Pharmacokinetic interactions between sildenafil and saquinavir/ritonavir

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Aims To investigate the effect of the antiretroviral protease inhibitors saquinavir (soft gelatin capsule) and ritonavir on the pharmacokinetic properties and tolerability of sildenafil and to investigate the effect of sildenafil on the steady-state pharmacokinetics of saquinavir and ritonavir.

Methods Two independent, 8 day, open, randomized, placebo-controlled, parallel-group studies (containing a double-blind crossover phase) were conducted at Pfizer Clinical research units (Canterbury, UK. and Brussels, Belgium). Twenty-eight healthy male volunteers entered each study. In each study, volunteers were randomized ($n = 14$ per group) to receive sildenafil on day 1 followed by a 7-day treatment period (days 2–8) with saquinavir or placebo (Study I) or ritonavir or placebo (Study II). Sildenafil or placebo (Study I and Study II) was administered alternately on day 7 or day 8, depending on initial randomization. The effect of saquinavir and ritonavir on the pharmacokinetics of sildenafil and its primary circulating metabolite (UK-103, 320) and the effect of single-dose sildenafil on the steady-state pharmacokinetics of saquinavir (1200 mg three times daily) and ritonavir (500 mg twice daily) were determined. The safety and tolerability of sildenafil coadministered with saquinavir or ritonavir were also assessed.

Results Both protease inhibitors significantly increased C_{max}, AUC, t_{max} and $t_{½}$ values for both sildenafil and UK-103, 320. Ritonavir showed a significantly greater effect than saquinavir with increases in sildenafil AUC and C_{max} of 11-fold (95% CI: 9.0, 12.0) and 3.9-fold (95% CI: 3.2, 4.9), respectively. This compared with increases of 3.1-fold (95% CI: 2.5, 4.0) and 2.4-fold (95% CI: 1.8, 3.3) for coadministration with saquinavir. In contrast, the steady-state pharmacokinetics of saquinavir and ritonavir were unaffected by sildenafil. The increases in systemic exposure to sildenafil and UK-103, 320 were not associated with an increased incidence of adverse events or clinically significant changes in blood pressure, heart rate or ECG parameters.

Conclusions These results indicate that both saquinavir and ritonavir modify the pharmacokinetics of sildenafil presumably through inhibition of CYP3A4. The more pronounced effect of ritonavir may be attributed to its additional potent inhibition of CYP2C9. No change in safety or tolerability was observed when sildenafil was coadministered with either protease inhibitor. However, given the extent of the interactions, a lower sildenafil starting dose (25 mg) should be considered for patients receiving saquinavir and it is recommended not to exceed a maximum single dose of 25 mg in a 48 h period for patients receiving ritonavir.

Keywords: antiretroviral protease inhibitors, cytochrome P450 2C9, cytochrome P450 3A4, pharmacokinetic interactions, ritonavir, saquinavir, sildenafil citrate

Introduction

Sildenafil (Viagra®, Pfizer) is an orally active phosphodiesterase-type-5 inhibitor that is effective in the treatment of male erectile dysfunction of organic, psychogenic or mixed aetiology [1]. Given the demonstrated efficacy, ease of use,

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good tolerability and positive impact on patients' quality of life with sildenafil, it has assumed a major role in the treatment of erectile dysfunction [2]. The drug is eliminated predominantly by hepatic metabolism, being subjected to extensive first pass *N*-demethylation via the cytochrome P450 isoenzymes CYP3A4 (major route) and CYP2C9 (minor route) [2, 3]. Thus, there is scope for pharmacokinetic interaction between sildenafil and coadministered drugs that act as substrates, inhibitors or inducers of these isoenzymes. Increased systemic exposure to sildenafil has been described following its coadministration with the established CYP3A4 inhibitor erythromycin [4].

Sexual (including erectile) dysfunction is a relatively common complaint in men with HIV infection [5–7], a population that routinely receives multiple medication most notably, antiretroviral drug combinations. The effectiveness of protease inhibitors in reducing the morbidity and mortality associated with advanced HIV infection is well established [8, 9]. However, these agents have been reported to interact with several other drugs, including azole antifungals, clarithromycin, rifamycins and non-nucleoside reverse transcriptase inhibitors [10, 11]. The protease inhibitors saquinavir, ritonavir, indinavir, nelfinavir and amprenavir are metabolized by and inhibit the CYP3A4 isoenzyme [12–18]. In addition, at therapeutic concentrations, ritonavir inhibits a number of other cytochrome P450 isoenzymes, including CYP2C9, CYP2C19 and CYP2D6 [19–21].

As effective antiretroviral therapy continues to improve physical wellbeing an increased awareness of erectile dysfunction is likely to emerge. Patients have voiced a need for advice on erectile dysfunction and concerns surrounding potential interactions between sildenafil and protease inhibitors have been raised [22]. Merry *et al.* investigated the interaction of sildenafil and indinavir in HIV-positive patients [23]. This study showed that coadministration of sildenafil 25 mg did not significantly alter plasma indinavir concentrations. However, plasma sildenafil AUC was markedly increased in the presence of indinavir compared with historical controls. This suggests that a lower starting dosage of sildenafil may be appropriate in this clinical setting.

Given the potential for indinavir to alter the kinetics of sildenafil, this study was undertaken to investigate the pharmacokinetic interaction between sildenafil and the protease inhibitors saquinavir and ritonavir in healthy male volunteers.

Methods

Subjects and study design

Two groups of 28 healthy male volunteers (18–45 years) weighing between 60 and 100 kg and with a body mass index within the permitted range (Quetelet's index range 18–28) were enrolled in two separate, randomised, open-label, placebo-controlled, parallel-group studies, each containing a double-blind crossover phase. Volunteers were excluded if they showed signs of clinical disease, orthostatic hypotension, drug abuse or excessive alcohol consumption (>21 units/week). Those with a history of allergy, abnormal laboratory results or recent use of a prescription drug (within 3 weeks) or investigational drug (within 4 months) were also excluded. Written informed consent was obtained from each subject prior to study entry and the study protocols were reviewed and approved by the relevant local ethics committees.

In each study, volunteers were randomised to receive oral sildenafil 100 mg on day 1 followed by oral saquinavir (soft gelatin capsule) or placebo (Study I) or oral ritonavir or placebo (Study II) for the next 7 days (days 2–8). In both studies sildenafil (100 mg) and placebo were administered alternately on days 7 and 8. This design allowed assessment of both the effect of saquinavir and ritonavir on the pharmacokinetics of single-dose sildenafil, and the effect of single dose sildenafil on the steady-state pharmacokinetics of saquinavir and ritonavir. Saquinavir (soft gelatin capsule) 1200 mg three times daily was administered on days 2–8 and ritonavir 300, 400 and 500 mg twice daily was administered on days 2, 3, and 4–8, respectively. The study schedule and treatment regimens are presented in Table 1.

Pharmacokinetic measurements

Blood samples (7 or 10 ml) for pharmacokinetic analyses were taken on days 1, 7, and 8 at time 0 (baseline premorning dose) and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 18 and 24 h after the morning dose.

Table 1 Study schedule and treatment regimens.

	Treatment			
Study/Group	*Day 1*	*Days 2–8*	*Day 7*	*Day 8*
Study I				
Sequence 1	Sildenafil	Saquinavir	Sildenafil	Placebo
Sequence 2	Sildenafil	Saquinavir	Placebo	Sildenafil
Sequence 3	Sildenafil	Placebo	Sildenafil	Placebo
Sequence 4	Sildenafil	Placebo	Placebo	Sildenafil
Study II				
Sequence 1	Sildenafil	Ritonavir	Sildenafil	Placebo
Sequence 2	Sildenafil	Ritonavir	Placebo	Sildenafil
Sequence 3	Sildenafil	Placebo	Sildenafil	Placebo
Sequence 4	Sildenafil	Placebo	Placebo	Sildenafil

Sildenafil administered as a single 100 mg dose; saquinavir administered at a dosage of 1200 mg three times daily; ritonavir administered at a dosage of 300, 400 and 500 mg twice daily on days 2, 3 and 4–8, respectively.

Table 2 Effect of saquinavir and ritonavir on the pharmacokinetics of single-dose sildenafil.

Treatment regimen		*Pharmacokinetic parameter*			
		AUC† *(ng ml^{-1} h)*	C_{max}† *(ng ml^{-1})*	t_{max}‡ (h)	λ_z‡ *(h^{-1})*
Study I ($n=27$)					
Saquinavir					
	Sildenafil (day 1)	1378	296	2.1	0.207
	Sildenafil (day 7/8)/saquinavir (day 2–8)	4261	609	4.8	0.156
Placebo					
	Sildenafil (day 1)	1468	302	3.1	0.199
	Sildenafil (day 7/8)/placebo (day 2–8)	1459	256	3.3	0.194
⋆*P* value		<0.0001	<0.0001	0.0012	0.012
Study II ($n=28$)					
Ritonavir					
	Sildenafil (day 1)	1419	321	2.0	0.183
	Sildenafil (day 7/8)/ritonavir (day 2–8)	13278	1063	5.6	0.127
Placebo					
	Sildenafil (day 1)	1502	325	2.3	0.193
	Sildenafil (day 7/8)/placebo (day 2–8)	1342	274	2.7	0.198
⋆*P* value		<0.0001	<0.0001	0.0018	0.0003

Sildenafil administered as a single 100 mg dose; saquinavir administered at a dosage of 1200 mg three times daily; ritonavir administered at a dosage of 300, 400 and 500 mg twice daily on days 2, 3 and 4–8, respectively. ⋆: intergroup comparison (saquinavir/ritonavir *vs* placebo) of change in sildenafil pharmacokinetics from day 1 to day 7/8; †: geometric mean; ‡: arithmetic mean.

Table 3 Effect of saquinavir and ritonavir on the pharmacokinetics of the sildenafil metabolite UK-103,320.

Treatment regimen		*Pharmacokinetic parameter*			
		AUC† *(ng ml^{-1} h)*	C_{max}† *(ng ml^{-1})*	t_{max}‡ *(h)*	λ_z‡ *(h^{-1})*
Study I ($n=27$)					
Saquinavir					
	Sildenafil (day 1)	569	116	2.5	0.121
	Sildenafil (day 7/8)/saquinavir (day 2–8)	1172	94	6.4	0.144
Placebo					
	Sildenafil (day 1)	659	118	3.5	0.111
	Sildenafil (day 7/8)/placebo (day 2–8)	582	95	3.5	0.148
⋆*P* value		<0.0001	0.995	0.002	0.352
Study II ($n=28$)					
Ritonavir					
	Sildenafil (day 1)	567a	132	2.0	0.141
	Sildenafil (day 7/8)/ritonavir (day 2–8)	840a	55	11.7	na
Placebo					
	Sildenafil (day 1)	731a	160	2.5	0.121
	Sildenafil (day 7/8)/placebo (day 2–8)	622a	129	2.5	0.124
⋆*P* value		<0.0001	0.0002	0.0001	–

Sildenafil administered as a single 100 mg dose; saquinavir administered at a dosage of 1200 mg three times daily; ritonavir administered at a dosage of 300, 400 and 500 mg twice daily on days 2, 3 and 4–8, respectively. a:AUC value from time zero to the final quantifiable concentration (AUC_t). ⋆: intergroup comparison (saquinavir/ritonavir *vs* placebo) of change in UK-103 320 pharmacokinetics from day 1 to day 7/8; †: geometric mean; ‡: arithmetic mean.

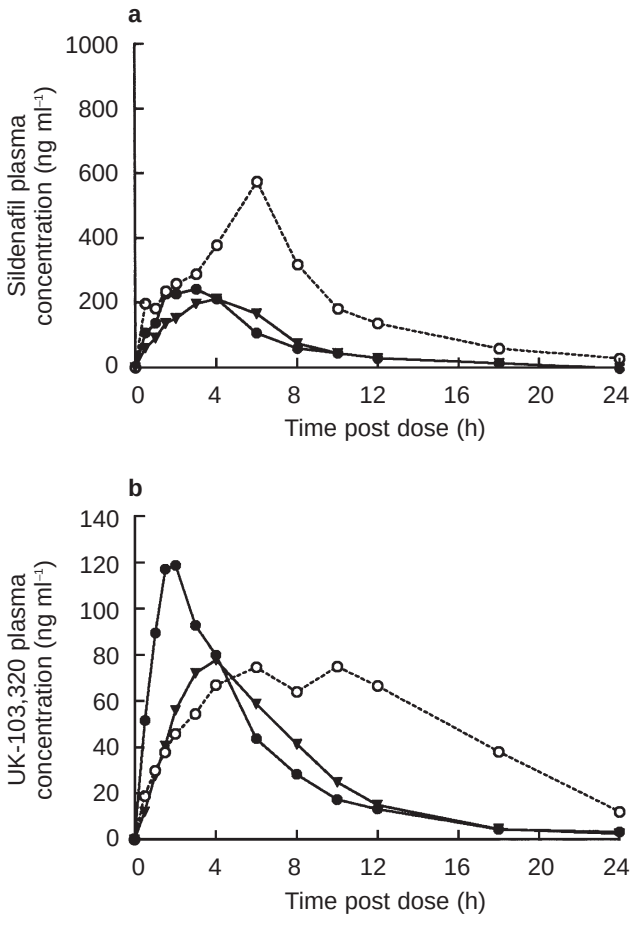

Figure 1 a) Mean plasma sildenafil concentrations (ng ml^{-1}) on day 1 (●), and in the presence of saquinavir (○) or placebo (▼) on day 7/8. b) Mean plasma UK-103 320 concentrations (ng ml^{-1}) on day 1 (●), and in the presence of saquinavir (○) or placebo (▼) on day 7/8.

The simultaneous determination of sildenafil (Viagra) and its metabolite (UK-103 320) were analysed using automated sequential trace enrichment of dialysates and high-performance liquid chromatography [24]. The limits of quantification were 1 ng ml^{-1} for both sildenafil and UK-103 320. The overall imprecision (CV) was 5.1, 3.2 and 3.0% for sildenafil and 3.4, 3.1 and 2.9% for UK-103 320 concentrations of 3.00, 125 and 200 ng ml^{-1}, respectively. The inaccuracy (bias) of the assay at all concentrations ranged from −2.3% to 3.5% for sildenafil and −7.0% to 4.8% for UK-103 320.

The analytical procedure for analysing saquinavir and ritonavir in plasma utilized a Sciex API 300 instrument with a heated nebuliser source. The analytes were isolated by solid phase extraction and the resultant sample extracts were injected onto the LC/MS/MS detection system. The limits of quantification were 1 ng ml^{-1} and 10 ng ml^{-1} for the saquinavir and ritonavir assays, respectively. The overall imprecision of the saquinavir assay (CV) was 7.8, 6.6 and 6.3% at target concentrations of 3.00, 120 and 799 ng ml^{-1}, respectively. The inaccuracy (bias) of the assay at all concentrations ranged from −5.4% to 0.8%. The overall imprecision of the ritonavir assay (CV) was 7.0, 4.7 and 5.6% at target concentrations of 30.0, 1001 and 8010 ng ml^{-1}, respectively. The inaccuracy (bias) of the assay at all concentrations ranged from −10.0% to 7.3%.

Table 4 Effect of single-dose sildenafil on the steady-state pharmacokinetics of saquinavir and ritonavir.

Treatment regimen	*Steady-state pharmacokinetic parameter* $AUC(\tau)$ (ng ml^{-1} h)	C_{max} (ng ml^{-1})	t_{max} (h)
Study I ($n=27$)			
Saquinavir			
Saquinavir/placebo	6005	1859	3.0
Saquinavir/sildenafil	6156	1800	3.1
⋆*P* value	0.755	0.749	0.749
Study II ($n=28$)			
Ritonavir			
Ritonavir/placebo	127058	15792	6.0
Ritonavir/sildenafil	121865	15441	6.3
⋆*P* value	0.439	0.684	0.753

Sildenafil administered as a single 100 mg dose; saquinavir administered at a dosage of 1200 mg three times daily; ritonavir administered at a dosage of 300, 400 and 500 mg twice daily on days 2, 3 and 4–8, respectively. AUC(τ):area under plasma concentration-time curve at steady state (Study I: $t=8$ h; Study II: $t=12$ h); ⋆: intergroup comparison (sildenafil vs placebo).

All pharmacokinetic parameters were calculated by noncompartmental analysis using WinNonlin™ Version 1.1 (Pharsight Corporation). The pharmacokinetic parameters calculated for sildenafil and its primary metabolite, UK-103,320, were: maximum observed plasma concentration (C_{max}), time to reach maximum plasma concentration (t_{max}), apparent terminal elimination phase rate constant (λ_z), area under the plasma concentration-time curve from time zero to the last measurable concentration (AUC(0,t)) and area under the plasma concentration-time curve extrapolated to infinity (AUC). On days 7 and 8, C_{max}, t_{max} and the area under the plasma concentration-time curve over the dosing interval (AUCτ) were calculated for saquinavir and ritonavir in the presence of sildenafil or placebo. Additional (premorning dose) blood samples were taken to determine trough plasma concentrations (C_{min}) of saquinavir and ritonavir.

Safety and tolerability

Laboratory safety tests, vital sign measurements and electrocardiograms (ECG) were performed at regular intervals during the study. The nature and severity of all adverse events were recorded throughout the study

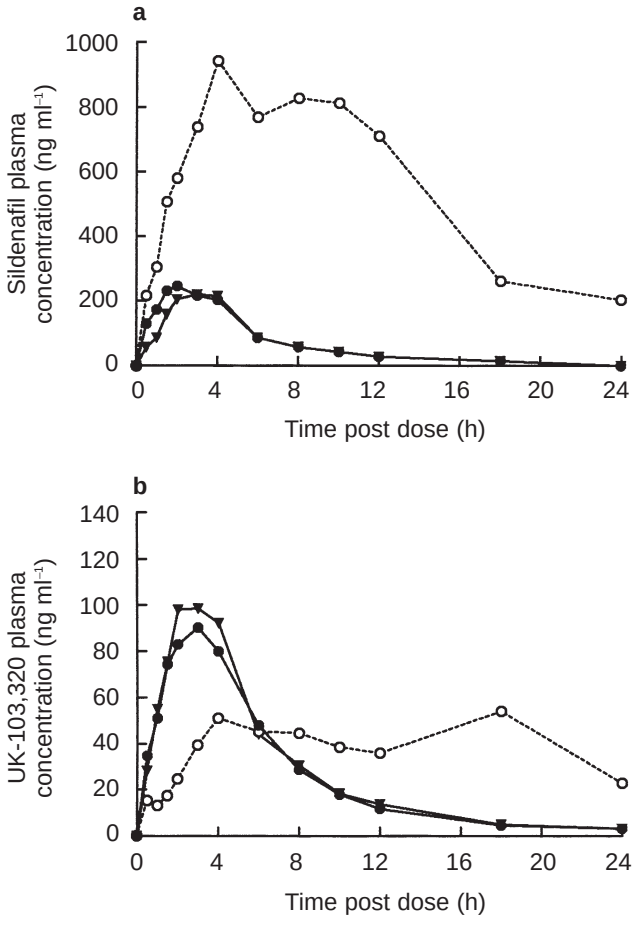

Figure 2 a) Mean plasma sildenafil concentrations (ng ml^{-1}) on day 1 (●), and in the presence of ritonavir or placebo (▼) on day 7/8. b) Mean plasma UK-103 320 concentrations (ng ml^{-1}) on day 1 (●), and in the presence of ritonavir (○) or placebo (▼) on day 7/8.

period. The investigators obtained all adverse events using non leading questions and recorded their opinion of the relationship to study treatment.

Statistical analysis

Sildenafil and UK-103 320 pharmacokinetic parameters An An analysis of variance (ANOVA) appropriate to the study design was conducted for sildenafil (natural-log transformed AUC and C_{max} and untransformed λ_z and t_{max}) and UK-103 320 (natural-log transformed AUC(0,t) and C_{max} and untransformed t_{max}). Differences between the mean pharmacokinetic values of sildenafil on day 1 and days 7/8 were calculated for each treatment group (i.e. saquinavir, ritonavir or placebo). The two active treatment groups (i.e. saquinavir and ritonavir) were compared with their corresponding placebo group by estimating the intergroup difference in change from day 1 to days 7/8, together with the corresponding standard error and 95% confidence interval (95% CI).

Saquinavir and ritonavir Cmin values were plotted to assess attainment of steady-state plasma levels. Steady-state saquinavir and ritonavir AUC(τ) and C_{max} values (both natural-log transformed) and t_{max} (untransformed) were subjected to an ANOVA appropriate to the study design. Differences between mean pharmacokinetic values recorded in the presence of sildenafil or placebo were estimated, together with the associated standard errors and 95% CIs.

Results

Demographic characteristics

A total of 27 and 28 subjects completed Studies I and II, respectively, and were included in the pharmacokinetic analysis; one subject withdrew from Study I with a nontreatment-related adverse event. All enrolled subjects were evaluated for safety. Subjects included in the two studies were predominantly Caucasian (96.4%) and ranged in age from 18 to 44 (mean 28.8) years.

Effect of saquinavir on sildenafil and UK-103, 320 pharmacokinetics

Pharmacokinetic analysis was performed on data from 27 of the 28 subjects in the saquinavir study. The mean plasma concentration profiles for sildenafil and UK-103, 320 on day 1, and in the presence of saquinavir or placebo on day 7/8, are shown in Figure 1a,b. Mean pharmacokinetic parameters for sildenafil and UK-103, 320 are summarized in Tables 2 and 3, respectively.

Multiple dosing with saquinavir produced significant increases in sildenafil AUC and C_{max} values (both $P<0.0001$) and significantly delayed t_{max} ($P=0.0012$) relative to placebo. There was a 3.1-fold (95% CI: 2.5, 4.0) increase in AUC and a 2.4-fold (95% CI: 1.8, 3.3) increase in C_{max}, while t_{max} was delayed by 2.6 h (95% CI: 1.1, 4.1 h). Multiple dosing with saquinavir also significantly ($P=0.012$) decreased λ_z of sildenafil by 0.046/h (95% CI: -0.8, -0.1), resulting in an increase in the $t_{½}$ of sildenafil of approximately 1 h. However, by 24 h postdose, mean plasma sildenafil concentrations had declined to levels comparable to those recorded in the placebo group (Figure 1a).

In the case of the metabolite UK-103,320, multiple dosing with saquinavir increased its AUC 2.3-fold (95% CI: 1.9, 2.9; $P<0.0001$) and delayed its t_{max} by 3.9 h (95% CI: 1.5, 6.3 h; $P=0.002$) relative to placebo, but had no significant effect on its C_{max} or λ_z values.

Table 5 Incidence of the most common treatment-related adverse events.
Study I

Adverse events	*Sildenafil* (n = 14)	*Saquinavir Sildenafil/ saquinavir* (n = 13)	*Placebo/ saquinavir* (n = 13)	*Sildenafil* (n = 14)	*Placebo Sildenafil/ placebo* (n = 14)	*Placebo/ placebo* (n = 14)
Total number of subjects with adverse events	8	10	3	5	11	4
Discontinued therapy due to adverse event	0	0	0	0	0	0
Headache	4	7	3	1	7	2
Vasodilation	3	1	0	2	2	0
Diarrhoea	0	1	0	0	0	0
Nausea	0	0	0	0	2	0
Dyspepsia	0	1	0	1	1	0
Paresthesia	0	1	0	1	0	0
Dizziness	1	3	0	2	2	0
Rhinitis	2	4	1	1	3	0
Abnormal vision	0	0	0	0	1	0

Study II

Adverse events	*Sildenafil* (n = 14)	*Ritonavir Sildenafil/ ritonavir* (n = 14)	*Placebo/ ritonavir* (n = 14)	*Sildenafil* (n = 14)	*Placebo Sildenafil/ placebo* (n = 14)	*Placebo/ placebo* (n = 14)
Total number of subjects with adverse events	9	12	11	11	8	2
Discontinued therapy due to adverse event	0	0	0	0	0	0
Headache	5	4	1	7	5	0
Asthenia	0	5	5	2	0	1
Vasodilation	6	6	1	3	4	1
Postural hypotension	0	3	0	0	2	0
Diarrhoea	0	3	2	0	0	0
Nausea	1	4	4	0	0	0
Dyspepsia	1	2	2	0	0	0
Paresthesia	0	4	5	0	0	0
Dizziness	0	3	1	0	1	0
Rhinitis	0	3	0	1	2	0
Abnormal vision	1	3	0	0	0	0

Effect of ritonavir on sildenafil and UK-103, 320 pharmacokinetics

Pharmacokinetic analysis was performed on data from all 28 subjects in the ritonavir study. The mean plasma concentration profiles for sildenafil and UK-103, 320 on day 1, and in the presence of ritonavir or placebo on day 7/8, are shown in Figure 2a,b. Mean pharmacokinetic parameters for sildenafil and UK-103,320 are summarized in Tables 2 and 3, respectively.

Multiple dosing with ritonavir produced significant increases in sildenafil AUC and C_{max} (both $P<0.0001$) and significantly delayed t_{max} ($P=0.0018$) relative to placebo. There was an 11-fold (95% CI: 9.0, 12.0) increase in AUC and a 3.9-fold (95% CI: 3.2, 4.9) increase in C_{max}, while t_{max} was delayed by 3.1 h (95% CI: 1.3, 4.9 h). In addition, multiple dosing with ritonavir significantly ($P=0.0003$) decreased λ_z of sildenafil by 0.06 h^{-1} (95% CI: -0.09, -0.03), resulting in an increase in its $t_{½}$ of 1.8 h. Mean plasma sildenafil concentrations remained elevated throughout the dosing interval, with mean concentrations 24 h postdose similar to the maximum mean concentrations observed in the absence of ritonavir (placebo group) (Figure 2a).

For the metabolite UK-103,320, multiple dosing with ritonavir significantly increased its AUC(0,t) 1.7-fold (95% CI: 1.4, 2.2; $P<0.0001$), decreased its C_{max} 0.5-fold (95% CI: 0.37, 0.71; $P=0.0002$) and delayed its t_{max} by 9.7 h

(95% CI: 5.8, 14 h; $P=0.0001$) relative to placebo. Due to the extent of the drug interaction, it was not possible to characterize the elimination phase, and thus the λ_z or $t_{½}$ values.

Effect of single dose sildenafil on the steady-state pharmacokinetics of saquinavir and ritonavir

Visual inspection of the predose (trough) plasma saquinavir and ritonavir concentrations during the course of the studies indicated that steady state was achieved by day 7 with both protease inhibitors. Mean steady-state pharmacokinetic values for saquinavir and ritonavir are summarized in Table 4.

Sildenafil had no significant effect on steady-state AUC(τ), C_{max} or t_{max} values of either saquinavir or ritonavir.

Safety and tolerability of sildenafil in combination with saquinavir or ritonavir

The most common adverse events reported with sildenafil were headache, facial flushing (vasodilatation), dizziness, abnormal vision and rhinitis. Coadministration of sildenafil with either saquinavir or ritonavir did not significantly affect the incidence or severity of these adverse events (Table 5). The increases in systemic bioavailability of sildenafil caused by these protease inhibitors, were not associated with clinically significant laboratory abnormalities or changes in blood pressure, heart rate or ECG parameters.

Discussion

Sildenafil is subject to extensive oxidative metabolism *in vitro*, undergoing *N*-demethylation to UK-103,320, a reaction mediated by the low-affinity, high-capacity CYP3A4 isoenzyme (major route) and the high-affinity, low-capacity CYP2C9 isoenzyme (minor route) [2, 3]. Coadministration of sildenafil with inhibitors of these isoenzymes can therefore be expected to affect its pharmacokinetics. This is substantiated by the approximately 3-fold increase in peak plasma sildenafil concentrations obtained in the presence of the CYP3A4 inhibitor erythromycin [25].

The protease inhibitors are subject to extensive first-pass metabolism by the hepatic cytochrome P450 system, in particular by the CYP3A4 isoenzyme [13], and in addition act as inhibitors of CYP3A4 [20, 22]. *In vitro* studies with human hepatic microsomes indicate that, of the currently available protease inhibitors, ritonavir is the most potent inhibitor of CYP3A4 and CYP2C9, while saquinavir is the least potent inhibitor of CYP3A4 with only weak *in vitro* inhibitory activity against CYP2C9 [20, 22, 26]. Thus, IC_{50} values for inhibition of CYP3A4-mediated testosterone 6β-hydroxylation by ritonavir and saquinavir were 0.034 and 2.14 $\mu mol\,l^{-1}$, respectively. Corresponding IC_{50} values for inhibition of CYP2C9-mediated tolbutamide hydroxylation were 4.2 and 53.9 $\mu mol\,l^{-1}$, respectively [19]. However, it should be recognized that the estimated IC_{50} for the effect of saquinavir on CYP2C9 is more than 10-fold higher than the mean maximum observed concentrations in patients treated with saquinavir soft gelatin formulation at a dose of 1200 mg three times daily [17]. Therefore, administration of saquinavir soft gelatin formulation is unlikely to result in significant inhibition of CYP2C9 *in vivo*.

In this study, there were significant increases in C_{max} and AUC of sildenafil (2.4-and 3.1-fold, respectively) and significant prolongation of its t_{max} (by 2.6 h) during multiple dosing with saquinavir 1200 mg three times daily in human volunteers. This indicates that saquinavir inhibits the CYP3A4-mediated first pass metabolism of sildenafil. The extent of the interaction with saquinavir appears comparable to that obtained with erythromycin (see above) and indinavir [23]. The modest increase in $t_{½}$ of sildenafil (by 1.0 h) indicates a limited effect of saquinavir on the systemic clearance of sildenafil. In addition, the significant increase in AUC (2.3-fold) and prolongation of t_{max} (by 3.9 h) of the sildenafil metabolite UK-103 320 suggests that saquinavir also inhibits the systemic metabolism of UK-103,320, a process also thought to be mediated by CYP3A4.

Ritonavir had a much greater effect than saquinavir on the pharmacokinetics of sildenafil. This provides further evidence for the involvement of the cytochrome P450 system in the sildenafil–protease inhibitor interaction. The 11-fold increase in sildenafil's AUC and high plasma concentrations, even at 24 h postdose, can be attributed to the potent inhibition by ritonavir of both the major (CYP3A4) and minor (CYP2C9) routes of sildenafil metabolism [20–22]. This effectively prevented the possibility of any compensatory shift in first pass metabolism of sildenafil through the secondary CYP2C9-mediated pathway and, in addition, inhibited the systemic clearance of sildenafil. These results are consistent with the marked effects of ritonavir on a range of other cytochrome P450 substrates [27, 28].

In vitro studies have indicated that sildenafil is not an inhibitor of CYP3A4 ($IC_{50} > 300\ \mu mol\,l^{-1}$) at clinically relevant concentrations ($\approx 1\ \mu mol\,l^{-1}$ after a 100 mg dose) [4]. Accordingly, sildenafil (100 mg) did not significantly affect the steady-state pharmacokinetics of either saquinavir or ritonavir in this study. This suggests that the antiretroviral efficacy of these protease inhibitors is unlikely to be affected by coadministered sildenafil.

The significant increase in systemic exposure to sildenafil, resulting from coadministration with the

protease inhibitors (in particular ritonavir), was not associated with an increase in the incidence of adverse effects or laboratory abnormalities. The pattern of reported adverse events in this volunteer study was consistent with that noted in large-scale clinical trials of sildenafil, in which headache, facial flushing, dyspepsia and nasal congestion occur most frequently [29]. Given that it is C_{max} which primarily influences the frequency and severity of sildenafil adverse events and that sildenafil is well tolerated upto doses of 800 mg [4] it is not surprising that the adverse event profile even in the presence of ritonavir was not significantly affected. The adverse effects of sildenafil, are described as dose-related over the range 50–200 mg, but are usually mild and transient in nature [2]. In view of this, consideration of a lower starting dose (25 mg) is an advisable precaution in patients concomitantly receiving protease inhibitors. Given the extent and duration of the effects of ritonavir, it is recommended not to exceed a maximum dose of 25 mg in a 48 h period.

In summary, saquinavir and ritonavir significantly modified the pharmacokinetics of sildenafil resulting in increased plasma concentrations of both drug and metabolite. The effect was considerably more pronounced with ritonavir than with saquinavir, although neither drug significantly altered the safety or tolerability of sildenafil. Given the extent of the interactions, a lower sildenafil starting dose (25 mg) should be considered for patients receiving saquinavir whilst it is recommended not to exceed a maximum single dose of 25 mg in a 48 h period for patients receiving ritonavir. As sildenafil did not affect the steady-state pharmacokinetic profile of either saquinavir or ritonavir, the clinical effectiveness of protease inhibitor therapy is unlikely to be compromised by coadministration of sildenafil.

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