Lack of sex-related differences in saquinavir pharmacokinetics in an HIV-seronegative cohort

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Aims

To examine the influence of sex on steady-state saquinavir pharmacokinetics in HIV-seronegative volunteers administered saquinavir without a concomitant protease inhibitor.

Methods

Thirty-eight healthy volunteers (14 female) received saquinavir soft-gel capsules 1200 mg three times daily for 3 days to achieve steady-state conditions. Following administration of the 10th dose, blood was collected serially over 8 h for measurement of saquinavir plasma concentrations. Saquinavir pharmacokinetic parameter values were determined using noncompartmental methods and compared between males and females. CYP3A phenotype (using oral midazolam) and MDR-1 genotypes at positions 3435 and 2677 were determined for all subjects in order to characterize possible mechanisms for any observed sex-related differences.

Results

There was no significant difference in saquinavir AUC₀₋₈ or any other pharmacokinetic parameter value between the sexes. These findings persisted after mathematically correcting for total body weight. The mean weight-normalized AUC₀₋₈ was 29.9 (95% confidence interval 15.5, 44.3) and 29.8 (18.6, 40.9) ng h⁻¹ mL⁻¹ kg⁻¹ for males and females, respectively. No significant difference in CYP3A phenotype was observed between the groups; likewise, the distribution of MDR-1 genotypes was similar for males and females.

Conclusion

In contrast to previous study findings, results from this investigation showed no difference in saquinavir pharmacokinetics between males and females. The discrepancy between our findings and those previously reported may be explained by the fact that we evaluated HIV-seronegative volunteers and administered saquinavir in the absence of concomitant protease inhibitors such as zidovudine. Caution must be exercised when extrapolating pharmacokinetic data from healthy volunteer studies (including sex-based pharmacokinetic differences) to HIV-infected populations or to patients receiving additional concurrent medications.

Introduction

Sex-based differences in drug efficacy, toxicity and pharmacokinetics continue to be appreciated for numerous medications. Pharmacokinetic differences between males and females probably result from a combination of mechanisms, including altered bioavailability, distribution, metabolism and elimination [1]. Sex-based differences in cytochrome P450 (CYP) activity, in particular CYP3A4, and the drug transport protein P-glycoprotein (P-gp) have been demonstrated, although results are not consistent among studies [1–7].

Saquinavir is an HIV protease inhibitor (PI) used in combination with other antiretroviral agents for the treatment of HIV infection. When given as a lone protease inhibitor, saquinavir has a high pill burden (18 soft-gel capsules per day) as a consequence of its poor absorption and extensive first-pass metabolism [8, 9]. Saquinavir's low and variable absorption is due to the coordinated efforts of intestinal P-gp efflux and CYP3A4 metabolism [8–10]. Saquinavir's pill burden is markedly reduced when it is coadministered with low-dose ritonavir, a potent inhibitor of CYP3A4 and a moderate inhibitor of P-gp *in vivo* [11, 12].

Previous studies in HIV-infected patients taking saquinavir in combination with ritonavir have observed significantly higher saquinavir plasma concentrations in females compared with males [13–16]. Proposed explanations for these findings include sex-based differences in CYP3A4 and P-gp activity, as well as greater boosting effects due to enhanced ritonavir exposure in females. In contrast, saquinavir pharmacokinetics did not differ between males and females when a single 1200-mg dose was given without concomitant ritonavir to HIV-seronegative volunteers [8]. It is unknown whether sex-based differences in saquinavir exposure exist under steady-state conditions when the drug is administered to HIV-seronegative adults in the absence of a pharmacoenhancing protease inhibitor (e.g. ritonavir). The purpose of this investigation was to determine the influence of sex on steady-state saquinavir pharmacokinetics in HIV-seronegative volunteers receiving the drug without a concomitant protease inhibitor. Secondary aims were to evaluate the roles of multidrug resistant protein (MDR-1) genotype and CYP3A phenotype in any observed sex-based differences in saquinavir exposure.

Methods

Subjects

A total of 24 men and 14 women between the ages of 18 and 50 were enrolled into the study. Evaluation of potential subjects included a medical history, physical

examination and laboratory analysis (serum electrolytes, liver function tests, creatinine and blood urea nitrogen) to rule out any abnormalities or medical conditions that could increase subject risk or affect study results. Participants were also required to have a negative HIV ELISA test. All subjects were within 10% of their ideal body weight (IBW). Subjects were not allowed to have taken any medication (including non-prescription, herbal supplements and oral contraceptives) within 20 days of study participation. Additional exclusion criteria included smoking, drug or alcohol abuse, history of intolerance to saquinavir and persistent diarrhoea. The only medications allowed during study participation were acetaminophen, ibuprofen and a multivitamin with minerals. Subjects refrained from taking any of these medications on saquinavir blood sampling days. Volunteers were also instructed to refrain from ingesting grapefruit or grapefruit juice during the study period. Females of child-bearing potential were required to use a nonhormonal method of contraception throughout the study. Informed consent was obtained from all participants and clinical research was conducted in accordance with guidelines for human experimentation as specified by the United States Department of Health and Human Services. The study was approved by the National Institute of Allergy and Infectious Diseases Investigational Review Board.

Study design

This study was a single-centre, open-label investigation to evaluate potential differences in steady-state saquinavir pharmacokinetics between males and females in an HIV-seronegative cohort.

After signing consent, subjects who met inclusion criteria had blood drawn for determination of MDR-1 genotypes at positions 3435 and 2677, which are in linkage disequilibrium with each other, for identification of polymorphisms previously shown to alter P-gp expression [17]. In addition, subjects received a single oral dose of midazolam followed by a single blood draw 4 h later to determine their serum midazolam concentration – a previously validated method of characterizing CYP3A phenotype *in vivo* [18].

Treatment and blood sampling

Enrolled subjects were administered a single oral dose of 8 mg (4 ml) midazolam syrup (Roche Laboratories, Nutley, NJ, USA) on an empty stomach (1 h before or 2 h after a meal). Four hours after midazolam ingestion a 7-ml blood sample was collected for determination of midazolam serum concentration. After collection, all samples were immediately centrifuged,

separated and frozen at -80°C until chromatographic analysis.

Three to 8 weeks after midazolam administration subjects began taking saquinavir soft-gel capsule (sgc) (Fortovase®; F. Hoffman-La Roche Ltd, Basel, Switzerland) 1200 mg ($6 \times 200\text{-mg}$ capsules), orally three times daily for 10 doses. Subjects were instructed to take their saquinavir doses with a full meal. Medication adherence was evaluated by pill counts and saquinavir plasma concentrations. Saquinavir pharmacokinetic analyses were conducted on day 4 of drug administration, following each subject's 10th and final dose. Blood samples were collected into heparinized tubes at times 0 (predose), 0.5, 1, 2, 3, 4, 5, 6 and 8 h postdose. Samples were centrifuged and the plasma separated and stored at -80°C until the time of analysis.

Drug analysis

Midazolam concentrations were determined with a validated high-performance liquid chromatography (HPLC) method that is a modification of that reported by Sautou *et al.* [19]. Percent errors, as a measure of accuracy, were $<10\%$. The inter- and intra-assay coefficients of variation were 3.25–8.48% and 2.54–6.92%, respectively, at three different drug concentrations. The limit of quantification was 2.0 ng ml^{-1} and the limit of detection was 1.0 ng ml^{-1} . Plasma saquinavir concentrations were also measured using a previously validated HPLC method [20]. Percent errors were $<10\%$ and inter- and intra-assay coefficients of variation for this assay were 1.35–5.05% and 1.72–4.2%, respectively, at three different concentrations. The lower limit of quantification was 20 ng ml^{-1} .

MDR-1 genotyping

MDR-1 genotype at positions 3435 and 2677 in exons 26 and 21, respectively, were determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), as previously described [21]. After venous blood samples were obtained from all subjects, DNA was isolated from peripheral leucocytes using the Qiamp system (Qiagen Inc., Valencia, CA, USA). Based on PCR-RFLP analysis, subjects were genotypically classified based on polymorphisms at C3435T (CC, CT or TT) and G2677T (GG, GT/A or TT).

Pharmacokinetic analysis

Saquinavir concentrations were analysed by noncompartmental methods using WinNonlin pharmacokinetic software (version 3.2, Pharsight Corp., Mountain View, CA, USA). The maximum plasma concentration (C_{max}) and time to reach C_{max} (T_{max}) were obtained by direct

inspection of the plasma concentration-time profiles. The elimination rate constant (λ_z) was determined by calculating the absolute value of the slope of the log-linear regression using at least three points on the plasma concentration-time plot. The plasma elimination half-life ($t_{1/2}$) was calculated from $(\ln 2)/\lambda_z$. The mean area under the plasma concentration vs. time curve (AUC) from 0 to 8 h (AUC_{0-8}) was determined by using the log-linear trapezoidal rule, and the apparent oral clearance (CL/F) was calculated by dividing the oral dose by AUC_{0-8} . The apparent volume of distribution was calculated as follows: $V_d/F = \text{oral dose}/(\lambda_z \times \text{AUC}_{0-8})$.

Statistical analyses

The target sample number was determined using a standard equation for continuous variables in which an α value of 0.05 was selected *a priori* and $\beta = 0.2$ and $\sigma = 0.88$ [8, 22]. Using these criteria, a total of 28 subjects (two groups of 14) were required to detect a 100% difference in saquinavir AUC between male and female study groups.

All statistical analyses of the data were performed using Statistica (2001, StatSoft Inc., Tulsa, OK, USA) and descriptive data were generated using Microsoft Excel 2002 (Microsoft Corp.). Results are reported as geometric means with 95% confidence intervals (CI). Saquinavir pharmacokinetic parameter values and midazolam concentrations were compared between males and females using an unpaired, two-tailed Student's *t*-test, except for T_{max} , which was analysed using Kruskal-Wallis test. Saquinavir pharmacokinetic parameter values were compared between MDR-1 genotypic groups using ANOVA with *post hoc* testing. Spearman rank correlation was used to test the correlation between midazolam exposure and saquinavir clearance. *P*-values <0.05 were considered statistically significant for all analyses.

Results

A total of 38 subjects (14 women) enrolled in and completed the study. The median age was 31.5 years (range 22–50) and 33.5 years (range 22–43) for males and females, respectively. Races were distributed as follows: males 58% caucasian, 21% Asian, 13% Hispanic and 8% African-American; and females 72% caucasian, 7% Hispanic and 21% African-American. There were no missed saquinavir doses reported by any of the volunteers. Adherence was further confirmed by pill counts and saquinavir plasma concentrations, which were comparable to steady-state values reported in other healthy volunteer studies [23–25].

Figure 1

Distribution of MDR-1 genotypes at positions 3435/2677. Males (□), females (■)

In most subjects MDR-1 genotype at position 3435 coincided with that at position 2677, with CC/GG, CT/GT and TT/TT alleles coexpressed. There was no apparent difference in the distribution of MDR-1 genotypes between the genders, as shown in Figure 1. There was no significant difference in saquinavir CL/F, $T_{1/2}$ or C_{min} among the different MDR-1 genotypic groups. However, saquinavir C_{max} was significantly higher among TT vs. GG subjects at position 2677, with a median of 1739 ng ml⁻¹ (range 317–2994) vs. 695 ng ml⁻¹ (range 275–1787) ($P = 0.05$). Although not statistically significant, saquinavir AUC values for 0–2, 0–4 and 0–8 h were greater for the following genotypes at positions 3435 and 2677, respectively: TT > CT > CC and TT > GT > GG [26].

Midazolam concentrations were available for 37 subjects (one female sample was lost in transport). There was no significant difference in 4-h postdose midazolam concentrations between males and females: 10.1 ng ml⁻¹ (6.79–13.5) vs. 11.0 ng ml⁻¹ (4.34–17.7) ($P = 0.57$). There was no correlation between the 4-h midazolam concentrations and saquinavir CL/F ($R^2 = 0.011$; $P > 0.05$), as previously presented [26].

Interpatient variability in saquinavir exposure was not significantly different between males and females after correcting for weight; coefficients of variation (CV) for weight-normalized AUC_{0–8} and CL/F values were 60 and 71% for females and 86 and 76% for males, respectively ($P > 0.05$, F -test). Analysis of the data further revealed that there were no single pharmacokinetic outliers that required exclusion. The largest deviating $t_{1/2}$ and AUC_{0–8} values were <3 SD from the mean. Mean steady-state saquinavir concentration vs. time profiles are shown in Figure 2. There was no statistically significant

Figure 2

Mean saquinavir concentrations ($\pm$ 95% CI) for male (—■—) and female (---▲---) subjects

difference in saquinavir AUC_{0–8} or any other pharmacokinetic parameter value between the sexes (Table 1). There was a statistically significant difference in weight, with males and females weighing a mean of 81 kg ($\pm$ 17.8) and 68 kg ($\pm$ 11.9), respectively ($P < 0.005$). The ratio of male:female AUC_{0–8} values was 1.00 after adjusting for weight.

Overall, saquinavir was well tolerated by the study participants and none of the volunteers discontinued study participation due to adverse effects. Side-effects reported by subjects were primarily grade I headache ($n = 4$) or grade I gastrointestinal complaints, including abdominal pain ($n = 5$), indigestion ($n = 5$), bloating/gas ($n = 3$), nausea ($n = 3$), vomiting ($n = 1$) and diarrhoea ($n = 1$). Other side-effects reported by two volunteers include flushing, fatigue, oedema, blurred vision, sore throat, muscle aches, feeling off-balance and difficulty achieving orgasm (all grade I). Laboratory abnormalities included minor elevations (grade I) in aspartate aminotransferase (AST) and alanine aminotransferase (ALT), which subsequently normalized in all subjects within 1–2 weeks of study cessation. Eleven subjects experienced AST elevations and six had elevations in ALT (four subjects had elevations of both enzymes). None of the increases in AST or ALT exceeded $2.2 \times$ the upper limit of normal (ULN) and most were not more than $1.2 \times$ ULN. All subjects remained asymptomatic and no therapeutic interventions were required for these liver enzyme elevations.

Discussion

In contrast to previous reports, results from this investigation showed no difference in saquinavir pharmacokinetics between males and females [13–16]. Although

Table 1

Pharmacokinetic parameter values of saquinavir

Parameter	Males ^a (n = 24)	Females ^a (n = 14)	Ratio of means ^b
Weight (kg)	81.0 (73.9, 88.1)	67.1 (60.9, 73.3)	1.21 ^c
4-h midazolam (ng ml ⁻¹)	10.1 (6.79, 13.5)	11.0 (4.34, 17.7)	0.918
AUC ₀₋₈ (ng h ⁻¹ ml ⁻¹)	2418.7 (1040.1, 3797.4)	1997.6 (1327.2, 2667.9)	1.21
AUC ₀₋₈ (normalized) (ng h ⁻¹ ml ⁻¹ kg ⁻¹)	29.9 (15.5, 44.3)	29.8 (18.6, 40.9)	1.00
C _{max} (ng ml ⁻¹)	736.3 (421.2, 1051)	721.1 (402.5, 1040)	1.02
C _{min} (ng ml ⁻¹)	70.6 (33.3, 108)	52.7 (35.3, 70.1)	1.34 ^d
T _{1/2} (h)	1.97 (1.59, 2.36)	1.89 (1.46, 2.33)	1.04
Cl/F (l h ⁻¹ kg ⁻¹)	6.12 (3.54, 8.71)	8.93 (4.91, 12.9)	0.685
V _d /F (l kg ⁻¹)	17.4 (2.58, 32.3)	24.4 (6.00, 42.8)	0.713

^aData are geometric means (95% confidence intervals). ^bGeometric mean ratio (GMR) (males/females). ^cP < 0.005. ^dP = 0.053.

not statistically significant, saquinavir exposure was slightly greater among males vs. females prior to adjusting for weight [geometric mean ratio (GMR) 1.21]. However, a significantly greater weight among males compensated for this difference, resulting in a weight-normalized AUC₀₋₈ GMR of 1.00. Likely reasons for the discrepancy between our findings and those of previous studies include differences in our study population (HIV-negative vs. HIV-infected subjects) and saquinavir treatment (unboosted vs. ritonavir boosted) (see Table 2).

Fletcher *et al.* characterized the influence of sex on saquinavir pharmacokinetics in HIV-positive patients taking saquinavir in combination with either ritonavir or nelfinavir [14]. The authors reported a 1.3-fold increase in saquinavir AUC in females vs. males ($P \leq 0.004$). Moreover, a significantly greater proportion of females had a viral load = 500 copies ml⁻¹ at week 16 compared with males. Another investigation evaluated saquinavir concentrations in a group of treatment-experienced patients on a multiple-PI regimen containing saquinavir, lopinavir and ritonavir [15]. Similar to findings by Fletcher *et al.*, saquinavir AUC, C_{max} and C_{min} were significantly higher in females than in males, with the average AUC 88% greater in females. Of note, ritonavir exposure was significantly elevated in females also. However, there was no significant difference in lopinavir pharmacokinetics between the sexes. A third study evaluated gender differences in 12 HIV-infected individuals treated with saquinavir plus low-dose ritonavir [13]. The mean saquinavir AUC in this cohort was 74% higher in females compared with males. In addition, ritonavir clearance was significantly reduced in women, resulting in a 144% increase in ritonavir AUC.

In contrast to results from these studies, no sex-related differences in saquinavir pharmacokinetics were observed when healthy volunteers were administered a single 1200-mg dose of saquinavir sgc [8]. A second healthy volunteer investigation evaluated saquinavir pharmacokinetics in subjects receiving the drug in combination with ritonavir or atazanavir [27]. In this study men were found to have significantly lower C_{max} and AUC values compared with women for all three protease inhibitors. Saquinavir AUC values were 2–2.3-fold greater in females, while atazanavir and ritonavir AUCs were 2.1–2.4-fold and 2.8-fold greater, respectively, in females. Proposed hypotheses for the disparity in saquinavir exposure between males and females include sex-based differences in the expression and/or activity of CYP3A and/or P-gp. In addition, greater ritonavir exposure in females, as shown in previous studies, may also contribute to observed differences in saquinavir pharmacokinetics between males and females when the drug is given in combination with ritonavir [13, 15, 27]. Ritonavir is a substrate of both CYP3A4 and P-gp; therefore, like saquinavir, observed variability in its exposure may be explained by sex-based differences in CYP3A and/or P-gp.

Several drugs that are CYP3A4 substrates have been found to have higher clearances in women compared with men following intravenous administration, including ciclosporin, verapamil and tacrolimus [5]. The majority of studies using oral or intravenous midazolam as a CYP3A probe found negligible differences in catalytic activity between males and females [1, 6]. Similarly, the oral midazolam phenotyping results from our study did not show a difference in CYP3A metabolizing capacity between the sexes. In contrast, studies using

Table 2

Studies evaluating saquinavir sex-based differences

Reference	Study population	M/F (n)	SQV regimen ^a	Differences in SQV PK
[13]	HIV+	5/5	SQV 1600 mg + RTV 100 mg QD × 14 days	Higher median AUC (1.7-fold) in females ($P = 0.036$)
[14]	HIV+	157/29	SQV ^b + RTV + DLV ($n = 28$) SQV + RTV + ADV ($n = 35$) SQV + RTV + DLV + ADV ($n = 32$) SQV ^c + NFV + DLV ($n = 30$) SQV + NFV + ADV ($n = 32$) SQV + NFV + DLV + ADV ($n = 29$)	Higher median AUC (1.3-fold) ($P = 0.004$) and $C_{\min}$ (2.3-fold) ($P = 0.0001$) in females 53% lower CL/F in females after adjusting for weight
[15]	HIV+	18/7	SQV 1000 mg + RTV 100 mg + LPV 400 mg BID for ≥ 30 days	Higher median AUC (1.9-fold), $C_{\max}$ (1.8-fold), and $C_{\min}$ (1.5-fold) in females (all $P < 0.05$). (Also, RTV AUC 2.1-fold higher in females)
[16]	HIV+	28/6	SQV 1000 mg + RTV 100 mg BID (Retrospective analysis of four PK studies)	Higher median AUC (1.8-fold), $C_{\min}$ (2.9-fold), and $C_{\max}$ (1.9-fold) in females (all $P = 0.05$). (Also, RTV AUC twofold higher in females)
[8]	HIV-	18/12	Single SQV 1200 mg dose	No difference
[27]	HIV-	6/7	Three-way cross-over: SQV 1600 mg + RTV 100 mg QD; SQV 1600 mg + ATV 400 mg QD; SQV 2000 mg + ATV 400 mg QD (each × 11 days)	Higher median AUC (2.3-2.7-fold) and $C_{\max}$ (2.0-2.6-fold) in females. (Also, ATV and RTV AUC and $C_{\max}$ values greater in females) (all $P < 0.05$)
This study	HIV-	24/14	SQV 1200 mg TID × 10 doses	No difference

^aSaquinavir (SQV), ritonavir (RTV), delavirdine (DLV), adefovir (ADV), nelfinavir (NFV), lopinavir (LPV), daily (QD), twice daily (BID), three times daily (TID). ^bSaquinavir dosing with ritonavir: SQV 400 mg + RTV 400 mg BID. ^cSaquinavir dosing with nelfinavir: SQV 800 mg + NFV 750 mg BID.

intravenous verapamil have shown higher drug clearance in females compared with males, while studies with oral verapamil have reported either no sex-related differences or greater oral clearances among males vs. females [28, 29]. Higher systemic clearances of intravenous verapamil and other CYP3A4 substrates in women are consistent with results from a liver biopsy study which demonstrated twofold higher CYP3A4 levels in female livers vs. male livers [30]. Other biopsy studies, however, were unable to detect any significant sex-related differences in CYP3A4 expression or function in the liver or the intestine [31–34].

Inconsistent sex-related differences among CYP3A4 substrates, such as found with midazolam and verapamil, may be explained by P-gp – verapamil is a substrate for both CYP3A4 and P-gp, while midazolam does not undergo P-gp-mediated transport. The most extensively studied MDR-1 protein, P-gp is an ATP-

dependent efflux pump that back-transport a heterogeneous group of substrates across biological membranes [35]. It has been proposed that observed sex-related differences in CYP3A4 substrate exposure may be due to sex-based differences in P-gp activity, as opposed to CYP3A metabolism, since the two proteins have very similar substrate specificity [5].

Schuetz *et al.* reported that men have approximately twofold greater P-gp content in their livers compared with women [36]. Wolbold and colleagues, however, were not able to confirm this finding after examining both P-gp levels and MDR-1 mRNA levels from human liver samples [30]. In the liver, P-gp is located on the canalicular (basolateral) membrane of hepatocytes. Drugs that are substrates of P-gp and CYP3A4 enter the liver by crossing the sinusoidal membrane of the hepatocyte, whereupon they undergo intracellular distribution and metabolism by CYP3A4 before being

actively excreted into the bile by P-gp [5]. Therefore, if indeed there is a sex-related difference in P-gp content in the liver, it may not appreciably influence plasma concentrations of drugs that are significantly metabolized by P450 enzymes, such as saquinavir. Heterogeneity in P-gp content at the site of the intestine, however, may possibly contribute to differences in substrate metabolism between the sexes; P-gp-mediated efflux in the intestinal tract increases drug contact with luminal CYP3A4, thereby limiting the systemic exposure of certain orally administered substrates [37, 38]. However, the only study to report intestinal P-gp content in humans found no significant difference in P-gp levels from male and female duodenal samples [34].

Genetic polymorphisms in MDR-1 at position 3435 in exon 26 have been shown to contribute to pharmacokinetic variability among P-gp substrates such as digoxin [39, 40]. Individuals with C/C, C/T and T/T genotypes at position 3435 express high, intermediate and low levels of P-gp, respectively [17]. In our cohort, the relationship between MDR-1 genotype and saquinavir exposure did not reach significance, except for C_{max} . Although this study was not designed to evaluate such a correlation, the trend suggests a larger study population may show a relationship between P-gp expression and saquinavir exposure. Relative P-gp expression may influence an individual's predisposition to drug interactions that result from modulation of P-gp. Studies evaluating the bioavailability of the P-gp substrate digoxin suggest that increases in digoxin exposure with concomitant P-gp inhibitors, such as clarithromycin and ritonavir, are greater in subjects with the 3435-CC genotype vs. TT genotype [41, 42]. A likely explanation for these findings is that CC individuals, who have higher intestinal P-gp content than TT individuals, have a comparatively greater increase in digoxin bioavailability when administered a P-gp inhibitor. This hypothesis conflicts with our study findings, however. If males do indeed have greater P-gp content than females (analogous to the difference in P-gp content between CC and TT individuals), then there should be a greater increase in saquinavir bioavailability in males vs. females when ritonavir is coadministered. In other words, we would expect to see a difference in saquinavir exposure between males and females without concomitant ritonavir, and a tempering of that difference with the addition of ritonavir. This inference, however, is only correct if CYP3A expression/function is not significantly different between males and females – a matter that is currently unclear. To complicate matters further, *in vitro* studies have shown that protease

inhibitors, including ritonavir, can actually induce, as opposed to inhibit, P-gp function [43, 44]. Thus, it is not entirely clear what effect concomitantly administered protease inhibitors have on P-gp-mediated efflux or how these effects may contribute to sex-related differences in saquinavir pharmacokinetics.

Differences in saquinavir exposure between HIV-infected and non-infected individuals is well documented, with a greater than twofold AUC observed in HIV-infected patients [8]. Potential explanations for this observation include downregulation or inhibition of P-gp and/or CYP3A4 in HIV-infected individuals. A number of reports have indicated that CYP-dependent drug metabolism is compromised during certain bacterial and viral infections [45]. Some studies have suggested lower CYP450 activity and greater variability in metabolism among HIV-infected subjects vs. that of healthy volunteers, while other studies have not been able to confirm these findings [46–48]. Similarly, studies describing P-gp function in HIV-infected patients have shown conflicting results. Some findings suggest that cytokines released as a part of HIV's pathogenesis downregulate P-gp activity, while other study results suggest that the opposite occurs [49–52]. However, as discussed above, sex-related differences in saquinavir exposure have been observed in both HIV-positive and HIV-negative volunteers administered saquinavir with a concomitant protease inhibitor. This suggests that the discrepancy between our results and those of previous studies is more likely due to the presence or absence of a concomitant PI, as opposed to HIV-negative serostatus.

Interestingly, sex-based differences in indinavir pharmacokinetics have not been observed when studied in both HIV-infected and non-infected populations [53, 54]. Although indinavir is a substrate of both P-gp and CYP3A4, unlike saquinavir, indinavir concentrations in the intestinal lumen far exceed the K_m for P-gp transport [55, 56]. When given at its typical dose, indinavir saturates intestinal P-gp and passive diffusion of drug through enterocytes exceeds P-gp-mediated efflux; as such, P-gp does not appear to play a role in limiting indinavir absorption. This difference in P-gp influence on indinavir and saquinavir exposure further supports a role for P-gp, as opposed to CYP3A, in explaining sex-related pharmacokinetic disparities between these two drugs.

We hypothesize that the addition of ritonavir unmasks a difference in P-gp function between the sexes, resulting in an observable difference in saquinavir exposure between males and females, as documented by previous studies. Results from our investigation suggest that differences in P-gp and CYP3A content or activity between

the sexes may not be as clear-cut as previously suggested. Confounding variables that may influence P-gp and/or CYP3A4 activity include concomitant exposure to CYP3A4 and P-gp-modulating drugs, HIV or other disease states, and genetic variation in CYP3A4/5, MDR-1 and other transport proteins. In summary, clinicians must be cautious when extrapolating sex-based pharmacokinetic findings from healthy volunteer studies to HIV-infected populations or to patients receiving concurrent medications.

Competing interests: None declared.

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