Ritonavir

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Chapter 5

Decreased exposure to saquinavir in HIV-1-infected patients after long term antiretroviral therapy including ritonavir and saquinavir

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Summary

Objective: To explore whether steady-state plasma pharmacokinetics of ritonavir and saquinavir change during long term treatment in HIV-1-infected patients on antiretroviral treatment including ritonavir and saquinavir.

Methods: The pharmacokinetics of ritonavir and saquinavir were assessed during an eight hour period on two occasions in six HIV-1 infected patients, on stable treatment with ritonavir 400 mg twice daily (BID), saquinavir 400 mg BID, stavudine 40 mg BID +/- lamivudine 150 mg BID.

Results: The first study day was 4-12 months (median 7 months) after start of the current regimen. The second study day was 9-15 months (median 10 months) later. No significant differences were observed for the ritonavir pharmacokinetics between the first and second study day. However, median change in plasma trough level of saquinavir between the two study days was -30% (range –79 to +11%, p = .06). Median change in maximum plasma concentration was -40% (range –62 to +34%, p = .09). The median change in area under the plasma concentration versus time curve from 0 to 8 hours was -33% (range –53 to +21%, p = .06).

Conclusion: The exposure to saquinavir decreased over time in HIV–infected patients on stable antiretroviral therapy. These data suggest that, even in apparently compliant patients, regular monitoring of plasma drug concentrations should become part of routine patient care.

Introduction

The introduction of the HIV-1 protease inhibitors has had great impact on the course of HIV infection. Beneficial effects on the suppression of viral replication and the progression to AIDS and death has been shown for ritonavir (RTV), saquinavir (SQV) and indinavir, when they were used in combination therapy [1-4]. In the long term, however, a rebound of HIV-1 RNA in blood is observed in a substantial proportion of patients [5].

In patients with adequate exposure to antiretroviral drugs viral rebound can occur if the prescribed regimen is not powerful enough to suppress HIV replication. Virological treatment failure in these patients can be due to ongoing, viral replication in sanctuary sites, where drug concentrations may be insufficient due to anatomical barriers, such as the blood brain barrier in the central nervous system [6].

Viral rebound in patients with low plasma drug levels can be caused by insufficient efficacy of the drug at that plasma concentration or by the development of resistant virus. Poor compliance, intercurrent diseases, or the use of co-medication may result in decreased exposure. However, there are also indications that exposure to antiretroviral drugs may gradually decrease over time in patients on stable antiretroviral therapy [7]. The objective of this study is to investigate whether the pharmacokinetic exposure to RTV and SQV decreases over time.

Methods

Six HIV-1 infected patients, who were on stable treatment with RTV (capsules) 400 mg twice daily (BID), SQV 400 mg BID, stavudine 40 mg BID +/- lamivudine 150 mg BID, volunteered to participate in this study. They were hospitalized in a pharmacokinetic unit during two study days, 9-15 months apart, to obtain pharmokinetic profiles of the used protease inhibitors. During the study days, patients attended the clinic in the morning after an overnight fast. Blood samples were taken just before, and ½, 1, 1 ½, 2, 2 ½, 3, 4, 5, 6, 7, and 8 hours after ingestion of RTV and SQV with a standardized breakfast. 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.

RTV and SQV concentrations were analyzed in 500 µl plasma by validated ion-pair high performance liquid chromatographic assays with ultraviolet detection as described before [8]. The lower limits of quantification for RTV and SQV in these assays were 50 ng/ml and 25 ng/ml respectively.

Plasma concentration (C) - time (T) data for RTV and SQV were analyzed by noncompartmental methods. The highest observed plasma concentration was defined as C_{max} with the corresponding sampling time as T_{max}. The plasma concentration 8 hours after observed ingestion of the drugs was defined as $C_{min(8hr)}$. The terminal, log-linear period (log_{10} C versus T) was defined by the last data points ($N \geq 4$) by visual inspection. The absolute value of the slope (β/ln 10) was calculated by least squares analysis. The elimination half-life ($T_{1/2}$) was estimated by the equation ln $2/\beta$. The area under the plasma concentration versus time curve ($AUC_{[0\text{-}8h]}$) was obtained using the trapezoidal rule from zero to eight hours. Differences in $AUC_{[0\text{-}8h]}$, C_{max}, $C_{min(8hr)}$, T_{max} and $T_{1/2}$ between the two study days were tested, to evaluate whether these differences were significantly different from zero, using the sign rank test. Data were analyzed using the SAS software package (version 6.12, SAS Institute, Cary, North Carolina, USA).

Results

Six chronically HIV-1-infected male patients, age 34-52 years and weight 68-92 kg, consented for this study. Four patients had experienced an AIDS-defining event before study entry, one had HIV-related symptoms (CDC B) and one patient was asymptomatic (CDC A). They were treated with RTV 400 mg BID, SQV 400 mg BID, stavudine 40 mg BID +/- lamivudine 150 mg BID. Co-medication consisted of cotrimoxazole 480 mg per day (QD) as PCP prophylaxis (n=3) and carbasalate calcium 38 mg QD (n=1). One patient used terbinafine and another temazepam only during the first study day. In one patient cotrimoxazole was initiated after the first study day.

The first study day was 4-12 months (median 7 months) after start of the current regimen. The second study day was 9-15 months (median 10 months) later.

All six patients had detectable serum HIV-RNA at the time the current regimen was initiated (range 3.7-4.9 log_{10} copies/ml). Serum HIV-RNA decreased to < 400 copies/ml in 5/6 patients during the first study day and remained undetectable until at least the second study day.

Median CD4+ cell count increased from 180/mm^3 (range 10-345) at the time patients started current therapy, to 280/mm^3 (150-480) during the first study day, and 360/mm^3 (190-610) during the second study day.

Alanin aminotransferase (ALT) levels were elevated during the first study day in two out of the six patients (ACTG grade 2 elevation) [9], whereas aspartate aminotransferase (AST) levels were normal. On the second study day one grade 2 elevation of ALT level became grade 1 and one grade 1 elevation became grade 2 elevation and AST levels remained normal.

Creatinine, alkaline phosphatase, bilirubin, hemoglobin, leukocyte and platelet concentrations were within normal ranges.

No significant differences were observed for the RTV pharmacokinetics between the first and second study day (Table 1). However, SQV pharmacokinetics were different between the first and second study day: the $AUC_{[0\text{-}8h]}$, C_{max} and $C_{min(8hr)}$ of SQV at the second study day were lower in five out of six patients compared to the first study day. The median value of the $AUC_{[0\text{-}8h]}$ during the first and second study day decreased from 5778 to 4656 h*ng/ml (p = .06). The median percentage change in $AUC_{[0\text{-}8h]}$ was -33% (range –53 to +21%). Median C_{max} decreased from 1384 to 1009 ng/ml (p = .09). $C_{min(8hr)}$ decreased from 400 to 259 ng/ml (p = .06). T_{max} and $T_{1/2}$ were not different between the first and second study day (Table 1). Individual results for the pharmacokinetics of SQV are shown in Figure 1.

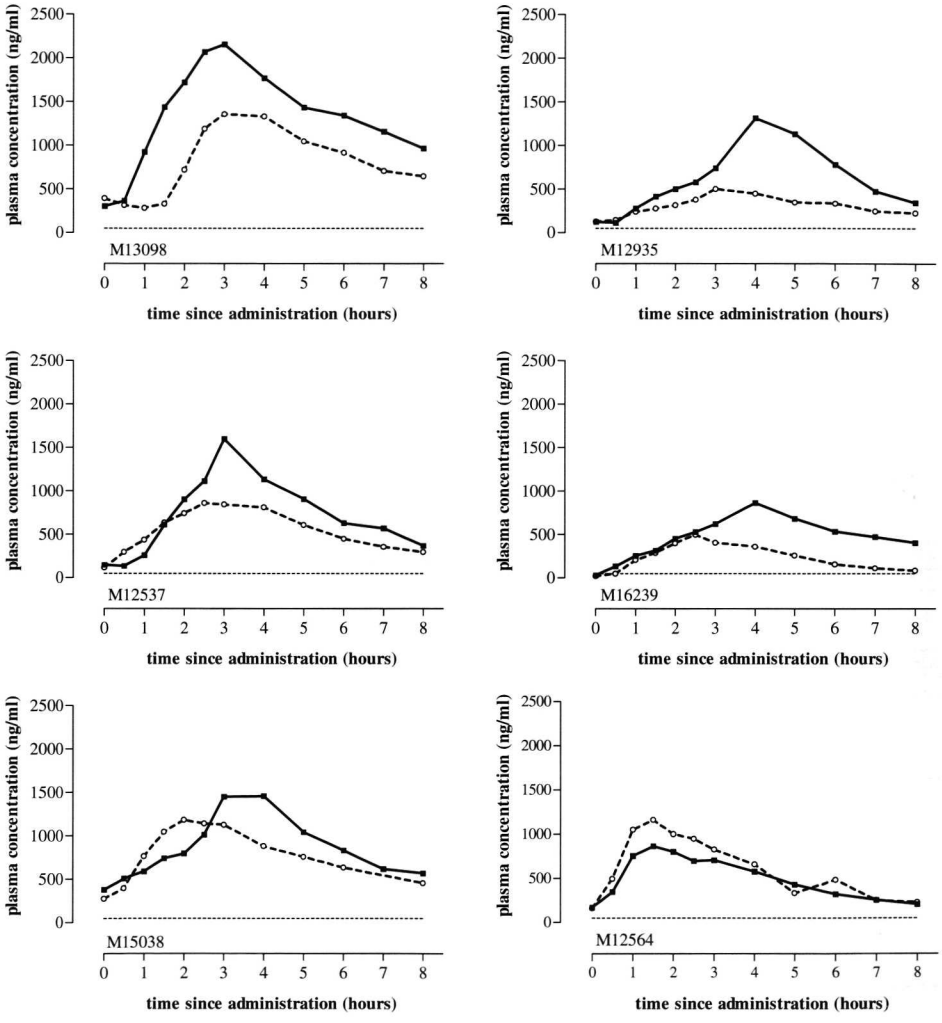
Table 1 Plasma pharmacokinetics of ritonavir and saquinavir on two separate study days 9-15 months apart of six HIV-1 infected patients on stable antiretroviral therapy.

ritonavir	**1st study day**		**2nd study day**		
	median	range	median	range	p-value*
$AUC_{[0\text{-}8h]}$ (h*ng/ml)	38,329	36,542-44,351	36,455	31,327-44,543	.48
C_{max} (ng/ml)	7,680	6,331-8,580	7,322	5,389-17,138	.46
$C_{min(8hr)}$ (ng/ml)	2,738	1,772-3,163	2,642	1,818-3,911	.79
T_{max} (h)	2.25	0.5-3	2	0.5-3	.82
$t_{½}$ (h)	3.3	2.3-9.3	4.5	2.9-5.1	.64

saquinavir	**1st study day**		**2nd study day**		
	median	range	median	range	p-value*
$AUC_{[0\text{-}8h]}$ (h*ng/ml)	5,778	3,975-11,138	4,656	1,944-6,844	.06
C_{max} (ng/ml)	1,384	861-2,155	1,009	496-1,357	.09
$C_{min(8hr)}$ (ng/ml)	400	203-966	259	83-646	.06
T_{max} (h)	3.5	1.5-4	3	1.5-4	1.00
$t_{½}$ (h)	3	1.7-4.2	4	1.9-4.3	0.44

$AUC_{[0\text{-}8h]}$ = area under the plasma concentration versus time curve from 0 to 8 hours, C_{max} = maximum plasma concentration, $C_{min(8hr)}$ = plasma concentration eight hours after observed intake, T_{max} = time to maximum plasma concentration, $t_{½}$ = plasma elimination half-life, h = hour, * difference between two study days

Figure 1 Pharmacokinetic profiles of saquinavir



Every figure represents the pharmacokinetic profiles of saquinavir of an individual patient on stable antiretroviral therapy including ritonavir 400 mg BID and saquinavir 400 mg BID. The first study day (solid line/solid squares) was 4-12 months after start of the current antiretroviral therapy, the second study day (dotted line/open circles) was 9-15 months later. The horizontal dotted lines represent the minimal recommended saquinavir trough concentration of 50 ng/ml.

Discussion

In this study, plasma concentrations of SQV decreased over time in five out of six HIV-1-infected patients on stable antiretroviral therapy, including RTV and SQV.

It is known that plasma concentrations of protease inhibitors can vary highly within and between HIV infected individuals [10]. The time interval between drug intake and blood sampling, food taken with the drugs, co-medication, and non-compliance influence plasma concentrations of protease inhibitors. In addition, there is a large interpatient variability. To minimize these effects, we obtained pharmacokinetic profiles during eight hours after observed intake of medication and standardized breakfast. No significant differences between the two study days were observed in the use of co-medication, that might explain the changes in SQV exposure over time. Also there were no changes in liver or kidney function between the two study days that can be expected to have influenced the pharmacokinetics. To correct for inter patient variability, pharmacokinetic profiles were obtained twice from the same six patients.

Protease inhibitors are found to be substrates for P-glycoprotein, an energy-dependent efflux pump of multiple structurally unrelated compounds [11-13]. P-glycoprotein is, among other places, found in mucosa of the gut. In-vitro studies showed that the addition of P-glycoprotein specific inhibitors to cells containing HIV-1 and protease inhibitors improved antiviral activity, suggesting that enhanced P-glycoprotein activity indeed has a negative effect on the antiretroviral activity of the protease inhibitors [11]. Induction of expression of P-glycoprotein has been described as a protective answer of cells to toxic concentrations of anti cancer drugs such as cyclosporine A [14]. The same mechanism may apply for SQV and result in the reduction of SQV plasma concentrations over time. Maybe other factors, such as other transport molecules or changes in the inhibition of cytochrome P-450 by RTV, are involved in the change of pharmacokinetic profile of SQV over time. For instance, in healthy volunteers, lower SQV exposure was found when compared to HIV-infected patients [15]. Maybe the same mechanism applies for the patients in this study, who were treated effectively for their HIV-infection. Further research to investigate the mechanism of decreased SQV exposure over time is warranted.

Patients in this study used SQV in combination with RTV, resulting in enhanced bioavailability of SQV and subsequently higher SQV plasma concentrations. In one patient, the SQV concentrations before observed drug intake during both study days were below 50 ng/ml, which has been found to be a minimal trough concentration needed for sustained virological efficacy [16]. This was also the only patient with a detectable HIV-RNA

concentration during both study days. All other SQV concentrations measured were above 50 ng/ml (Figure 1).

Despite the median 33% reduction in SQV plasma concentrations over time, AUCs of SQV during the second study day were still higher when compared to SQV 1200 mg three times a day (TID) or SQV 600 mg TID in combination with nelfinavir 750 mg TID [17]. In patients treated with SQV in combination with nelfinavir or with SQV as the only PI, a similar reduction of the SQV levels over time as found in this study can lead to SQV concentrations below the recommended trough levels for a prolonged period of the dosing interval, which may result in insufficient viral suppression and the development of resistant virus.

We showed that SQV plasma levels during stable antiretroviral therapy decreased substantially over time in 5/6 of the patients studied. These data suggest that, even in apparently compliant patients, regular monitoring of drug concentrations should become part of routine patient care. Thus, timely dose adjustment if necessary, may prevent viral rebound as a result of sub-therapeutic plasma drug concentrations.

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References

1. Cameron DW, Heath-Chiozzi M, Danner S, et al: **Randomised placebo-controlled trial of ritonavir in advanced HIV-1 disease.** *Lancet* 1998, **351:**543-549.
2. Hammer SM, Squires KE, Hughes MD, et al: **A controlled trial of two nucleoside analogues plus indinavir in persons with human immunodeficiency virus infection and CD4 cell counts of 200 per cubic millimeter or less.** *N.Engl.J.Med.* 1997, **337:**725-733.
3. Gulick RM, Mellors JW, Havlir D, et al: **Treatment with indinavir, zidovudine, and lamivudine in adults with human immunodeficiency virus infection and prior antiretroviral therapy.** *N.Engl.J.Med.* 1997, **337:**734-739.
4. Collier AC, Coombs RW, Schoenfeld DA, et al: **Treatment of human immunodeficiency infection with saquinavir, zidovudine, and zalcitabine.** *N.Engl.J.Med.* 1996, **334:**1011-1017.
5. Wit FWNM, van Leeuwen R, Weverling GJ, et al: **Outcome and predictors of failure of highly active antiretroviral therapy: one-year follow-up of a cohort of human immunodeficiency virus type 1-infected persons.** *J.Infect.Dis.* 1999, **179:**790-798.
6. Enting RH, Hoetelmans RMW, Lange JMA, Burger DM, Beijnen JH, Portegies P: **Antiretroviral drugs and the central nervous system.** *AIDS* 1998, **12:**1941-1955.
7. Khaliq Y, Gallicano K, Sahai J, et al: **Effect of nelfinavir on short and long term plasma exposure of saquinavir in hard gel capsule during tid and bid dosing regimens.** *AIDS* 1998, **12 (suppl 4):**S28

8. van Heeswijk RPG, Hoetelmans RMW, Harms R, et al: **Simultaneous quantitative determination of the HIV protease inhibitors amprenavir, indinavir, nelfinavir, ritonavir, and saquinavir in human plasma by ion-pair high-performance liquid chromatography with ultraviolet detection.** *J.Chromatogr.B.* 1998, **719:**159-168.
9. ACTG criteria, division of AIDS. Table for grading severity of adult adverse experiences. 1992.
10. Hoetelmans RMW, Reijers MHE, Wit FWNM, et al. **Saquinavir pharmacokinetics in combination with nelfinavir in the ADAM study.** *6th European Conference on Clinical Aspects and Treatment of HIV-Infection, Hamburg October 11-15.*[Abstract 255].
11. Lee CGL, Gottesman MM , Cardarelli CO, et al: **HIV-1 protease inhibitors are substrates for the MDR1 multi drug transporter.** *Biochemistry* 1998, **37:**3594-3601.
12. Washington CB, Duran GE, Man MC, Sikic BI, Blaschke TF: **Interaction of anti-HIV protease inhibitors with the multidrug transporter P-glycoprotein (P-gp) in human cultured cells.** *J.Acquir.Immune Defic.Syndr.Hum.Retrovirol.* 1999, **19:**203-209.
13. Profit L, Eagling VA, Back DJ: **Modulation of P-glycoprotein function in human lymphocytes and Caco-2 cell monolayers by HIV-1 protease inhibitors.** *AIDS* 1999, **12:**1623-1627.
14. Hauser IA, Koziolek M , Hopfer U, Thevonod F: **Therapeutic concentrations of cyclosporin A, but not FK506, increase P-glycoprotein expression in endothelial and renal tubule cells.** *Kidney Int.* 1998, **54:**1139-1149.
15. Noble S, Faulds D: **Saquinavir. A review of its pharmacology and clinical potential in the management of HIV infection.** *Drugs* 1996, **52:**93-112.
16. Hoetelmans RMW, van Heeswijk RPG, Meenhorst PL, Mulder JW, Scheele WA, Beijnen JH. **Plasma concentrations of saquinavir determine HIV-1 RNA response over a 48-week period.** *12th World AIDS Conference, Geneva, Switzerland, June 28-July 3, 1998.*[Abstract 42261].
17. Merry C, Barry MG, Mulcahy F, Halifax KL, Back DJ: **Saquinavir pharmacokinetics alone and in combination with nelfinavir in HIV-infected patients.** *AIDS* 1997, **11:**F117-F120.