be necessary for a long period. Antiretroviral therapy often requires complicated dosing schedules, and dietary restrictions, and is causing toxicity, both on the short- and long-term. Especially for the considerable part of the HIV-1 infected patients coping with poverty, mental illness, or drug and/or alcohol use,[12,13] these factors might interfere with the use of the drug combinations and foster the development of drug-resistance due to inadequate use of the antiretroviral agents.[14-19]

Induction-maintenance therapy seemed a promising treatment strategy to reduce the complexity and long-term toxicity of antiretroviral drug regimens. Three different studies, were started to investigate the feasibility of such a strategy.[20,21] In the ADAM study, described in this thesis, patients were treated with maintenance therapy consisting of two agents (stavudine plus nelfinavir or saquinavir plus nelfinavir) following a quadruple drug induction therapy of 26 or 50 weeks [Chapter 2 and 3]. The ACTG 343 and Trilège studies used an induction therapy consisting of zidovudine, lamivudine, and indinavir for 24 and 12 weeks, respectively. Subsequently, patients in the ACTG 343 study were randomised to maintenance therapy consisting of either indinavir mono-therapy, or zidovudine plus lamivudine or to continued triple drug therapy.[20] The Trilège study randomised patients to either zidovudine plus lamivudine, or zidovudine plus indinavir or to continued triple drug therapy.[21] Although all three studies were significantly different in design (see Table 1), each study concluded that with the currently available antiretroviral agents, induction-maintenance therapy was not resulting in a durable suppression of viral replication in HIV-1 infected patients. It did not matter whether the induction period consisted of a triple (ACTG 343, Trilège) or a quadruple drug regimen (ADAM) or whether the maintenance therapy consisted of two nucleoside analogue RT inhibitors (ACTG 343, Trilège), a single protease inhibitor (ACTG 343), one nucleoside RT plus one protease inhibitor (Trilège, ADAM), or two protease inhibitors (ADAM) . One could argue that the induction period in the Trilège study was too short for attaining low HIV-1 RNA concentrations in plasma (27% had a

HIV-1 RNA concentration >50 copies/mL at time of randomisation). But even in patients with an induction therapy for fifty weeks and an HIV-1 concentration <50 copies/mL (ADAM), viral rebound was found more frequently during maintenance therapy than during prolonged induction therapy. Patients participating the ACTG 343 study were mostly experienced with zidovudine. Pretreatment with zidovudine did predispose for virological failure during maintenance therapy compared to continued triple drug therapy. However this was not the only factor contributing to virological failure during maintenance therapy, since the antiretroviral therapy naive patients in the Trilège and the ADAM study were having viral rebound during maintenance therapy as well.

Table 1

	ADAM	ACTG 343	Trilège
Inclusion criteria:			
HIV-1 RNA in plasma (copies/mL)	≥1000	≥1000	3500-100000
CD4⁺T-cell count ($x10^6$cells/mL)	≥ 200	≥ 200	<600
treatment experience	naïve	naïve to 3TC, abacavir, PI	naïve
Induction regimen			
agents	d4T+3TC+NFV+SQV	ZDV+3TC+IDV	ZDV+3TC+IDV
duration	26 or 50 weeks	24 weeks	12 weeks
criteria for randomisation	<LLQ[a] at weeks 24 and 25 or 48 and 49	<200 copies/mL at weeks 16, 20 and 24	<500 copies/mL at weeks 8 and 12
Maintenance regimen			
agents	d4T+NFV or NFV+SQV	IDV or IDV+3TC	ZDV+IDV or ZDV+3TC
median duration of follow-up after randomisation	10 weeks	8 weeks	24 weeks
Virological failure			
definition	>LLQ[a] copies/mL at week 36	>200 copies/mL (confirmed)	>500 copies/mL (confirmed)
% failure during maintenance	64% [b]	23%	27%
% failure during prolonged induction	9% [b]	4%	9%

[a] LLQ =the lower limit of quantification (<50 or variable quantification limit of an ultrasense HIV-1 RNA assay)

[b] after 26 weeks of induction therapy

These three studies point out that in most patients, a simple maintenance regimen can not continue to suppress viral replication after an induction period in which a plasma HIV-1 RNA concentration below 50 copies/mL was achieved.

Why did induction-maintenance therapy fail? In the period these studies were conducted, evidence accumulated that even in patients with sustained viral suppression below a plasma HIV-1 RNA concentration of 50 copies/mL, viral replication was still present.[22-26] In line with this evidence, Grossman et al. hypothesised that antiretroviral drug therapy only reduces ongoing virus production bursts and diminishes their frequency but fails to completely block them.[27] Viral decay rates are therefore not a reflection of half lifes of different cells infected before the initiation of therapy, but of cells infected after the initiation of therapy. The mathematical model describing the viral decay with these assumptions results in an equilibrium between infected cells with a certain reproduction ratio and a certain number of infected cells. This model fits to observations in earlier clinical studies, that triple drug combinations may not provide full suppression of viral replication in individual patients. The plasma HIV-1 RNA concentration was observed to decline below the quantification limit of an ultrasensitive assay more rapidly with a five-drug- than a three-drug-regimen.[28] But even with five agents, suppression appears to be less than 100%: HIV-1 RNA could still be found in peripheral blood mononuclear cells.[29]

The model also provides an explanation for the increase in plasma HIV-1 RNA concentration during maintenance therapy [Chapter 2 and 3]. With a reduction in the number of drugs during maintenance therapy, virus production bursts may increase in frequency and result in a new equilibrium at a detectable plasma HIV-1 RNA level. This is supported by observations in the ACTG 343 and Trilège studies.[30,31] Both studies investigated whether viral rebound during maintenance therapy was associated with the selection of resistant mutant virus. The presence of resistant mutant virus with a decreased susceptibility to protease inhibitors was preceded by an increase in viral replication of wild-type virus or viral mutants resistant to other agents than the protease inhibitors, indicating that insufficient antiretroviral potency or poor compliance enabled the occurrence of viral replication and the subsequent development of resistant mutant virus.[30,31]

In addition to incomplete inhibition of viral replication, the existence of a long-lived latent reservoir of infected cells may be another reason for virological failure during maintenance therapy.[10,11,32] Using mathemathical modelling, the time needed

to eradicate this reservoir was first estimated to be 2 to 3 years.[9] Finzi et al. measured the decay rate of the latent infected resting $CD4^+$ T-cells to result in a mean T½ of 43.9 months. With a reservoir of 1×10^5 cells, the time required for eradication would be 60.8 years.[25] Some trials already aimed at selectively activate the latently infected cells in this reservoir.[26,33,34] OKT3 (a monoclonal antibody against CD3) and IL-2 (interleukine-2) indeed activated T-cells and viral replication, but the effect on the size of the pool of latently infected cells was not clear. Stimulation of these cells with incomplete suppression of viral replication by antiretroviral agents, is even suggested to result in newly infected cells.[27] Moreover, the use of these agents was accompanied with considerable side effects.[26]

So, with ongoing viral replication in the presence of potent antiretroviral therapy and the existence of long-lived latently infected cells, it is likely that a reduction of the antiretroviral pressure during maintenance therapy will result in viral rebound.

If viral replication during maintenance therapy can expand, replication might further benefit from the increased availability of target cells (e.g. proliferating $CD4^+$T-cells). For both resistant mutant viruses as wild type virus, an increase in the availability of activated $CD4^+$ T-cells can contribute to the level of rebound of HIV-1 RNA in plasma.[35-37] After induction therapy, at time of the randomisation to maintenance regimens, increased target cell availability was likely. Indeed, the ACTG 343 study found this 'predator-prey' mechanism to be of significance to the viral rebound during maintenance therapy.[20] Fleury et al. recently showed that target cells were not expected to decline to normal levels before week 72,[38] indicating that even after an induction therapy for 50 weeks as given in the ADAM study (Table 1), replication might have been facilitated by increased target cell availability [Chapter 3].

Another suggested threat to the efficacy of induction-maintenance therapy is the continued replication of viruses in the anatomical sanctuaries, such as the central nervous system (CNS) and the genital tract.[39-42] Since it is difficult to obtain material from the CNS or the genital tract, cerebrospinal fluid (CSF) and semen are used as the accessible representatives of these respective compartments. Several studies have shown discrepancies between HIV-1 RNA concentrations in plasma and CSF or semen,[43,44] both in treated as untreated patients. Moreover, the evolution of the viral gene differed in virus obtained from cells in the CSF or the semen and virus obtained form peripheral mononuclear cells in plasma.[45-47] Most studies however show prompt decreases of HIV-1 RNA concentration in CSF and semen,[42,48-50] suggesting that triple drug combinations do effect these sites. Still, as shown in

Chapter 8 and by others, penetration of antiretroviral agents, is usually lower than in plasma.[42,51-54] Especially the penetration of some of the protease inhibitors in these compartments is limited by high protein binding, high molecular weight, and the presence of transport molecules such as P-glycoprotein (PgP).[53,55,56] This may have clinical consequences, as was shown by for example Gisolf et al. They observed less consistent decreases of HIV-1 RNA concentrations in CSF of patients treated with only saquinavir and ritonavir compared to patients treated with stavudine and the same protease inhibitor combination. In addition, a slower decay rate of virus was observed in CSF compared to plasma,[57] and the development of drug resistant mutants in CSF and semen differed from that in plasma.[40] Therefore, caution is warranted for insufficient efficacy of antiretroviral therapy in these sanctuary sites on the long-term. If necessary, the penetration of antiretroviral agents into the sanctuary sites might be improved upon, as is indicated by recent publications. Van Praag et al. found increased indinavir concentrations with the combined use of ritonavir and indinavir compared to indinavir alone.[58] In addition, in Chapter 9, the concentrations of stavudine were described to be higher in patients using ritonavir and/or indinavir containing regimens than in regimens without these specific protease inhibitors. In vivo studies have demonstrated the possible use of PgP molecule blockers in order to increase drug concentrations in the CSF. At the annual meeting of the American Society for Clinical Pharmacology and Therapeutics, Dr. Choo et al. presented data from a mouse model, indicating that nelfinavir concentrations in the CSF and the testis could be increased by blocking the PgP molecules.[59]

In the past few years the drawbacks of antiretroviral therapy only have become more pronounced. In addition to the toxicity on the short term, such as diarrhoea, nephrolithiasis, and hepatotoxicity, toxicity on the long-term, such as lipodystrophy and metabolic disorders, are limiting for the use of antiretroviral therapy.[16,17,60,61] Although mitochondrial toxicity is suggested to be crucial in the majority of these side effects, the exact pathogenesis of most side effects is not yet unravelled,[62,63] nor the impact of these side effects on morbidity on the long-term.[64,65] With the awareness that eradication is not to be accomplished in several decades,[23,25,27] more and more patients and doctors are inclined to delay treatment. Induction-maintenance regimens or other treatment strategies that minimise toxicity and improve tolerance and compliance are therefore more warranted than ever.

Induction-maintenance therapy with the currently available agents has not been successful. However, the concept may not be lost for antiretroviral therapy. The use of agents that decrease the availability of target cells, e.g. activated $CD4^+$ T cells, such as mycophenolic acid or hydroxy urea might reduce the number of drugs required for suppression of viral replication during maintenance therapy.[37,66,67] However, the side effects of these agents are considerable, and may therefore outweigh the benefit of this strategy.

The induction-maintenance strategy is not the only option to simplify therapy. A triple drug therapy may be simplified as well. Recently, equal virological efficacy of triple drug combinations containing protease inhibitors compared to triple drug combinations containing non-nucleoside RT inhibitors has been shown.[68,69] So, patients may profit from the advantages of the non-nucleoside RT inhibitors over the protease inhibitors with respect to dosing and toxicity. Furthermore, the pharmacokinetic properties of agents may be used to improve drug intake schedules and pill burden.[70-74] For example, the addition of a low dose ritonavir can facilitate the use of indinavir by changing the regular dosing schedule of two tablets thrice daily on an empty stomach, to two tablets twice daily without dietary restrictions.[74]

As indicated in Chapter 5 and 6, both efficacy and toxicity may be associated with the level of drug exposure. Although there is a large inter- and intra individual variability in drug exposure (at least for the protease inhibitors [Chapter 6]) and plasma drug concentrations are only a rough representative of drug exposure, the monitoring of drug levels of antiretroviral agents, might help to identify the optimal drug level per patient with respect to efficacy and toxicity. Furthermore it can be used to gain further insight into the pharmacokinetic interactions which may be useful for establishing more convenient dosing and intake schedules, or for the selective improvement of drug exposure in certain cell or body compartments (see above).

Some case histories, like 'the Berlin patient',[75,76] in which a vigorous mainly cellular, HIV-1 specific immune response was capable of controlling HIV-1 replication after discontinuation of antiretroviral therapy, has stimulated the use of immunological control of viral replication (with or without the stimulation of latently infected cells) in the treatment of HIV-1 infection. Although these case reports of recently infected patients were promising, in chronically infected patients this strategy usually led to HIV-1 RNA rebound.[77] Nevertheless, strategies involving treatment interruptions are initiated to investigate the benefits of inducing an HIV-1 specific immune response by an increase in viral replication during treatment

interruptions.[78-80] Vaccination, with for example recombinant gp160, in combination with antiretroviral therapy was capable of inducing HIV-1 specific immune responses, however, no additional clinical benefit was observed so far.[81,82]

For now, eradication of HIV-1 infection seems beyond the current treatment options. However, it should not be forgotten that major advances have already been made in the treatment of HIV-1 infection, and further progresses are expected to be made in the future. With long-term treatment assumed to be necessary, elucidating the pathogenesis of the metabolic disorders and lipodystrophy syndrome associated with the long-term use of antiretroviral agents is pivotal. In the meanwhile therapy in the Western world needs to become more individualised, carefully timing the initiation of therapy and balancing the pros en cons of long-term therapy for the HIV-1 infected patient.

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Summary

Treatment with highly active antiretroviral therapy (HAART) has led to significant health benefits for HIV-1 infected patients. However, long-term-use of multi-drug regimens is difficult to sustain. Simplifying antiretroviral treatment regimens would increase patient-adherence and minimise toxicity. In the Amsterdam Duration of Antiretroviral Medication (ADAM) study the feasibility of an induction-maintenance strategy in HAART was investigated.

In the **introduction** of this thesis, the background to the design of the study is outlined. HIV-1 infected patients with ≥ 200 $CD4^+$ cells/mm^3, ≥ 1000 HIV-1 RNA copies/mL in plasma and no previous exposure to antiretrovirals were enrolled in this multi-centre, randomised, open-label study. After 26 weeks of induction therapy (stavudine (d4T) + lamivudine (3TC) + saquinavir (SQV) + nelfinavir (NFV)), patients were randomised to maintenance therapy (either d4T + NFV or SQV + NFV) or prolonged induction therapy, if the plasma HIV-1 RNA level at weeks 24 and 25 had been < 50 copies/mL. The patients randomised to prolonged induction therapy were subsequently randomised at week 50 to continued quadruple drug therapy or one of the two maintenance therapies, if the plasma HIV-1 RNA level at weeks 48 and 49 had been the lower limit of quantification of the ultrasensitive assay.

In **Chapter 2,** the results of an interim analysis of the ADAM study are described. The quadruple induction regimen provided a rapid suppression of viral replication to below 50 copies/mL. Nevertheless, this level of suppression was not sustained in a considerable number of patients randomised to maintenance therapy at week 26. A sustained suppression of viral replication during maintenance therapy was associated with a relatively high initial viral clearance rate. In the light of these findings, and those reported by others, randomisation at week 26 was discontinued. Randomisation after 50 weeks of induction therapy was not discontinued.

The patients randomised at week 50 to induction or maintenance therapy were compared for the proportion of patients with a viral rebound (**Chapter 3**). Treatment failure was observed in 50% of the patients on maintenance therapy compared to only 20% of the patients on continued quadruple drug therapy (not statistically significant). Subsequently, the time to > 400 HIV-1 RNA copies/ml in plasma during maintenance therapy was compared between patients randomised at week 26 and at week 50. Time to >400 HIV-1 RNA copies/ml was comparable between patients randomised to maintenance therapy after 26 and 50 weeks of induction therapy. Apparently, a longer period of quadruple drug therapy does not postpone viral rebound during maintenance therapy.

Before discussing the association between the exposure to the protease inhibitors and toxicity and efficacy of the quadruple drug regimen in Chapter 5 and 6, the steady-state plasma pharmacokinetics of nelfinavir (Viracept®) and saquinavir (Invirase®) during a quadruple antiretroviral drug regimen are described in **Chapter 4**. In eighteen patients participating in the ADAM study, who used the quadruple antiretroviral drug regimen for at least 4 weeks, plasma concentrations of nelfinavir and saquinavir were quantified during a full (8-hour) dosing interval. Plasma pharmacokinetics of both protease inhibitors were calculated using noncompartmental methods. The positive pharmacokinetic interaction between nelfinavir and saquinavir was further explored by comparing saquinavir pharmacokinetics to those observed in historical controls treated with a saquinavir dosage of 1,200 mg tid (Invirase®), without the use of nelfinavir. Observed interindividual variation in pharmacokinetic parameters was approximately 4-fold for nelfinavir, and approximately 6-fold for saquinavir. Nelfinavir increased saquinavir plasma concentrations at least 2-fold, and reduced intrapatient fluctuation of saquinavir plasma concentrations. Interpatient variability in saquinavir pharmacokinetics was not reduced by concomitant administration of nelfinavir.

By means of these plasma drug concentrations during a full 8h-dosing interval, plasma drug concentration ratios were calculated as a measure for drug exposure. Using these ratios, plasma drug concentrations were adjusted for the time interval between drug ingestion and drawing of the sample. In addition, the elimination rate constant (k) for HIV-1 clearance was calculated during the first two weeks of treatment in 29 patients. It appeared that the variation in the rate of decline of HIV-1 RNA in plasma between patients after the initiation of therapy could be explained by differences in exposure to nelfinavir or saquinavir.

These results, described in **Chapter 5**, could be used to further optimise antiretroviral drug therapy and may be a first tool to assess antiretroviral activities of new or increasing doses of drugs administered in combination regimens. Furthermore, these data suggest that exposure to antiretroviral drugs should be incorporated in mathematical models to describe HIV-1 dynamics in more detail.

In **Chapter 6,** again the exposure to nelfinavir (NFV) and saquinavir (SQV) as part of the quadruple drug regimen are described, but now for the whole 26 weeks of induction therapy. The exposure to the protease inhibitors was relatively low, although the virological efficacy of the regimen used was satisfactory. In addition, the toxicity of the quadruple induction regimen was described. The quadruple drug regimen was rather well tolerated. Diarrhea was frequently reported but could be

relieved by the use of antidiarrheal agents. Lipid abnormalities in plasma were infrequent and mild. Subsequently, the association between toxicity and drug exposure was described. Except for diarrhea, all gastrointestinal complaints observed were found to be associated with the level of exposure to nelfinavir or saquinavir. Experiencing abdominal pain was associated with a relatively high exposure to nelfinavir, while the complaints nausea and abdominal distension were associated with a low exposure to the two protease inhibitors.

One of the sub-studies in which patients of the ADAM study could participate concerned Quality of Life (**Chapter 7**). The patients randomised at week 26 to prolonged induction therapy or maintenance therapy were compared for their quality of life (QoL). QoL declined more during maintenance therapy than during prolonged quadruple induction therapy. Most likely, inferior viral suppression associated with maintenance therapy added to this negative impact on QoL, that outweighed the burden of the quadruple regimen.

12 patients of the ADAM study were willing to participate in another sub-study, described in **Chapter 8**, in which cerebrospinal fluid (CSF) and semen were sampled after 26 or 50 weeks of continuous treatment with the quadruple drug regimen. The concentrations of stavudine, lamivudine, nelfinavir, and saquinavir, were assessed in plasma, CSF and semen. Of the protease inhibitors, only saquinavir was detected at a low concentration in seminal plasma. Neither nelfinavir nor saquinavir was detected in the CSF. The concentrations of stavudine and lamivudine in seminal plasma were higher than in CSF. For lamivudine, seminal plasma/blood plasma ratios were significantly higher than CSF/blood plasma ratios. These data support the hypothesis that poor penetration of the central nervous system and the male genital tract by antiretroviral drugs can contribute to differences in viral dynamics in these compartments.

The plasma- and CSF concentrations of stavudine were further investigated (**Chapter 9**). The metabolism of this nucleoside analogue reverse transcriptase inhibitor (NRTI), is partly unknown. In order to investigate the pharmacokinetics of stavudine, stavudine concentrations in paired samples of plasma and cerebrospinal fluid (CSF) of patients using four different treatment regimens were compared. Patients using stavudine without the addition of a protease inhibitor or in combination with nelfinavir and saquinavir were found to have significantly lower stavudine concentrations in plasma and CSF than patients using ritonavir and/or indinavir. CSF/plasma concentration ratios of stavudine were comparable. In a multivariate linear regression analysis, the use of ritonavir and/or indinavir and the baseline $CD4^+$ T-cell count were significantly associated with the stavudine

concentrations in plasma and CSF. This unexpected finding suggested that stavudine metabolism may, at least in part, be inhibited by ritonavir and/or indinavir.

In conclusion, in **Chapter 10**, the results of three studies, the Trilège, ACTG 343, and ADAM study investigating induction-maintenance strategies are discussed. Probably, several factors such as insufficient potency of antiretroviral therapy, target cell availability, and the presence of cellular- and anatomical reservoirs, may contribute to viral rebound during maintenance therapy. Although simplified treatment regimens are still warranted, the Trilège, ACTG 343, and ADAM study have indicated that induction-maintenance therapy with the currently available agents is not advocated for antiretroviral therapy.

Samenvatting

Een infectie met het humane immunodeficiëntie virus (HIV) leidt tot een vermindering van de afweer van de patiënt tegen bacteriën, schimmels en andere virussen. Het virus veroorzaakt namelijk een afname van $CD4^+$-cellen (een type afweercel). Wanneer de $CD4^+$ cellen dusdanig afnemen, dat de patiënt infecties krijgt waartegen een niet HIV-geïnfecteerde patiënt voldoende afweer heeft, spreekt men van AIDS (aquired immunodeficiency syndrome). De behandeling van een infectie met HIV bestaat op dit moment uit een combinatie van tenminste drie antiretrovirale middelen. Hiermee wordt de vermenigvuldiging van HIV geremd, zodat het aantal virusdeeltjes in het bloed (HIV-RNA concentratie in copieën/mL) onmeetbaar wordt. Hierdoor kan het immuunsysteem zich herstellen of raakt het niet verder beschadigd. Dit heeft geleid tot een enorme verbetering van de prognose van HIV-geïnfecteerde patiënten. De behandeling is echter voor veel patiënten moeilijk vol te houden, zeker op de lange termijn. De medicijnen moeten vaak volgens strikte regels worden ingenomen, zowel wat betreft het tijdschema, als wat betreft het te volgen dieet. Bovendien kan de behandeling gepaard gaan met aanzienlijke bijwerkingen, zowel op de korte als de lange termijn. Voor patiënten zou het daarom een verbetering betekenen wanneer simpele behandelingen zouden kunnen volstaan voor de behandeling van HIV infectie. Zowel de belasting met betrekking tot de pilleninname als de bijwerkingen zou hiermee kunnen worden verminderd. In de 'Amsterdam Duration of Antiretroviral Medication' of kortweg de ADAM-studie werd bestudeerd of het mogelijk is na een periode van intensieve behandeling (inductiebehandeling) te volstaan met een eenvoudige onderhoudsbehandeling, die mogelijk beter is vol te houden.

In **Hoofdstuk 1**, de introductie van dit proefschrift, worden de achtergronden van deze studie uiteengezet. HIV-1 geïnfecteerde patiënten met meer dan 200 $CD4^+$ cellen/mm^3 en meer dan 1000 HIV-1 RNA copieën/mL in het bloed en geen eerdere blootstelling aan antiretrovirale middelen konden meedoen aan deze studie. Gedurende 26 weken werd een inductiebehandeling gegeven bestaande uit 4 geneesmiddelen: twee nucleoside reverse transcriptase remmers (NRTI's): stavudine + lamivudine en twee protease remmers: saquinavir en nelfinavir. Vervolgens werd met behulp van loting bepaald of patiënten een onderhoudsbehandeling met stavudine + nelfinavir of saquinavir + nelfinavir kregen of de inductiebehandeling voortzetten. Loting was alleen toegestaan als de concentratie HIV-1 RNA in het bloed op week 24 en op week 25 lager was dan 50 copieën/mL. De patiënten die na week 26 de inductiebehandeling voortzetten kregen vervolgens op week 50 opnieuw een behandeling volgens loting toegewezen: opnieuw voortzetting van de inductiebehandeling of een van de twee

onderhoudsbehandelingen. Alleen patiënten waarbij de concentratie HIV-1 RNA op week 48 en 49 lager was dan de onderste meetgrens van een ultra-gevoelige assay kwamen in aanmerking voor loting.

In **Hoofdstuk 2** worden de resultaten van een tussentijdse analyse van de ADAM-studie beschreven. De inductiebehandeling met vier middelen was in staat een snelle onderdrukking van de virusvermenigvuldiging te bewerkstelligen, waarbij de concentratie HIV-1 RNA in het plasma tot minder dan 50 copieën/mL daalde. Desalniettemin bleek 10 weken na de loting 64% van de patiënten op onderhoudsbehandeling en slechts 9% van de patiënten op voortgezette inductiebehandeling een meetbare concentratie HIV-1 RNA (<50 copieën/mL) in het bloed te hebben. Gezien deze bevindingen werd de loting op week 26 gestaakt. Patiënten op onderhoudsbehandeling die wel een goede onderdrukking van de virusvermenigvuldiging behielden hadden een relatief snellere daling van de concentratie HIV-1 RNA in het plasma in de eerste twee weken van inductiebehandeling dan patiënten bij wie dit niet het geval was. Dit is een aanwijzing dat de snelheid van daling van HIV-1 RNA in het bloed in de eerste twee weken van behandeling een maat kan zijn voor de effectiviteit van de behandeling. De loting na 50 weken inductiebehandeling werd wel voortgezet.

De patiënten die op week 50 volgens loting een van de mogelijke behandelingen kregen toegewezen werden vergeleken met betrekking tot het deel van de patiënten dat weer een meetbare hoeveelheid virus in plasma had. Dit wordt gezien als een teken van onvoldoende onderdrukking van de vermenigvuldiging van het virus (virologisch falen) (**Hoofdstuk 3**). Na week 50 werd virologisch falen geobserveerd in slechts 20% van de patiënten op voortgezette inductiebehandeling vergeleken met 50% van de patiënten op onderhoudsbehandeling. Wederom lijkt onderhoudsbehandeling onvoldoende onderdrukking van virusvermenigvuldiging te geven. Om te bestuderen of een langere periode van inductiebehandeling invloed heeft op het voorkómen van virologisch falen werd de tijd tot virologisch falen gedurende de onderhoudsbehandeling vergeleken tussen patiënten waarbij loting plaatsvond op week 26 en op week 50. De tijd tot een HIV-1 RNA concentratie >400 copieën/mL tijdens onderhoudsbehandeling was vergelijkbaar bij deze groepen. Blijkbaar kan een langere periode van inductiebehandeling het virologische falen gedurende onderhoudsbehandeling niet uitstellen.

Aangezien de farmacokinetiek (d.w.z. de verdeling van de ingenomen medicatie over het lichaam ofwel de blootstelling aan een geneesmiddel) van de proteaseremmers nelfinavir en saquinavir belangrijk is voor het begrip van hoofdstuk 5 en 6, wordt in **Hoofdstuk 4** eerst de zogenaamde 'steady-state' farmacokinetiek

van nelfinavir en saquinavir gedurende de inductiebehandeling beschreven. Bij achttien deelnemers aan de ADAM-studie, die de inductiebehandeling met vier antiretrovirale geneesmiddelen ten minste vier weken gebruikten, werden plasmaconcentraties van nelfinavir en saquinavir bepaald gedurende een doseringsinterval van acht uur. De farmacokinetische parameters van de beide proteaseremmers in plasma werden berekend en vervolgens voor saquinavir vergeleken met de farmacokinetiek van saquinavir in patiënten die behandeld werden met saquinavir (Invirase®) in een dosering van 1,200 mg drie maal daags zonder het gebruik van nelfinavir. De variatie in de blootstelling aan nelfinavir tussen de verschillende patiënten was ongeveer vier maal, en aan saquinavir ongeveer zes maal. Nelfinavir verhoogde de saquinavir plasmaconcentraties ten minste twee maal en verminderde de variatie van saquinavir plasma concentraties binnen een patiënt. De variatie in de blootstelling aan saquinavir tussen de verschillende patiënten werd niet verminderd door het gelijktijdig gebruiken van nelfinavir.

In hoofdstuk 5 en 6 werden als maat voor de blootstelling aan nelfinavir en saquinavir plasmaconcentratieratios berekend. Deze ratio (de gemeten concentratie van een geneesmiddel in het bloed gedeeld door de bijbehorende referentie concentratie voor het tijdsinterval tussen inname van de medicatie en het moment van bloedafname) corrigeert voor het tijdsinterval tussen het moment van inname van de medicatie en het afnemen van bloed. Referentie concentraties werden verkregen uit de plasmaconcentraties van nelfinavir en saquinavir bepaald gedurende een doseringsinterval van acht uur. Zoals al in Hoofdstuk 2 besproken is, zou de snelheid van daling van de concentratie HIV-1 RNA in het plasma gedurende de eerste twee weken (k) een maat kunnen zijn voor de effectiviteit van de behandeling. Deze daling werd berekend in 29 patiënten van de ADAM-studie. Het bleek dat de variatie in deze snelheid van daling na het starten van de behandeling gedeeltelijk kon worden verklaard door verschillen in blootstelling aan nelfinavir en saquinavir. Deze resultaten, beschreven in **Hoofdstuk 5**, zouden kunnen worden gebruikt om de antiretrovirale behandeling verder te optimaliseren. Het meten van de daling van HIV in plasma zou een snelle methode zijn om de antiretrovirale activiteit van nieuwe of hogere doses van geneesmiddelen binnen een combinatiebehandeling te bepalen. Bovendien suggereren deze gegevens dat wanneer de blootstelling aan geneesmiddelen als factor in rekenmodellen zou worden meegenomen de dynamiek van HIV in het lichaam tijdens behandeling meer gedetailleerd zou kunnen worden beschreven.

In **Hoofdstuk 6** wordt de blootstelling aan nelfinavir en saquinavir gedurende de eerste 26 weken inductie-behandeling beschreven. De blootstelling aan proteaseremmers in de 65 patiënten was lager dan verwacht. Desondanks was de virologische effectiviteit gedurende de eerste 26 weken naar wens; slechts bij één patiënt daalde de concentratie HIV-1 RNA in plasma niet onder de 400 kopieën/mL. Verder worden in dit hoofdstuk de bijwerkingen van deze combinatiebehandeling beschreven. De vier antiretrovirale middelen werden tezamen redelijk goed verdragen. Als bijwerking werd diarree het meest frequent gemeld, maar deze klacht kon meestal worden verlicht door het gebruik van anti-diarree middelen. De meest voorkomende reden voor het staken van de geneesmiddelen combinatie (in vier van de zeven patiënten) was het vóórkomen van leverenzymstijgingen in het bloed. Een abnormale verdeling van de lipiden in het plasma werd niet frequent gezien. Verder werd getracht het vóórkomen van bijwerkingen te relateren aan de blootstelling aan geneesmiddelen. Met uitzondering van diarree, waren alle klachten van het maag-darmstelsel geassocieerd met de blootstelling aan nelfinavir of saquinavir. Het vóórkomen van buikpijn was geassocieerd met een relatief hoge blootstelling aan nelfinavir of saquinavir, terwijl klachten van misselijkheid of een opgeblazen gevoel juist geassocieerd waren met een relatief lage blootstelling aan deze proteaseremmers.

Een van de substudies waaraan patiënten in de ADAM-studie konden deelnemen betrof een onderzoek naar de kwaliteit van leven tijdens deze behandelingsstrategie (**Hoofdstuk 7**). De kwaliteit van leven van patiënten die op week 26 de inductiebehandeling voortzetten of een onderhoudsbehandeling kregen werd vergeleken. Kwaliteit van leven verslechterde meer gedurende onderhouds-behandeling dan gedurende het voortzetten van de inductiebehandeling. Kwaliteit van leven bleek geassocieerd te zijn met de de mate van onderdrukking van de virusvermenigvulding. Patiënten waarbij een suboptimale onderdrukking van de virusvermenigvuldiging was gevonden (met name bij patiënten met onderhoudsbehandeling) hadden minder goede kwaliteit van leven. Waarschijnlijk heeft de wetenschap een suboptimale onderdrukking van de virusvermenigvuldiging te hebben meer effect op kwaliteit van leven dan de belasting van het slikken van vier in plaats van twee geneesmiddelen.

In een andere substudie, beschreven in **Hoofdstuk 8**, werd na 26 of 50 weken continue behandeling met de vier antiretrovirale geneesmiddelen hersenvocht (liquor) en sperma (semen) verzameld. In zowel deze vloeistoffen als in het plasma werden de concentraties van stavudine, lamivudine, nelfinavir en saquinavir bepaald. Van de proteaseremmers was alleen saquinavir in lage concentraties

aanwezig in het sperma. Nelfinavir en saquinavir waren niet meetbaar in de liquor. De concentraties van stavudine en lamivudine in sperma waren hoger dan in liquor. Deze gegevens ondersteunen de veronderstelling dat het matig doordringen van geneesmiddelen in het centrale zenuwstelsel en het mannelijk genitaal stelsel kan bijdragen aan de verschillen tussen de onderdrukking van virusvermenigvuldiging in deze weefsels en in het bloed.

De concentraties van stavudine in plasma en liquor worden ook besproken in **Hoofdstuk 9**. Het metabolisme van stavudine is nog gedeeltelijk onbekend. Om de farmacokinetiek van dit middel te onderzoeken werden de stavudineconcentraties in gepaarde afnames van plasma en liquor van patiënten met verschillende combinaties van antiretrovirale geneesmiddelen vergeleken. Patiënten die naast stavudine geen proteaseremmer of nelfinavir of saquinavir gebruikten hadden een lagere stavudineconcentratie in zowel plasma als liquor dan patiënten die stavudine combineerden met ritonavir en/of indinavir. Het bleek dat de hoogte van het $CD4^+$ celaantal op het moment van starten van therapie en het gebruik van ritonavir en/of indinavir geassocieerd waren met de stavudineconcentraties in plasma. Waarschijnlijk hebben patiënten met een laag $CD4^+$ cel aantal een verder gevorderde HIV-infectie. Daarbij hoort over het algemeen een lager gewicht ten opzichte van de lichaamslengte, waardoor stavudine zich over minder lichaamsweefsel hoeft te verdelen en de concentraties dus hoger zijn. Daarnaast suggereren de hoge stavudineconcentraties bij patiënten die ook ritonavir/indinavir gebruiken dat deze proteaseremmers waarschijnlijk het metabolisme van stavudine remmen.

In de conclusie, in **Hoofdstuk 10**, worden de resultaten van drie studies die inductie-onderhoudsstrategieën voor de behandeling van de HIV-infectie hebben onderzocht besproken. Deze drie studies, de Trilège-, de ACTG 343- en de ADAM-studie, suggereren dat er verschillende factoren aanwijsbaar zijn voor de teleurstellende resultaten van inductie-onderhoudsstrategieën voor de behandeling van de HIV-infectie. Zelfs antiretrovirale therapie zoals die standaard wordt gegeven blijkt niet krachtig genoeg voor 100% onderdrukking van de virusvermenigvuldiging. Waarschijnlijk wordt er slechts een evenwicht bereikt tussen onderdrukking van virusvermenigvuldiging door medicijnen enerzijds en de mogelijkheid van het virus zich toch te vermenigvuldigen anderzijds. Tijdens onderhoudsbehandeling lijkt het evenwicht verstoord en kan virusvermenigvuldiging weer toenemen. Daarnaast zou een verhoogde beschikbaarheid van te infecteren cellen of de aanwezigheid van cellulaire maar ook anatomische reservoirs voor HIV bij kunnen dragen aan het virologisch falen van inductie-onderhoudsbehandeling. Ook al zijn eenvoudige

behandelingsstrategieën nog steeds vereist, de ADAM-studie heeft samen met de Trilège- en de ACTG 343-studie aangetoond dat met de huidige antiretrovirale geneesmiddelen inductie-onderhoudsbehandeling niet geadviseerd moet worden.

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Stellingen

1. Het meten van concentraties van saquinavir en nelfinavir in het bloed van patiënten na een standaardontbijt geeft een overschatting van deze concentraties in het dagelijks leven van patiënten. *(dit proefschrift)*
2. Drie is niet altijd één te veel. *(dit proefschrift)*
3. Het feit dat de duur van inductiebehandeling geen invloed lijkt te hebben op de tijd tot virologisch falen gedurende onderhoudsbehandeling suggereert, dat met antiretrovirale behandeling slechts een evenwicht wordt bereikt tussen suppressie en replicatie van HIV. *(dit proefschrift)*
4. Indien er inderdaad sprake is van accumulatie van geneesmiddelen in semen, kan onthouding van ejaculaties door patiënten met antiretrovirale medicatie op twee manieren bijdragen aan het voorkomen van de transmissie van HIV. *(dit proefschrift)*
5. De meeste patiënten hechten meer aan het onmeetbaar zijn van het aantal virussen in het bloed dan aan het verminderen van het aantal te slikken pillen. *(dit proefschrift)*
6. Het feit dat de schuilplaats van HIV wordt vertaald door 'sanctuary site', doet vermoeden dat voor sommige onderzoekers HIV heilig is.
7. Veel Nederlanders zien hun onverschilligheid ten opzichte van hun medemens aan voor tolerantie.
8. Als je niet weet hoe leuk het is om een eenling te zijn, kan je niet bepalen of het leuker is om een tweeling te zijn.
9. De toegenomen kennis van patiënten over de behandeling van hun ziekte betreft zelden wat er NIET kan.
10. Sommige mensen hebben een gat in hun hand, sommige artsen hebben geen handen.