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Pharmacokinetics of saquinavir hard gel/ritonavir (1000/100 mg twice daily) when administered with tenofovir diproxil fumarate in HIV-1-infected subjects

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Aims
To investigate whether the administration of tenofovir diproxil fumarate 300 mg once daily alters the plasma pharmacokinetics of the saquinavir hard gel/ritonavir combination in HIV-1 infected individuals.

Methods
On day 1, 12 h pharmacokinetic profiles for saquinavir/ritonavir (1000/100 mg given twice daily) were obtained for 18 subjects. All subjects were receiving ongoing treatment with a saquinavir/ritonavir-containing regimen. Tenofovir diproxil fumarate 300 mg given once daily was then added to the regimen and blood sampling was repeated at days 3 and 14. Saquinavir and ritonavir concentrations were measured by HPLC–MS/MS, and tenofovir concentrations by HPLC with UV detection.

Results
Following the addition of tenofovir diproxil fumarate to the regimen, saquinavir and ritonavir plasma concentrations were not significantly different compared with day 1. Thus the geometric mean ratios (95% confidence intervals) for the area under the concentration-time curve were 1.16 (0.97, 1.59) and 0.99 (0.87, 1.30) for saquinavir and 1.05 (0.92, 1.28) and 1.08 (0.97, 1.30) for ritonavir, on days 3 and 14, respectively.

Conclusions
Tenofovir diproxil fumarate did not alter the pharmacokinetics of saquinavir hard gel/ritonavir.

Introduction

When antiretroviral drugs are combined, clinically important drug–drug interactions may occur and lead to a decrease in drug plasma concentrations and consequently to virological failure [1].

Saquinavir (SQV), in either soft and hard gel capsule formulations, is routinely administered in combination with low-doses of ritonavir (RTV) plus two nucleoside/nucleotide reverse transcriptase inhibitors (RTIs), one of which could often be tenofovir diproxil fumarate (tenofovir DF), a pro-drug of tenofovir.

Clinically relevant drug interactions between tenofovir and protease inhibitors have been reported, most notably that caused by a decrease in the concentration of atazanavir (ATV) by the former drug (both with and without RTV boosting) [2]. Moreover, no change in

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lopinavir/ritonavir (LPV/RTV) concentrations but an increase in tenofovir plasma exposure (32%) has been observed when LPV/RTV and tenofovir DF are co-administered [3, 4].

Tenofovir is an acyclic nucleoside phosphate excreted by glomerular filtration and active tubular secretion, as are cidofovir and adefovir. These have been shown to be substrates of different renal transporter proteins, such as human renal organic anion transporter 1 (hOAT1) and multidrug resistance protein 2 (Mrp-2) [5]. RTV is a potent inhibitor of Mrp-2-mediated transport [6], which may lead to an increase in tubular concentrations of tenofovir by reducing its efflux from the kidneys. Therefore, RTV treatment in patients on tenofovir could be an explanation for the development of tubular dysfunction described in several case reports [7–11].

The aim of this study was to investigate the effect of tenofovir on the pharmacokinetics of RTV and SQV in a hard gel formulation, as it is not known if a drug–drug interaction occurs when these agents are co-administered in HIV-1 infected individuals.

Methods

Subjects

Subjects eligible for this study were HIV-1 antibody-seropositive adults receiving ongoing treatment with two nucleoside RTIs and SQV/RTV. Approval for the study was obtained from the local ethics committee (Riverside Research Ethics Committee, London, UK) and all subjects gave written informed consent to participate in the study.

Study design

On day 1, subjects attended the unit for blood sampling to characterize the pharmacokinetics of SQV/RTV. On day 2, tenofovir DF 300 mg once daily was added to their treatment regimen and on days 3 and 14 they returned to the unit for further sampling.

On the three assessment days, participants received a 20 g-fat-standardized breakfast immediately before ingestion of their morning dose of drugs. A Y-can cannula (Y-CAN, Beldico, Marche-en-Famenne, Belgium) was inserted into a vein and blood was collected predose and at 0.5, 1, 2, 3, 4, 6, 8, 10, 12, and 24 h following drug ingestion.

Clinical assessment

A clinical assessment of the subjects was performed at each study visit on the basis of clinical adverse events (using the ACTG toxicity grading scale [12] to characterize abnormal findings), laboratory tests, vital signs and physical examinations.

Drug analysis

Plasma concentrations of SQV and RTV were analyzed by a fully validated method adapted from Reynolds *et al.* [13]. The limit of quantification for SQV and RTV was 50 ng ml^{-1}. The inter and intra-assay variability was less than 8% at each of three concentrations. Accuracy was 98.5–102.0% and 94.1–99.3% for SQV and RTV, respectively.

The laboratory has participated in an external quality control program (KKGT – Association for Quality Assessment in TDM and Clinical Toxicology) twice yearly since its initiation in December 1999.

Plasma tenofovir concentrations were determined by an HPLC method with UV detection at 259 nm [14]. The limit of quantification for tenofovir was 10 ng ml^{-1}. All analyses were performed in duplicate and the inter and intraday precision ranged between 1.19 and 6.66% and 3.22 and 8.72%, respectively.

Data analysis

The pharmacokinetic parameters determined for SQV, RTV and tenofovir were the maximum observed plasma concentration (C_{max}), the time to reach C_{max} (t_{max}), the trough plasma concentration observed 12 (SQV/RTV) or 24 (tenofovir) h after ingestion (C_{trough}), the apparent terminal half-life ($t_{1/2}$), and the area under the concentration–time curve (AUC(0,12 h) for SQV/RTV and AUC(0,24 h) for tenofovir). Noncompartmental analysis was used to derive the AUC and $t_{1/2}$ for each patient over the dosing interval (Topfit software, version 2.0; Gustav Fischer Verlag, Stuttgart, Germany).

Statistics were performed using Arcus QuickStat Biomedical (Longman Software Publishing, Cambridge, UK). Geometric mean ratios (GMRs) and associated 95% confidence intervals (CIs) were estimated for the SQV/RTV pharmacokinetic parameters, using day 1 values as references. CIs were first determined using logarithms of the individual GMR values and then expressed as linear values. The changes in pharmacokinetic parameters were considered significant when the CI for the GMR did not include unity.

The distribution of the data did not exhibit any evidence of non-normality (Shapiro-Wilkes test). Hence, a paired *t*-test was performed to compare the pharmacokinetic parameters measured on the different study days.

The coefficient of variation (CV) was calculated to express interpatient and intrapatient variability in the SQV and RTV pharmacokinetic parameters [(SD/mean) × 100].

Table 1
Saquinavir (SQV) and ritonavir (RTV) pharmacokinetic parameters on day 1 when given alone and on days 3 and 14 when given with tenofovir and 95% confidence intervals (CI) and geometric mean ratios (GMR)

Parameter	Day 1 SQV/RTV 1000/100 mg twice daily SQV	RTV	Day 3 SQV/RTV 1000/100 mg twice daily plus TDF 300 mg once daily SQV	RTV	Day 3/Day 1 GMR (95% CI) SQV	RTV	Day 14 SQV/RTV 1000/100 mg twice daily plus TDF 300 mg once daily SQV	RTV	Day 14/Day 1 GMR (95% CI) SQV	RTV
AUC (ng ml^{-1} h)										
Geometric mean	12952	9352	14996	9794	1.16	1.05	12777	10140	0.99	1.08
(95% CI)	(10606, 19473)	(8254, 12214)	(12781, 21891)	(8434, 12970)	(0.97, 1.59)	(0.92, 1.28)	(10987, 19555)	(9036, 12643)	(0.87, 1.30)	(0.97, 1.30)
CV (%)	59	39	53	43			56	33		
C_{max} (ng ml^{-1})										
Geometric mean	2309	1567	2355	1439	1.02	0.92	2157	1602	0.93	1.02
(95% CI)	(1846, 3432)	(1354, 2059)	(1690, 3340)	(1215, 1893)	(0.89, 1.29)	(0.80, 1.15)	(1853, 3096)	(1430, 1978)	(0.82, 1.22)	(0.88, 1.32)
CV (%)	60	42	54	44			50	32		
C_{trough} (ng ml^{-1})										
Geometric mean	310	252	453	313	1.46	1.24	361	321	1.16	1.27
(95% CI)	(258, 622)	(215, 389)	(387, 864)	(251, 563)	(0.98, 2.04)	(1.00, 1.56)*	(317, 720)	(274, 495)	(0.94, 2.02)	(1.07, 1.74)*
CV (%)	83	58	77	77			78	58		
$t_{1/2}$ (h)										
Geometric mean	3.03	3.17	3.35	3.87	1.11	1.26	2.81	3.26	0.92	1.03
(95% CI)	(2.65, 3.71)	(2.82, 3.79)	(2.89, 4.31)	(3.23, 5.09)	(0.93, 1.52)	(0.99, 1.72)	(2.52, 3.28)	(2.84, 3.98)	(0.81, 1.11)	(0.85, 1.32)
CV (%)	34	30	40	44			26	32		

**P = 0.06, paired t-test. TDF = tenofovir diproxil fumarate; AUC = area under the concentration vs time curve; C_{max} = highest observed plasma concentration; C_{trough} = trough concentration at 12 h; $t_{1/2}$ = elimination half-life; CV = coefficient of variation.*

Results

The sample size required to show a 25% difference in mean SQV AUC(0,12 h) with 90% and 80% power (at the 5% level) would have been 62 and 48 patients, respectively. However, this was an exploratory study and a total of 18 subjects was considered sufficient for reliable conclusions to be drawn. All 18 subjects completed all phases of the study. Median (range) age, body mass index and CD4 cell count at screening were 45.5 (22–62) years, 23 (14–31) and 471 (157–968) cells/mm^3. Two of the eighteen subjects had a detectable HIV load (69 and 199 copies ml^{-1}). Concurrent antiretroviral medication administered during the study included zidovudine ($n = 7$), lamivudine ($n = 8$), stavudine ($n = 3$), didanosine ($n = 5$), abacavir ($n = 8$), zalcitabine ($n = 1$). One subject was on an efavirenz (EFV) containing regimen. This subject had been stable on this regimen for more than 6 months with therapeutic plasma SQV concentrations confirmed by drug monitoring. EFV intake was maintained throughout the study period (without and with tenofovir DF).

No grade 3 or 4 adverse events were reported during the study period and the combination of SQV/RTV and tenofovir DF was well tolerated. Adverse events that were potentially drug related and occurred when tenofovir was added to the SQV/RTV regimen were nausea and vomiting (mild, $n = 1$), headache (mild, $n = 2$), diarrhoea (mild, $n = 1$) and abdominal pain (mild, $n = 1$). Laboratory parameters remained stable over the study period.

Pharmacokinetic parameters for SQV and RTV on the three sampling days are summarized in Table 1, and the pharmacokinetic parameters for tenofovir are presented in Table 2. On days 3 and 14, the AUC(0,12 h), C_{trough}, C_{max} and $t_{1/2}$ were not significantly different compared with day 1. Similar findings were observed for RTV. When comparing day 3 and day 14 Ctrough values with day 1 values, a 24 and 27% increase in C_{trough} for RTV was observed during treatment with tenofovir DF. However, in both cases the changes were not statistically significant ($P = 0.06$).

No differences in SQV and RTV t_{max} were observed after the addition of tenofovir DF to the regimen.

The interpatient variability for SQV, RTV (Table 1) and tenofovir (Table 2) AUC, C_{max}, and C_{trough} values was high, despite all subjects having been administered a standardized meal on all three study days. Thus, the mean (± SD) intrapatient variability (considering the determinations assessed on days 1, 3 and 14) for AUC(0,12 h) was 31% (± 14) and 22% (± 9), for C_{max}, 43% (± 25) and 25% (± 11), and for C_{trough} 27% (± 14) and 32% (± 13) for SQV and RTV, respectively.

Table 2
Tenofovir pharmacokinetic parameters measured on days 3 and 14 of the study during treatment with saquinavir/ritonavir (SQV/RTV)

Parameter	Day 3 SQV/RTV 1000/100 mg twice daily plus TDF 300 mg once daily	Day 14 SQV/RTV 1000/100 mg twice daily plus TDF 300 mg once daily
AUC (ng ml^{-1} h)		
Geometric mean (95% CI)	2701 (2333–3423)	3120 (2739–3875)
CV (%)	40	37
C_{max} (ng ml^{-1})		
Geometric mean (95% CI)	271 (235–351)	292 (253–369)
CV (%)	42	39
C_{trough} (ng ml^{-1})		
Geometric mean (95% CI)	55 (49–79)	69 (59–91)
CV (%)	50	45
$t_{1/2}$ (h)		
Geometric mean (95% CI)	14.9 (11.7–20.8)	13.4 (12.2–15.4)
CV (%)	54	23

TDF = tenofovir diproxil fumarate; AUC = area under the concentration vs. time curve; C_{max} = highest observed plasma concentration; C_{trough} = trough concentration at 12 h; $t_{1/2}$ = apparent terminal half-life; CV = coefficient of variation.

Discussion

The pharmacokinetics of SQV/RTV in the formulation and regimens used were not affected by the addition of tenofovir DF in patients with HIV.

Both SQV and RTV are metabolized by cytochrome P450 (CYP), mainly the isoform CYP3A4. Therefore, drug–drug interactions are likely to occur when drugs that share the same metabolic pathway are co-administered [15]. Howvever, tenofovir is not a substrate nor does it inhibit or induce CYP enzymes *in vitro* [16]. Thus, the potential for CYP–mediated drug interactions involving tenofovir should be low. However, drug–drug pharmacokinetic interactions have been described between tenofovir DF and hepatically metabolized PIs (indinavir [17], LPV/RTV [3, 4], atazanavir [2]).

The combination of SQV/RTV and tenofovir DF was well tolerated, with adverse events limited to a small number of subjects who reported grade 1 nausea, vomiting, headache and/or diarrhoea after the addition of tenofovir DF to the SQV/RTV regimen.

As shown for other drugs [18], there was marked intrapatient variability in the pharmacokinetics of the three drugs. However, only two subjects on day 1, none on day 3 and two on day 14 had a SQV C_{trough} lower than the minimum concentration proposed for treatment naïve patients [19].

In summary, tenofovir DF appeared to have no effect on the pharmacokinetics of SQV and RTV in HIV infected patients.

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