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Interindividual variability of once-daily ritonavir boosted saquinavir pharmacokinetics in Thai and UK patients

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***Objectives*: Differential exposure to saquinavir/ritonavir may lead to therapy failure. The objective was to identify factors that influence variability of saquinavir/ritonavir plasma concentrations.**

***Methods*: Saquinavir/ritonavir data, dosed as 1600/100 mg once daily, from three separate pharmacokinetic studies, in 45 patients from Thailand and the UK, were pooled. Pharmacokinetic parameters were based on non-compartmental analysis. Univariate analysis was performed with saquinavir as the dependent variable, and ritonavir area under the curve (AUC), gender, body weight, body mass index (BMI) and study site as independent variables. Variables with a P value <0.10 were included in a multivariate linear regression analysis.**

***Results*: Higher saquinavir AUCs, maximum concentrations (C_{max}) and minimum concentrations (C_{min}) were seen in Thai patients than in UK patients. Univariate analysis showed associations between body weight, gender, study site and ritonavir AUC and saquinavir AUC ($P < 0.05$), whereas BMI ($P = 0.13$) did not. In the multivariate analysis, ritonavir AUC ($P = 0.0001$) and study site ($P = 0.0021$) were significantly related to saquinavir AUC ($R^2 = 0.50$).**

***Conclusions*: The ritonavir AUC and study site appeared to be related to exposure of saquinavir. Study site should be viewed as the total of country- and study-specific differences—such as differences in lifestyle, environment, genetic background and dietary composition—between the analysed studies.**

Keywords: HIV, clinical pharmacology, protease inhibitors, Thailand, United Kingdom

Introduction

The favourable pharmacokinetic profile of the HIV protease inhibitors saquinavir/ritonavir may allow for once-daily dosing. The US Food and Drug Administration (FDA) approved twice-daily dosing of saquinavir/ritonavir of 1000/100 mg. Once-daily dosing has not been approved, but dosing of 1600/100 mg once daily has been studied in clinical trials.[1–3]

The minimum recommended effective concentration for saquinavir is 0.1 mg/L.[4] Ritonavir is able to boost the plasma

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concentration of saquinavir. Therefore, currently ritonavir is added to saquinavir regimens in doses of 100 mg once or twice daily. This is a relatively low dose in order to minimize adverse events related to higher doses of ritonavir. In the dose range of 100–400 mg, the boosting effect is independent of the dose of ritonavir.[5] The pharmacological action of boosting saquinavir by ritonavir is based on inhibition of hepatic and intestinal cytochrome P-450 isoenzyme 3A4, and it has been suggested that the inhibition of P-glycoprotein, an ATP-dependent drug efflux pump, contributes to the boosting effect. Several *in vitro* studies have supported this mechanism of action, which *in vivo* has yet to be established conclusively.[6,7]

When treating HIV with a saquinavir-containing regimen, drug concentrations should be considered, because high saquinavir plasma concentrations are related to better virological outcome.[8,9] Dosing of saquinavir/ritonavir at 1600/100 mg once daily leads to lower plasma concentrations than when dosed at 2000/100 mg once daily or 1000/100 mg twice daily, and low plasma concentrations can subsequently lead to low exposure. Extensive variability of saquinavir plasma concentrations could also result in suboptimal exposure.[10–13] Low exposure to saquinavir may lead to the selection of antiretroviral-resistant HIV strains and therefore to therapy failure.

Factors that can potentially influence the clinical pharmacokinetic properties of a drug and the pharmacokinetic variability are patient characteristics such as age, gender, body composition and ethnic background.[4] In terms of gender, Fletcher *et al*.[9] showed that female HIV-infected patients have higher saquinavir concentrations than their male counterparts, and that high concentrations in female patients were correlated with better virological response. For other protease inhibitors such as indinavir, it has been shown that Thai patients—whose body weight is generally lower than Caucasians—had higher maximum concentrations.[14] In addition, lower clearance of the non-nucleoside reverse transcriptase inhibitor efavirenz has been described in Black or Hispanic HIV-patients than in their Caucasian counterparts.[15]

It is not yet clear which variables are responsible for the non-uniformity of boosted saquinavir concentrations. The objective of this analysis was to investigate the factors that determine saquinavir plasma concentrations and the heterogeneity of saquinavir plasma concentrations when saquinavir is combined with low-dose ritonavir in a once-daily regimen at 1600/100 mg in two different ethnic populations of HIV-infected patients.

Material and methods

Study design, setting and patients

Data sets of three studies were combined. One study (study 1) was performed at Chelsea and Westminster Hospital in the UK,[10] and two studies (study 2 and study 3) were performed at the HIV Netherlands Australia Thailand Research Collaboration (HIV-NAT), the Thai Red Cross AIDS Research Centre in Thailand.[11,16]

All studies were single-centre, open-label, pharmacokinetic studies and included adult female or male patients with asymptomatic HIV infection. Viral load or CD4 restrictions were not applied. In study 1, 18 patients with a minimum of 2 weeks of saquinavir/ritonavir intake, were included. Study 2 included 14 patients and study 3, 20 patients with long-term (>4 weeks) saquinavir/ritonavir intake. All patients from studies 1 and 3 were included in the final analysis; data from one patient in study 2 was excluded because of incorrect intake of the medication. Six patients subsequently participated in studies 2 and 3; however, only data from study 3 were used in this analysis, because the actual measured concentrations at 24 h were available for patients in study 3 (see section entitled Pharmacokinetic methods). In the current analysis, only data from patients who received 1600/100 mg saquinavir/ritonavir were included.

Detailed descriptions of the study criteria can be reviewed in the previously indicated publications.[10,11,16] Studies were approved by the local ethical committees, and all patients gave informed consent.

Pharmacokinetic methods

All patients were treated with hard gel capsules of saquinavir (200 mg/capsule) and ritonavir (100 mg/capsule), and a nucleoside reverse transcriptase backbone. Co-medication that could interfere with the pharmacokinetics of saquinavir or ritonavir was not allowed.

Medications were taken on the study days with standardized meals (study 1: 40 g of fat; study 2: 10–15 g of fat, 400–700 kcal, study 3: 12 g of fat, ~500 calories). In study 1, blood sampling was performed just before intake, and at 30 min, 1, 2, 3, 4, 6, 8, 10, 12 and 24 h after intake of the medications. In study 2, blood was sampled before intake, and at 30 min, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10 and 12 h after intake of the medications. The 24 h time-point was extrapolated (see details in next paragraph). Sampling in study 3 was scheduled before intake, and at 2, 4, 6, 8, 10, 12 and 24 h after intake. Plasma concentrations of saquinavir and ritonavir were measured by validated reversed-phase high-performance liquid chromatography (HPLC). HPLC measurements were performed at the pharmacokinetic laboratories of the participating centres. These laboratories participated in the same international quality control and quality assessment (QA/QC) pharmacokinetic programme, and therefore have cross-validation with each other. In study 1, HPLC with tandem mass spectrometry was used with a lower limit of quantification (LLOQ) of 0.01 mg/L for both protease inhibitors. In studies 2 and 3, UV detection was used with an LLOQ of 0.04 mg/L. In study 1, slightly lower values could be detected for low levels; however, this has a minimal effect on the AUC from 0–24 h.

Full concentration–time data sets of saquinavir and ritonavir for all three studies were available. Non-compartmental analyses of pharmacokinetic parameters were performed using the same approach in the three studies. Pharmacokinetic calculations were performed with WinNonlin (version 4.1, Pharsight Corporation, Paola Alto, CA, USA) software. The area under the curve, AUC_{0-12} or AUC_{0-24}, was defined as the area under the plasma concentration–time curve until the last measurable plasma concentration calculated with the log-linear trapezoidal method. In study 2, the 24 h time-point or minimum concentration (C_{min}) was extrapolated, using the last concentration that was measured at 12 h, with the following formula: $C_{24} = C_{12} \times e^{-\beta \times 12}$. In this formula, a linear relationship is assumed between the saquinavir concentration at 12 and 24 h in the log-linear time–concentration profile. To estimate the rate of the decline of concentration from 12 to 24 h, the elimination rate constant (β) was derived from the following relationship: $\beta = -\text{slope} \times \ln 10$. The slope was calculated using least square linear regression analysis. C_{24} is the estimated C_{min}. In studies 1 and 3, the C_{min} was the observed concentration just before the next dosing of the medications. The peak concentration, defined as C_{max}, was the highest concentration during the dosing interval. The $t_{1/2}$ (h) was calculated using $\ln(2/\lambda)$. The definition of $t_{1/2}$ was the apparent elimination half-life associated with the terminal slope of a semi-logarithmic concentration–time curve in which λ is the elimination rate constant.

Statistical analysis

Baseline characteristics and pharmacokinetic parameters of the patients were summarized as medians plus (interquartile) ranges or means and standard deviations.

The variability of the pharmacokinetic parameters was expressed as coefficient of variance (CV).

Comparisons between the three studies were tested with the Kruskal–Wallis Test or the Fisher Exact Test, whichever was appropriate. Comparisons between the UK and Thai studies were tested by the Mann–Whitney *U*-test. Univariate linear regression analysis was performed with the log-transformed saquinavir AUC as the dependent variable. Ritonavir AUC, body weight, body mass index, study site and gender were the independent variables.

Parameters with P values <0.10 in univariate analysis were included in a multivariate linear regression model. Correlations between some of the independent variables were described by the Spearman correlation coefficient. Data were analysed by SPSS statistical software (version 11.5.2, SPSS Inc, Chicago, IL, USA).

Results

In total, data from 45 patients were available. Patient characteristics and history are summarized in Table 1. All baseline characteristics differed per study.

Pharmacokinetic data

The saquinavir and ritonavir pharmacokinetic parameters are listed in Table 2. The median saquinavir AUCs were different across the studies, with an AUC of 17.88 in study 1, 67.05 in study 2 and 42.42 mg·h/L in study 3 ($P < 0.001$). Comparing the AUCs between Thai and UK patients, saquinavir AUCs were notably higher in patients from Thailand (<0.001, respectively). Also, the ritonavir AUCs differed between the three studies but less markedly than the saquinavir AUCs ($P = 0.047$). The ritonavir AUCs between the UK and Thai patients differed significantly (0.019).

Comparisons of the other saquinavir pharmacokinetic parameters showed significant differences between saquinavir C_{min} and C_{max} of the three studies, but not for $t_{1/2}$. Saquinavir C_{min} and C_{max} between the UK and Thai groups were higher in patients from Thailand (P values 0.001 and 0.003, respectively).

For ritonavir, the C_{max} concentrations reached statistical difference but C_{min} concentrations were in the same range (Table 2). The ritonavir $C_{max,}$ were higher and $t_{1/2}$ lower in the Thai patients (P values 0.014 and 0.001, respectively).

Table 1. Patient characteristics, median [range]

	Study 1 (n = 18)	Study 2 (n = 7)	Study 3 (n = 20)	P value
Age, years	42 [22–61]	32 [25–48]	35 [30–42]	<0.001
Gender, M/F	15/3	5/2	4/16	<0.001
Ethnicity[a], number	Caucasian, 16; Black, 2	Thai, 7	Thai, 20	<0.001
Body weight, kg	68 [49–85]	46 [35–75]	54 [49–68]	<0.001
BMI, kg/m^2	22.34 [14.55–31.10]	19.5 [15.43–26.26]	21.09 [18.04–25.00]	0.025
CD4, cells/mm^3	362 [99–967]	644 [601–964]	593 [298–825]	<0.001
Viral load, copies/mL	91 [<50–14508]	<50 [<50–<50]	<50 [<50–9780]	0.003
Prior use of SQV/RTV, months	31 [1–92]	15 [14–15]	34 [32–46]	0.011
Prior use of NRTI, months	73 [4–162]	56 [55–56]	75 [64–87]	0.013

n, number of patients; M, male; F, female; BMI, body mass index; SQV, saquinavir; RTV, ritonavir; NRTI, nucleoside reverse transcriptase inhibitors.
[a]Self-reported ethnicity.

Table 2. Pharmacokinetic parameters of saquinavir 1600 mg once daily and ritonavir 100 mg once daily, median [range]

	Saquinavir				Ritonavir			
	study 1	study 2	study 3	P value	study 1	study 2	study 3	P value
AUC (mg·h/L)	17.88 [8.92–63.16]	67.05 [46.56–92.00]	42.42 [10.53–105.07]	<0.001	6.97 [4.88–19.56]	11.61 [3.22–14.42]	11.21 [4.60–21.74]	0.05
CV[a] (%)	66	25	57		48	40	39	
C_{max} (mg/L)	2.84 [0.98–9.21]	7.55 [5.37–9.92]	5.68 [1.31–12.36]	0.002	0.95 [0.39–2.27]	1.22 [0.53–2.51]	1.38 [0.57–3.08]	0.04
CV	64	21	57		49	52	47	
C_{min} (mg/L)	0.09 [<0.01–0.64]	0.38 [0.10–1.09]	0.25 [0.06–1.07]	0.003	0.06 [<0.01–0.32]	0.03 [<0.04–0.36]	0.04 [<0.04–0.32]	0.59
CV	99	81	79		92	137	112	
$t_{1/2}$ (h)	4.62 [3.26–6.30]	4.47 [2.96–6.96]	4.43 [3.84–6.44]	0.86	4.85 [3.47–6.53]	3.74 [1.50–11.34]	3.63 [1.80–14.03]	0.006
CV	18	32	16		18	70	58	

C_{min}, minimum observed concentration; C_{max}, maximum observed concentration; AUC, area under the plasma concentration-time curve.
[a]Coefficient of variation (CV) expressed as standard deviation divided by the mean.

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Interestingly, in study 1, nine patients had a concentration below the minimum recommended trough concentration for effective treatment of 0.10 mg/L. In study 2, there were two patients with exactly 0.1 mg/L, and in study 3 there were three patients with low concentrations (see Figure 1).

Univariate analysis and multivariate analysis

In the univariate analysis, body weight, gender, study site and ritonavir AUC were significantly related to the log AUC of saquinavir ($P < 0.05$), whereas body mass index was not ($P = 0.13$). In the final multivariate model, ritonavir AUC ($P = 0.0001$) and study site ($P = 0.0021$) were significantly associated with the log-transformed saquinavir AUC ($R^2 = 0.50$) (Table 3). In particular, patients with higher ritonavir AUCs and patients at the Thai study site had higher saquinavir AUCs (Figure 2). AUCs of common time-points of the samples were calculated and fitted in the univariate and multivariate model, which gave the same results.

The variables of the study sites may consist of several factors, of which one could be body weight. Thai people are smaller in posture and accordingly have a lower body weight than the Caucasian population. To investigate whether the association with study site could be attributed to body weight, further analysis was performed between these variables.

A strong correlation existed between study site and weight ($R = 0.69$, $P < 0.0001$). A further multivariate analysis, in which study site is replaced by body weight, resulted in a model ($R^2 = 0.43$) that included ritonavir AUC ($P = 0.00005$) and body weight ($P = 0.0379$). Patients with higher ritonavir AUCs and patients with lower body weights had higher saquinavir AUCs.

Study site was also correlated with gender ($R = 0.49$, $P = 0.0006$). However, replacing study site by gender in the multivariate model did not result in inclusion of gender in the model.

Weight differences between male and females reached statistical significance ($P < 0.001$, median body weight for males: 64.4 kg and females: 47.0 kg), but not for body mass index ($P = 0.176$, median body mass index for males: 22.1 and females: 20.0). Therefore, study site was replaced by both gender and body weight, which did not result in a significant model.

Multivariate analysis with other pharmacokinetic parameters, the log transformed saquinavir C_{min} and C_{max}, showed similar results, i.e. ritonavir AUC (C_{min} $P = 0.0001$, C_{max} $P = 0.003$) and study site (C_{min} $P = 0.012$, C_{max} $P = 0.021$) were significantly associated with saquinavir C_{min} ($R^2 = 0.47$) and C_{max} ($R^2 = 0.35$). Replacing weight for site resulted in models with a slightly worse fit (data not shown; R. S. Autar, E. Hassink, U. Siangphoe, F. W. N. M. Wit and D. M. Burger).

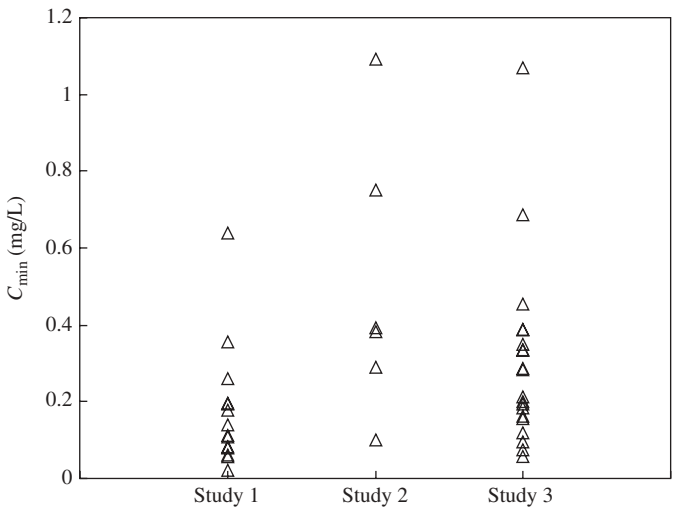

Figure 1. Minimum observed concentration (C_{min}) of saquinavir for individual patients. Open triangle, individual saquinavir C_{min} concentration.

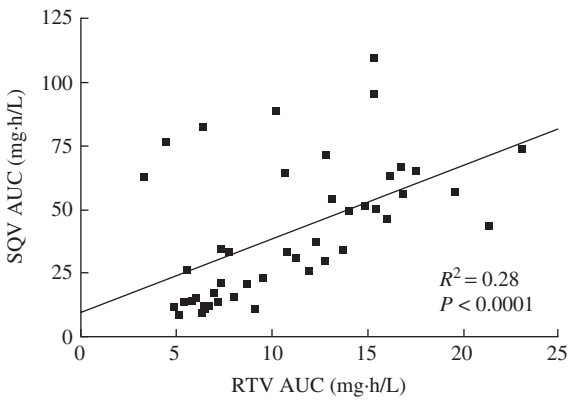

Figure 2. Correlation between ritonavir (RTV) area under the curve (AUC) and saquinavir (SQV) AUC. Filled square, individual patient. Data is plotted for individual patients. Linear equation: 2.86 RTV AUC + 10.15 = SQV AUC.

Table 3. Univariate and multivariate analysis with saquinavir AUC as the dependent variable

	Univariate			Multivariate			
Variable	parameter estimate	R^2	P value	parameter estimate[a]	P value	parameter estimate[b]	P value
BMI (kg/m^2)	–0.02016	0.05	0.1308				
Gender	0.20249	0.10	0.0307	–0.04430	0.6143		
Weight (kg)	–0.01160	0.24	0.0007	–0.00153	0.7055		
RTV AUC (mg·h/L)	0.03909	0.37	<0.0001	0.03116	0.0004	0.03111	0.0001
Study site	–0.34388	0.29	0.0001	–0.23664	0.0222	–0.24288	0.0021

BMI, body mass index; RTV AUC, ritonavir area under the curve.
[a]$R^2 = 0.50$.
[b]$R^2 = 0.50$.

Discussion

All patients in this analysis were using saquinavir/ritonavir 1600/100 mg once daily. By performing this analysis in patients who were taking the same dosages, it was possible to investigate other variables that determine saquinavir exposure. This analysis showed wide-ranging variability of saquinavir pharmacokinetic parameters between and within the studies. Patients with higher ritonavir AUC and patients at the Thai study site had higher saquinavir exposure. However, in the multivariate analysis, after correction for ritonavir exposure, study site was more strongly associated with saquinavir AUC than ritonavir AUC.

The finding that exposure to ritonavir influences exposure to saquinavir has not been reported yet. Previous studies investigated the relationship between the dose of ritonavir and saquinavir AUC. These studies showed that in healthy volunteers and HIV-infected patients the dose of ritonavir (ranging from 100–400 mg) does not affect saquinavir plasma exposure.[5,17] Ritonavir inhibits intestinal and hepatic isoenzyme CYP 3A4. While the dose of ritonavir will be of importance for the absorption of saquinavir in the gut, the ritonavir AUC will mainly have an effect on inhibition in the liver.

The saquinavir AUCs in the UK study were lower than the AUCs of the Thai patients.

A study with Caucasian patients, in which saquinavir soft gel capsules were administrated at 1600 mg with low dose ritonavir, showed comparable median saquinavir AUCs of 18.13 mg·h/L.[18] The difference between the saquinavir AUCs of the two Thai studies probably reflects the variability that is seen with saquinavir plasma concentrations, for which one explanation might be lower body weight in study 2. Saquinavir plasma concentrations can be higher in patients with small body size and body composition.[3] Higher drug concentrations may be expected in subjects with small volumes of distribution or low body weight. Thai patients in this study had, relatively, low body weight and high exposure to saquinavir and ritonavir. Relationships with body weight have been seen with other protease inhibitors such as for indinavir.[19] Nevertheless, this relationship has not led to body weight-dependent dose recommendations, as for some of the nucleoside analogue reverse transcriptase inhibitors. Body fat percentage could also affect the plasma concentration because of the lipophilicity of saquinavir.

Gender may affect the bioavailability of a drug by differential expression of CYP3A and P-glycoprotein in the gastrointestinal tract. Metabolism in the liver might be faster in men, but the total clearance of CYP3A substrates may be faster in women.[20] In our multivariate analysis, an effect of gender was not observed. However, another study showed that females, compared with men, have higher exposure to saquinavir.[9]

The patient group in study 1 was more diverse in terms of CD4. Studies 2 and 3 were immunologically more similar at the start of the pharmacokinetic study. Differences in disease stage may result in different pharmacokinetic profiles due to impaired absorption, variation in protein binding or altered clearance. We performed regression analyses including CD4 cell count; however, this did not add significance to the model (data not shown; R. S. Autar, E. Hassink, F. W. N. M. Wit and D. M. Burger).

An alternative explanation to describe the relationship between saquinavir and ritonavir exposure is that individuals with good pharmacokinetic profiles are able to absorb both drugs efficiently. These individuals will present with high concentrations for several other drugs. In this case, the association between ritonavir and saquinavir exposure does not necessarily implicate a causal relationship.

Retrospective analysis and small sample size are limitations in this exploratory study. The third limitation is that the UK study patients received study medication with a breakfast that contained a higher fat percentage than in the Thai studies. Saquinavir can be facilitated by high fat percentage and high gastric pH.[21,22] However, patients with the highest fat content in their meal—patients from the UK—had lower saquinavir pharmacokinetic parameters than patients from Thailand. If saquinavir was taken with a standard breakfast in the UK study perhaps even lower pharmacokinetic parameters would have been seen.

Fourthly, ethnic differences between the studies cannot be excluded. Ethnic differences concern dissimilarities between pharmacogenomic background, lifestyle, and environmental factors. About 40 variants of the gene coding for CYP3A4 have been described. *CYP3A4*1B* is present in Caucasians but not in East Asians (Japanese and Chinese). Higher tacrolimus doses are needed to reach target trough concentration in patients with the *CYP3A4*1B* allele. However, the functionality of *CYP3A4*1B* is not clear. *In vitro* studies have shown an association with increased enzyme activity, while clinical studies show decreased enzymes and no association was seen in microsomal studies.[23,24] Pharmacogenomic data were not available for our analysis. Lifestyle and environmental factors have not been studied extensively because of the complexity around defining and measuring these factors.

These limitations and the differences between the Thai and English data stress the need to investigate the effect of the above mentioned factors on saquinavir exposure further.

In the literature, ritonavir-boosted saquinavir regimens show lower inter-individual variability than unboosted saquinavir regimens in healthy volunteers.[5,25] However, looking at data from this analysis and previous studies performed in HIV-infected patients, high variability was seen for boosted saquinavir regimens.[26] In an attempt to reduce variability, twice-daily dosing has been used instead of once-daily dosing. A study with lopinavir/ritonavir showed lower inter-individual variability in twice-daily dosing compared with once-daily dosing.[27] As opposed to lopinavir/ritonavir, saquinavir/ritonavir twice-daily regimens (1000/100 mg) are not associated with lower variability.[10–12]

Variability may lead to low saquinavir plasma concentrations and low saquinavir exposure. Therapeutic drug monitoring of saquinavir pharmacokinetic parameters can be used to detect low concentrations. However, which pharmacokinetic parameter (i.e. AUC, C_{min} or perhaps another parameter) should be monitored in relationship to clinical outcome is still a matter of debate. The C_{min}, as a single time point concentration, is a practical pharmacokinetic parameter to measure. Although C_{min} has been shown to predict virological response for some protease inhibitors, analyses in two studies were not able to show a significant relationship between saquinavir C_{min} and virological outcome.[26,28] Conversely, total exposure to saquinavir was correlated with virological outcome; however, measurement of AUC is difficult to realize in clinical practice.[8,9] Further studies are required to investigate the relationships between clinical outcome and saquinavir pharmacokinetic parameters.

The present analysis was conducted to identify which factors have a role in saquinavir plasma exposure and variability. The outcomes of this study indicate that exposure to ritonavir and study site are related to the exposure of saquinavir, where study site might reflect differences in body weight, food intake,

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pharmacogenomic, environmental and other ethnic differences. Regarding ritonavir, further investigations are needed to verify the role of ritonavir AUC in other saquinavir/ritonavir dose combinations. To our knowledge, this is the first study in which the relationship between ritonavir AUC and saquinavir is reported. To identify ethnic, environmental and pharmacogenomic variables, and to investigate the effect of body weight and different food intake on saquinavir pharmacokinetics, further studies in heterogeneous patient populations are necessary.

References

1. Cardiello P, Srasuebkul P, Hassink E *et al*. The 48-week efficacy of once-daily saquinavir/ritonavir in patients with undetectable viral load after 3 years of antiretroviral therapy. *HIV Med* 2005; **6**: 122–8.

2. Lamotte C, Landman R, Peytavin G *et al*. Once-daily dosing of saquinavir soft-gel capsules and ritonavir combination in HIV-1-infected patients (IMEA015 study). *Antivir Ther* 2004; **9**: 247–56.

3. Cardiello PG, van Heeswijk RP, Hassink EA *et al*. Simplifying protease inhibitor therapy with once-daily dosing of saquinavir soft-gelatin capsules/ritonavir (1600/100 mg): HIVNAT 001.3 study. *J Acquir Immune Defic Syndr* 2002; **29**: 464–70.

4. Gibaldi M. Pharmacokinetic variability—body weight, age, sex and genetic factors. In: Gibaldi M., ed. *Biopharmaceutics and Clinical Pharmacokinetics*. Philadelphia: Lea & Febiger, 1984; 206–27.

5. Kilby JM, Hill A, Buss N. The effect of ritonavir on saquinavir plasma concentration is independent of ritonavir dosage: combined analysis of pharmacokinetic data from 97 subjects. *HIV Med* 2002; **3**: 97–104.

6. Chaillou S, Durant J, Garraffo R *et al*. Intracellular concentration of protease inhibitors in HIV-1-infected patients: correlation with MDR-1 gene expression and low dose of ritonavir. *HIV Clin Trials* 2002; **3**: 493–501.

7. Perloff MD, Von Moltke LL, Marchand JE *et al*. Ritonavir induces P-glycoprotein expression, multidrug resistance-associated protein (MRP1) expression, and drug transporter-mediated activity in a human intestinal cell line. *J Pharm Sci* 2001; **90**: 1829–37.

8. Gieschke R, Fotteler B, Buss N *et al*. Relationships between exposure to saquinavir monotherapy and antiviral response in HIV-positive patients. *Clin Pharmacokinet* 1999; **37**: 75–86.

9. Fletcher CV, Jiang H, Brundage RC *et al*. Sex-based differences in saquinavir pharmacology and virologic response in AIDS Clinical Trials Group study 359. *J Infect Dis* 2004; **189**: 1176–84.

10. Boffito M, Dickinson L, Hill A *et al*. Pharmacokinetics of once-daily saquinavir/ritonavir in HIV-infected subjects: comparison with the standard twice-daily regimen. *Antivir Ther* 2004; **9**: 423–9.

11. Autar RS, Ananworanich J, Apateerapong W *et al*. Pharmacokinetic study of saquinavir hard gel caps/ritonavir in HIV-1-infected patients: 1600/100 mg once-daily compared with 2000/100 mg once-daily and 1000/100 mg twice-daily. *J Antimicrob Chemother* 2004; **54**: 785–90.

12. Veldkamp AI, van Heeswijk RP, Mulder JW *et al*. Steady-state pharmacokinetics of twice-daily dosing of saquinavir plus ritonavir in HIV-1-infected individuals. *J Acquir Immune Defic Syndr* 2001; **27**: 344–9.

13. van Heeswijk RP, Veldkamp AI, Mulder JW *et al*. Once-daily dosing of saquinavir and low-dose ritonavir in HIV-1-infected individuals: a pharmacokinetic pilot study. *AIDS* 2000; **14**: F103–10.

14. Burger D, Boyd M, Duncombe C *et al*. Pharmacokinetics and pharmacodynamics of indinavir with or without low-dose ritonavir in HIV-infected Thai patients. *J Antimicrob Chemother* 2003; **51**: 1231–8.

15. Pfister M, Labbe L, Hammer SM *et al*. Population pharmacokinetics and pharmacodynamics of efavirenz, nelfinavir, and indinavir: Adult AIDS Clinical Trial Group study 398. *Antimicrob Agents Chemother* 2003; **47**: 130–7.

16. Cardiello PG, Monhaphol T, Mahanontharit A *et al*. Pharmacokinetics of once-daily saquinavir hard-gelatin capsules and saquinavir soft-gelatin capsules boosted with ritonavir in HIV-1-infected subjects. *J Acquir Immune Defic Syndr* 2003; **32**: 375–9.

17. Kurowski M, Arslan A, Moecklinghoff C *et al*. Effects of ritonavir on saquinavir plasma concentration: a preliminary analysis of 271 patients in routine clinical practice. Fifth International Congress on Drug Therapy in HIV Infection, Glasgow, UK, 2000. Abstract P261A. *AIDS* 2000; **14** Suppl 4: S90.

18. Luber A, Anderson D, Stryker R *et al*. Can ritonavir (RTV) once daily boost saquinavir (SQV) twice daily? A pilot study. The XV International AIDS Conference. Bangkok, Thailand, 2004. MedGenMed. 2004, 11; 6 (3): TuPeB4638 [eJIAS. 2004 11; **1**(1):TuPeB4638].

19. Boyd MA, Aarnoutse RE, Ruxrungtham K *et al*. Pharmacokinetics of indinavir/ritonavir (800/100 mg) in combination with efavirenz (600 mg) in HIV-1-infected subjects. *J Acquir Immune Defic Syndr* 2003; **34**: 134–9.

20. Schwartz JB. The influence of sex on pharmacokinetics. *Clin Pharmacokinet* 2003; **42**: 107–21.

21. European Medicines Agency (EMEA). Invirase product information. EMEA-H-C-113-II-44-PI. http://www.emea.eu.int/humandocs/Humans/EPAR/invirase/invirase.htm (29 June 2005, date last accessed).

22. Perry CM, Noble S. Saquinavir soft-gel capsule formulation. A review of its use in patients with HIV infection. *Drugs* 1998; **55**: 461–86.

23. Kim K, Johnson JA, Derendorf H. Differences in drug pharmacokinetics between East Asians and Caucasians and the role of genetic polymorphisms. *J Clin Pharmacol* 2004; **44**: 1083–1105.

24. Hesselink DA, van Schaik RH, van der Heiden IP *et al*. Genetic polymorphisms of the CYP3A4, CYP3A5, and MDR-1 genes and pharmacokinetics of the calcineurin inhibitors cyclosporine and tacrolimus. *Clin Pharmacol Ther* 2003; **74**: 245–54.

25. Plosker GL, Scott LJ. Saquinavir: a review of its use in boosted regimens for treating HIV infection. *Drugs* 2003; **63**: 1299–1324.

26. Schutz M GJ, Keh-Wei C, Montaner J. Lack of correlation between saquinavir trough levels and adverse events or clinical outcomes in the saquinavir cohort of the FOCUS Study. In: *Program and Abstracts of the Forty-first Annual Meeting of the Infectious Diseases Society of America, San Diego, CA, USA, 2003*. Abstract 654, p. 138. Infectious Diseases Society of America, Alexandria, VA, USA.

27. Eron JJ, Feinberg J, Kessler HA *et al*. Once-daily versus twice-daily lopinavir/ritonavir in antiretroviral-naive HIV-positive patients: a 48-week randomized clinical trial. *J Infect Dis* 2004; **189**: 265–72.

28. Justesen U, FOX Z, Pedersen C *et al*. Pharmacokinetics from a 48-week randomised trial to evaluate safety and efficacy of indinavir/ritonavir 800/100 mg versus saquinavir/ritonavir 1000/100 mg: the MaxCmin1 trial. In: *Ninth European AIDS Conference, Warsaw, Poland, 2003*. Oral presentation and Abstract F2/5. http://www.eacs.ws/conference/index_ns.htm (29 June 2005, date last accessed)