Pharmacokinetic interactions between protease inhibitors and statins in HIV seronegative volunteers: ACTG Study A5047

Carl J. Fichtenbaum\textsuperscript{a,b}, John G. Gerber\textsuperscript{c}, Susan L. Rosenkranz\textsuperscript{d}, Yoninah Segal\textsuperscript{d}, Judith A. Aberg\textsuperscript{e}, Terrence Blaschke\textsuperscript{f}, Beverly Alston\textsuperscript{g}, Fang Fang\textsuperscript{h}, Bradley Kosel\textsuperscript{e}, Francesca Aweeka\textsuperscript{e} and the NIAID AIDS Clinical Trials Group

Objective: Lipid lowering therapy is used increasingly in persons with HIV infection in the absence of safety data or information on drug interactions with antiretroviral agents. The primary objectives of this study were to examine the effects of ritonavir (RTV) plus saquinavir soft-gel (SQVsgc) capsules on the pharmacokinetics of pravastatin, simvastatin, and atorvastatin, and the effect of pravastatin on the pharmacokinetics of nelfinavir (NFV) in order to determine clinically important drug–drug interactions.

Design: Randomized, open-label study in healthy, HIV seronegative adults at AIDS Clinical Trials Units across the USA.

Methods: Three groups of subjects (arms 1, 2, and 3) received pravastatin, simvastatin or atorvastatin (40 mg daily each) from days 1–4 and 15–18. In these groups, RTV 400 mg and SQVsgc 400 mg twice daily were given from days 4–18. A fourth group (arm 4) received NFV 1250 mg twice daily from days 1–14 with pravastatin 40 mg daily added from days 15–18. Statin and NFV levels were measured by liquid chromatography/tandem mass spectrometry.

Results: Fifty-six subjects completed both pharmacokinetic study days. In arms 1–3, the median estimated area under the curves (AUC\textsubscript{0–24}) for the statins were: pravastatin (arm 1, \( n = 13 \)), 151 and 75 ng·h/ml on days 4 and 18 (decline of 50% in presence of RTV/SQVsgc), respectively (\( P = 0.005 \)); simvastatin (arm 2, \( n = 14 \)), 17 and 548 ng·h/ml on days 4 and 18 (increase of 3059% in the presence of RTV/SQVsgc), respectively (\( P , 0.001 \)); and total active atorvastatin (arm 3, \( n = 14 \)), 167 and 289 ng·h/ml on days 4 and 18 (increase of 79% in the presence of RTV/SQVsgc), respectively (\( P , 0.001 \)). In arm 4, the median estimated AUC\textsubscript{0–8} for NFV (24 319 versus 26 760 ng·h/ml; \( P = 0.58 \)) and its active M6 metabolite (15 565 versus 14 571 ng·h/m; \( P = 0.63 \)) were not statistically different from day 14 to day 18 (without or with pravastatin).

Conclusions: Simvastatin should be avoided and atorvastatin may be used with caution in persons taking RTV and SQVsgc. Dose adjustment of pravastatin may be...
necessary with concomitant use of RTV and SQVsgc. Pravastatin does not alter the NFV pharmacokinetics, and thus appears to be safe for concomitant use.

AIDS 2002, 16:569–577

Keywords: HIV infection, HMG-CoA reductase inhibitors, statins, protease inhibitors, drug interactions

Introduction

A number of metabolic complications including hyperlipidemia, insulin resistance, and fat redistribution disorders have emerged with the use of potent antiretroviral therapy for HIV infection [1–5]. Individual protease inhibitors may cause metabolic disturbances by different mechanisms. For example, in healthy HIV-seronegative persons, ritonavir (RTV) induces hypertriglyceridemia and hypercholesterolemia [6]. In animal models, RTV increases free fatty acid synthesis [7]. Conversely, indinavir induces insulin resistance in HIV-seronegative persons [8]. Population based studies suggest there is reason to be concerned that these metabolic abnormalities may lead to the development of premature coronary artery disease [1–5, 9]. Reports of premature myocardial infarctions and hyperlipidemia have been noted in HIV-infected persons on protease inhibitor-based potent antiretroviral therapy [4–5, 9]. Thus, there is a need to provide safe and effective treatment for hyperlipidemia in persons with HIV infection.

Several classes of medications are used to treat hyperlipidemia including the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins). Several statins decrease the risk of myocardial infarction in persons with hyperlipidemia [10–13]. The use of these drugs has increased in persons with HIV infection [14–17]. Cytochrome P4503A4/5 isozymes located within the liver and the gastrointestinal tract are responsible for the metabolism of most statins [10, 18, 19]. These isozymes are also responsible for the metabolism of protease inhibitors (PI) and non-nucleoside reverse transcriptase inhibitors. In addition, all of the clinically available PI are inhibitors of the cytochrome P450A isoforms. Thus, the possibility exists for significant drug–drug interactions when statins and PI are used concomitantly.

Elevated levels of statins have been reported with the use of other drugs metabolized by the cytochrome P450 3A4 isozyme (CYP3A4) [20–25]. Rhabdomyolysis has been associated with higher levels of statins typically when used in combination with other drugs that inhibit CYP3A4 [26–32]. Therefore, we investigated the pharmacokinetic interactions between three commonly prescribed statins and the PI RTV, saquinavir (SQV) and nelfinavir (NFV) to determine if there are clinically important drug–drug interactions.

Methods

AIDS Clinical Trials Group (ACTG) study A5047 was a randomized, phase I, open-label, multiple dose, pharmacokinetics drug interaction study. The primary objectives were to examine the effects of RTV plus SQV soft-gel capsules (sgc) on the pharmacokinetics of pravastatin, simvastatin, and atorvastatin, and to investigate the effect of pravastatin on the pharmacokinetics of NFV. At the time this study was designed there was no published information about whether statins would alter PI concentrations, an important clinical question. NFV was the most commonly prescribed PI in the USA. Based upon the known metabolism of pravastatin, we hypothesized that pravastatin would not alter the pharmacokinetics of NFV. A secondary objective of the study was to examine the effects of lipid-lowering agents on the pharmacokinetics of RTV and SQVsgc as compared to historic controls. This study was designed and conducted prior to the widespread use of low dose RTV to pharmacologically enhance antiretroviral treatment regimens.

Subjects

Study subjects were HIV-seronegative adult volunteers. HIV-seronegative volunteers were selected to avoid unnecessary exposure to PI in suboptimal regimens and to avoid the use of concomitant drugs known to affect CYP3A4. The planned sample size was 56 subjects, 14 per treatment arm. Subjects who did not complete both pharmacokinetic evaluations were replaced. Accrual began in July 1999 and ended in October 1999.

Study design

The study consisted of four treatment arms. Subjects in arm 1 began taking pravastatin 40 mg every morning on study day 1. These subjects underwent intensive pharmacokinetic sampling on day 4 (after steady-state was achieved) with blood samples taken just prior to the statin dose and 0.5, 1, 2, 3, 4, 6, 8, 12 and 24 h. At the end of the 24 h sampling, subjects discontinued pravastatin and began taking RTV 300 mg twice daily and SQVsgc 400 mg twice daily. The RTV dose was
increased to 400 mg on day 8. Pravastatin was added back to the RTV/SQVsc regimen on day 15. Subjects again underwent intensive pharmacokinetic sampling on day 18 (after steady-state was achieved) using the same schedule as was used on day 4. Subjects took RTV and SQVsc 30 min after taking the pravastatin. Arms 2 and 3 were identical to arm 1 except that simvastatin 40 mg/day and atorvastatin 40 mg/day, respectively, were the lipid-lowering agents.

Subjects in arm 4 began taking NFV 1250 mg every 12 h on day 1 and underwent intensive pharmacokinetic sampling on day 14 (steady-state for NFV). Blood samples were collected prior to the NFV dose and 0.5, 1, 2, 3, 4, 6 and 8 h. On day 15, pravastatin 40 mg/day was added to the NFV regimen. Subjects underwent intensive pharmacokinetic sampling on day 18 using the same schedule as was used on day 14. The NFV was dosed 30 min after dosing of the pravastatin.

Drug assays

Statin assays were performed at Advanced Bioanalytical Services, Inc. (Ithaca, New York, USA). Arm 1 samples were assayed to quantify concentrations of pravastatin and a minor active metabolite SQ-31906. In the assay procedure, pravastatin, d5-pravastatin, SQ-31906, d5-SQ-31906, and pravastatin lactone along with their respective internal standards, d3-pravastatin and d3-pravastatin lactone, were extracted from human serum samples using a solid-phase extraction procedure. The solid-phase extraction eluent was evaporated to dryness and reconstituted. Aliquots were analyzed by turbo ion spray LC/MS/MS in the positive ion mode. The LOQ for this assay was 0.5 ng/ml with a coefficient of variation of 10% for all analytes. Inter- and intra-assay precision and accuracy was within 8% (deviation from nominal) [33].

Arm 2 samples were assayed for simvastatin and simvastatin acid using an internally validated LC/MS/MS technique. Lovastatin was used as the internal standard for simvastatin and lovastatin acid was used as the internal standard for simvastatin acid. Serum samples were acidified and extracted by a solid-phase extraction procedure to isolate simvastatin, simvastatin acid and their respective internal standards. Sample extracts were reconstituted and separated by reversed-phase chromatography on a 2 × 50 mm BDS Hypersil C18 column (Keystone Scientific, Inc., Bellefonte, Pennsylvania, USA) with an initial mobile phase of 40% Eluent A (acetonitrile) and 60% Eluent B (4 mM ammonium acetate, pH 4.5). Aliquots were analyzed by turbo ion spray LC/MS/MS in the positive ion mode. The LOQ for this assay was 0.5 ng/ml of simvastatin and simvastatin acid with a coefficient of variation of 5% for simvastatin and simvastatin acid. The inter- and intra-assay precision was 8% for both analytes and the accuracy was within 8% (deviation from nominal).

Arm 3 samples were assayed for atorvastatin and the following metabolites: atorvastatin lactone, 2-hydroxy atorvastatin, 2-hydroxy atorvastatin lactone, 4-hydroxy atorvastatin and 4-hydroxy atorvastatin lactone. These compounds and their respective internal standards (d5-atorvastatin, d5-atorvastatin lactone, d5-2-hydroxy atorvastatin, d5-2-hydroxy atorvastatin lactone, d5-4-hydroxy atorvastatin, and d5-4-hydroxy atorvastatin lactone) were extracted from serum samples using a liquid–liquid extraction procedure. The supernatant was evaporated to dryness and reconstituted. Aliquots were analyzed by turbo ion spray LC/MS/MS in the positive ion mode. The LOQ for this assay was 0.5 ng/ml with a coefficient of variation of 10% for all analytes. The inter- and intra-assay precision and accuracy was ≤ 10% for all analytes [34].

NFV assays were performed at the ACTG Pharmacology Unit at San Francisco General Hospital. NFV and M8 metabolite isolated from plasma were measured by a new validated LC/MS/MS method. A simple acetonitrile precipitation of plasma proteins and centrifugation of samples after addition of internal standard (methyl-indinavir) was performed. Aliquots of the supernatant were analyzed by the LC/MS/MS. The standard curve of the assay is from 5 ng/ml to 4000 ng/ml for both NFV and M8 metabolite with an LOQ of 5 ng/ml for both analytes. The precision of the inter- and intra-assay was ≤ 9% for both analytes. The ACTG Pharmacology Quality Control Program approved the use of these assays for NFV and M8 metabolite after demonstration of appropriate validation.

The concentrations of RTV and SQV were determined by high-pressure liquid chromatography (HPLC) with ultraviolet (UV) detection at the Stanford ACTG Pharmacology Unit. After solid-phase extraction, a SUPERCOSIL LC-DP column with a phosphate mobile phase was used to chromatograph the samples. RTV, SQV and internal standards were detected by UV absorbance at 205 and 240 nm. The linear dynamic range of the assays was 200 to 24 000 ng for RTV, and 84 to 12 000 ng for SQV. The LOQ was 200 ng/ml and 8 ng/ml for RTV and SQV respectively. The inter- and intra-assay variation was within 15% for both analytes. The ACTG Pharmacology Quality Control Program approved the use of these HPLC assays for RTV and SQV after demonstration of appropriate validation.

Calculation of area under the curve (AUC)

Systemic exposure to the statins was quantified by calculating the AUC of the drugs from pre-dose to the
end of the dosing interval. Concentrations below the LOQ were assigned a value of zero. Steady-state AUC over a dosing interval were estimated according to the linear trapezoidal rule using the non-compartmental analysis component of WinNonlin (version 3.1, Pharsight Corporation, Cary, North Carolina, USA). Actual, rather than scheduled, sample times were used. Extrapolation beyond the dosing interval was not carried out because data was analyzed at steady state. For subjects in arm 2, AUC were calculated for simvastatin acid (the main active metabolite of simvastatin). For subjects in arm 3, AUC were calculated for atorvastatin and also for the ‘total active atorvastatin’ activity. The latter was calculated using the sum of the time-specific concentrations of atorvastatin, 2- and 4-hydroxy atorvastatin at each time point, and calculating an AUC based on the summed concentrations.

Systemic exposure to NFV was quantified by calculating the AUC from time 0 to 8 h. AUC calculations for NFV and its M8 metabolite proceeded as described for the statins. Twelve-hour AUC for RTV and SQV were calculated using the same method described for the statins. Nominal 12 h samples actually occurred between 10.8 and 12.0 h post-dose. No adjustment was made to normalize AUC to 12 h. Subjects with fewer than seven quantifiable concentrations or those with missing nominal 12 h samples were omitted from analysis.

Historic controls
The analysis of RTV and SQV levels was based upon data from historic controls kindly provided to us by K. Jorga (Roche Pharmaceuticals, Inc., Branchberg, New Jersey, USA). In this project, HIV-seronegative subjects were enrolled in a multiple-dose study of the tolerability and pharmacokinetics of SQVsgc and RTV when dosed separately and in combination. Twelve-hour AUC for RTV and SQV were calculated using the same method described for the statins. Nominal 12 h samples actually occurred between 10.8 and 12.0 h post-dose. No adjustment was made to normalize AUC to 12 h. Subjects with fewer than seven quantifiable concentrations or those with missing nominal 12 h samples were omitted from analysis.

Historic controls
The analysis of RTV and SQV levels was based upon data from historic controls kindly provided to us by K. Jorga (Roche Pharmaceuticals, Inc., Branchberg, New Jersey, USA). In this project, HIV-seronegative subjects were enrolled in a multiple-dose study of the tolerability and pharmacokinetics of SQVsgc and RTV when dosed separately and in combination. Twelve-hour AUC for RTV and SQV were calculated using the same method described for the statins. Nominal 12 h samples actually occurred between 10.8 and 12.0 h post-dose. No adjustment was made to normalize AUC to 12 h. Subjects with fewer than seven quantifiable concentrations or those with missing nominal 12 h samples were omitted from analysis.

Statistical analyses
The primary pharmacokinetic statin endpoints were: pravastatin AUC (arm 1), simvastatin acid AUC (arm 2), and atorvastatin and total active atorvastatin AUC (arm 3). The maximum concentration (C_{max}) for statins is reported. For each endpoint, day-specific medians and ranges are reported. Within-subject differences are calculated and expressed as both raw differences and as a percent of the first pharmacokinetic day value. Medians of these within-subject comparative measures are reported. The Wilcoxon signed rank test was used to test the hypothesis of no difference in the statin AUC before the initiation of the RTV/SQVsgc versus after dosing to steady state [35]. The primary pharmacokinetic endpoints for NFV are the AUC of NFV and of its active M8 metabolite. The minimum concentration (C_{min}) parameter was also analyzed statistically. Within-subject comparisons are based on differences between day 18 and day 14 AUC, and statistical inference based on the Wilcoxon signed rank test. RTV/SQV AUC in subjects from arms 1, 2, and 3 are compared to historical controls not receiving statins. For each agent (RTV and SQV), the Wilcoxon rank sum test was used to compare the AUC between treatments. Median values for C_{min} in arms 1, 2, and 3 are reported. All reported Wilcoxon P values are two-sided and were not adjusted for multiple comparisons.

The human studies committees of each participating institution and the regulatory branch of the Division of AIDS, National Institute of Allergy and Infectious Diseases approved this study and informed consent was obtained from the participants.

Results
Sixty-seven HIV-seronegative subjects were randomized. Eleven subjects failed to complete the second pharmacokinetic analysis because of protocol-defined toxicities. Discontinuations occurred while subjects were taking only the PI during study days 6–11. Diarrhea and gastrointestinal upset were the most common complaints. Subjects were discontinued from the pravastatin (n = 4), atorvastatin (n = 4), simvastatin (n = 2) and NFV (n = 1) arms and were considered ineligible for analysis of pharmacokinetic endpoints. One additional subject was excluded from the pharmacokinetic analysis of pravastatin after there were no measurable levels of this drug on study day 4. This subject was retained in the analysis of RTV and SQV AUC. Thus, a total of 56 subjects were used in one or more of the pharmacokinetic analyses. The median age was 32 years (range, 19–56 years). Women constituted 59% of the study population; 57% were white, 20% were Latino, 13% were African–American and 10% were Asian/Pacific Islanders.

Pharmacokinetic analyses for arms 1–3 are shown in Table 1 and Fig. 1. Pravastatin AUC declined (P = 0.005) while atorvastatin (P < 0.001), total active atorvastatin (P < 0.001) and simvastatin acid AUC (P < 0.001) increased with concomitant use of RTV and SQVsgc. The pharmacokinetic analyses for NFV and its M8 metabolite are shown in Table 2. The changes in NFV and its M8 metabolite AUC with the concomitant use of pravastatin were not significant.
Thirty-three subjects from arms 1, 2 and 3 had pharmacokinetic analyses completed for RTV and SQV (Table 3). There were no statistically significant changes in the levels of RTV and SQV compared to historic controls. There were no significant demographic differences in the subjects analyzed for RTV/SQV levels and the entire cohort (data not shown).

**Adverse events**

Grade 2 events were reported in 25% of the subjects. Clinical events were noted in 14% of the subjects and laboratory events were noted in 11% (Table 4). There were no cases of rhabdomyolysis or clinically significant hepatitis. One subject had shoulder and back pain not associated with elevation in creatine phosphokinase (CPK) levels. The relationship to statin use was unclear. One subject with an elevated CPK level was asymptomatic. Arm-specific sample sizes were too small and adverse events too infrequent to evaluate statistical differences between the groups.

**Discussion**

Elevated lipid levels occur in a significant percentage of patients taking potent antiretroviral therapy. Recent data suggests that specific PI may induce hyperlipidemia directly (increased free fatty acid synthesis) or indirectly (insulin resistance) [6–8]. Clinicians are increasingly prescribing statins to persons with hyperlipidemia and HIV infection. It is important to understand whether significant drug–drug interactions occur and might result in adverse reactions in persons taking statins and PI. Our study was designed to establish whether several statins in clinical use could be safely prescribed with RTV/SQV gc without concern for major drug–drug interactions. The choice of RTV/SQV gc was based on the following: the drug combination was commonly used in clinical practice; many statins utilize CYP3A4 for oxidative metabolism; and RTV is the most potent inhibitor of CYP3A4 of all the HIV PI [36]. HIV-seronegative subjects were chosen to avoid suboptimal exposure to PI in HIV-infected persons and to minimize the presence of other concomitant medications that might complicate the interpretation of drug–drug interactions. Although there may be differences in the absolute concentrations of some drugs (e.g., PI) between the HIV-infected and seronegative adults, there are no data to suggest that the nature of drug–drug interactions would be different.

This study demonstrated significant drug–drug interactions between some of the statins and RTV/SQV gc. Simvastatin acid concentrations increased 30-fold in persons taking RTV/SQV gc. Atorvastatin and its active metabolite concentrations were also increased though to a lesser degree than simvastatin acid. Conversely, pravastatin levels declined in subjects taking RTV/SQV gc. Pravastatin had no significant effect on the concentration of NFV or its M8 metabolite. Atorvastatin, pravastatin, and simvastatin did not significantly reduce the concentrations of RTV and SQV compared to historic controls in subjects who were not taking statins. Finally, there were few clinical adverse events associated with the short-term use of statins and NFV or RTV and SQV gc in this healthy HIV-seronegative population.

The differences in the known metabolism of the drugs investigated may explain the results of this study. The primary route of metabolism for most statins is via the CYP3A4 isozymes. Some statins are administered as inactive lactone pro-drugs and require hydrolysis by

---

**Table 1.** Pharmacokinetic parameters for pravastatin, atorvastatin, and simvastatin [median (range)].

<table>
<thead>
<tr>
<th></th>
<th>AUC day 4 (ng·h/ml)</th>
<th>AUC day 18 (ng·h/ml)</th>
<th>P</th>
<th>Cmax day 4 (ng/ml)</th>
<th>Cmax day 18 (ng/ml)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pravastatin (n = 13)</td>
<td>151 (79–227)</td>
<td>75 (25–205)</td>
<td>0.005</td>
<td>57 (35–150)</td>
<td>33 (9–174)</td>
<td>0.09</td>
</tr>
<tr>
<td>Atorvastatin (n = 14)</td>
<td>72 (34–197)</td>
<td>283 (174–1155)</td>
<td>&lt;0.001</td>
<td>13 (7–35)</td>
<td>56 (18–272)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total active atorvastatin (n = 14)</td>
<td>167 (69–338)</td>
<td>289 (178–1217)</td>
<td>&lt;0.001</td>
<td>23 (12–71)</td>
<td>57 (19–283)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Simvastatin acid (n = 14)</td>
<td>17 (5–59)</td>
<td>548 (134–2820)</td>
<td>&lt;0.001</td>
<td>3 (1–6)</td>
<td>93 (20–555)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

AUC, Area under the curve; Cmax, maximum concentration.

---

**Fig. 1.** Percent change in area under the curve of statin concentrations on pharmacokinetic days. Atorvastatin + metabolites is the same as the total active atorvastatin concentration (sum of the AUC of atorvastatin, 2-hydroxy- and 4-hydroxy-atorvastatin).
non-CYP esterases to the active hydroxy-acid (e.g., simvastatin $\rightarrow$ simvastatin acid) while others are administered directly as the active hydroxy-acid (e.g., atorvastatin, cerivastatin, and pravastatin) [20]. Lactone pro-drugs are metabolized by intestinal and liver CYP3A4 to active and inactive metabolites. When CYP3A4 is inhibited, more of the lactone pro-drug is available for non-CYP hydrolysis to the active hydroxy-acid. In addition, the role of gastrointestinal metabolism of the statins via CYP3A4 has been investigated and confirms that pravastatin clearance as compared to lovastatin (similar to simvastatin) is minimally affected by intestinal CYP3A4 [37]. Thus, the generation of simvastatin acid would always appear to be greater when CYP3A4 is inhibited than for drugs that are already in the hydroxy-acid form because of this shunting towards hydrolysis.

The more lipophilic lactone pro-drugs probably have a higher affinity for CYP3A4 than the hydroxy-acid forms. Known inhibitors of CYP3A4 have been shown to increase the concentration of selected statins [21–25]. One example is the pharmacokinetic interaction of itraconazole, an inhibitor of CYP3A4 that affects drug metabolism in both the gastrointestinal tract and the liver, with several statins [21–22]. Simvastatin acid and lovastatin acid concentrations are increased 20–30 fold with the addition of itraconazole, while itraconazole has only a relatively small effect on the pharmacokinetics of pravastatin [22]. For the former two statins, itraconazole seems to reduce the formation of active and inactive metabolites during first pass metabolism (requiring CYP3A4 activity in the gastrointestinal tract) resulting in increased bioavailability of the drugs. A similar mechanism probably occurs in the context of RTV coadministration that explains our present findings. Within the active acid group of statins, atorvastatin is more lipophilic than pravastatin, and pravastatin is not a substrate for CYP3A4 [20]. Multiple enzymes are involved in the metabolism of pravastatin but glucuronidation appears to be the predominant pathway [38]. As the primary pathway of elimination of pravastatin is by conjugation and RTV is a known inducer of glucuronidation, this may explain the decrease in pravastatin exposure [38].

This difference in metabolism explains, in part, the differences observed between atorvastatin and pravastatin concentrations when used concomitantly with RTV/SQVsgc. Finally, although most statins are metabolized by CYP3A4, they are not themselves significant inhibitors of CYP3A4 [20]. This could explain why RTV, SQV, and NFV concentrations were not altered significantly by the use of statins despite being substrates for CYP3A4.

There are several important clinical implications of our study. Simvastatin should not be used as a hypolipidemic agent in patients taking RTV/SQVsgc. Lovastatin, which is metabolized similarly to simvastatin, should also be avoided. These statins should probably also be avoided in patients using other PI that inhibit CYP3A4 activity. This recommendation is similar to what is suggested for azole antifungal drugs. Atorvastatin can probably be used with caution in patients taking RTV/SQVsgc. Although we have no clinical data, we suggest that atorvastatin should be initiated at doses of 10 mg/day and probably should not exceed 40 mg/day. Dose escalation should be based on clinical indication with careful monitoring for the development of toxicity. Pravastatin appears to be safe for use with RTV/SQVsgc. It is not clear whether the efficacy of pravastatin will be diminished when used concomitantly with RTV/SQVsgc. Higher doses of pravastatin may be necessary in the presence of certain PI. It would be

### Table 2. Pharmacokinetic parameters of nelﬁnavir and its M8 metabolite [median (range)].

<table>
<thead>
<tr>
<th></th>
<th>AUC day 14 (ng·h/ml)</th>
<th>AUC day 18 (ng·h/ml)</th>
<th>P</th>
<th>Cmin day 14 (ng/ml)</th>
<th>Cmin day 18 (ng/ml)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nelﬁnavir (n = 14)</td>
<td>24 319 (7899–64 905)</td>
<td>26 760 (7272–50 255)</td>
<td>0.58</td>
<td>1106 (212–5616)</td>
<td>1778 (410–2828)</td>
<td>0.76</td>
</tr>
<tr>
<td>M8 metabolite (n = 14)</td>
<td>15 565 (3441–40 561)</td>
<td>14 571 (2969–23 410)</td>
<td>0.63</td>
<td>499 (50–3787)</td>
<td>654 (201–1434)</td>
<td>0.76</td>
</tr>
</tbody>
</table>

AUC, Area under the curve; Cmin, minimum concentration.

### Table 3. Pharmacokinetic parameters of ritonavir and saquinavir after 14 days of administration [median (range)].

<table>
<thead>
<tr>
<th></th>
<th>Ritonavir AUC (ng·h/ml)</th>
<th>P</th>
<th>Ritonavir Cmin (ng/ml)</th>
<th>Saquinavir AUC</th>
<th>P</th>
<th>Saquinavir Cmin (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pravastatin co-drug (n = 11)</td>
<td>55 548 (16 013–82 153)</td>
<td>0.21</td>
<td>1957 (0–5143)</td>
<td>20 942 (10 509–34 660)</td>
<td>0.90</td>
<td>769 (0–1553)</td>
</tr>
<tr>
<td>Atorvastatin co-drug (n = 11)</td>
<td>56 440 (11 135–132 380)</td>
<td>0.60</td>
<td>2088 (292–5434)</td>
<td>18 919 (6333–38 982)</td>
<td>0.84</td>
<td>588 (181–1375)</td>
</tr>
<tr>
<td>Simvastatin co-drug (n = 11)</td>
<td>44 526 (11 801–95 854)</td>
<td>0.78</td>
<td>1515 (346–4314)</td>
<td>10 890 (7173–34 660)</td>
<td>0.31</td>
<td>500 (155–4308)</td>
</tr>
<tr>
<td>Historic controls (no statin) (n = 8)</td>
<td>48 591 (35 767–58 968)</td>
<td>–</td>
<td>–</td>
<td>20 883 (11 722–38 985)</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

*Test of significance of ritonavir and saquinavir AUCs in the presence of statin co-drug versus historic controls.
inappropriate to conclude that the relatively mild drug–drug interaction between RTV/SQV_{sgc} and pravastatin or atorvastatin implies they are safe for use in persons with HIV infection. Moyle recently demonstrated moderate hypolipidemic effects of pravastatin 40 mg daily along with dietary restriction in persons with HIV infection [18]. There were few adverse events attributed to pravastatin in that trial. Overall, there were few adverse events attributed to any of the statins used in our study. The safety and efficacy of statins remains to be established in HIV-infected persons taking PI.

There is a paucity of data on the safety of statins when used concomitantly with PI. Several small series have reported that statins are generally safe for the treatment of hyperlipidemia in persons with HIV infection [15–18]. However, two cases of rhabdomyolysis were recently reported with the use of statins in the setting of PI [39]. One patient was taking simvastatin 40 mg daily and began using indinavir 800 mg and RTV 200 mg twice daily 1 month prior to the onset of rhabdomyolysis. The other patient added atorvastatin 10 mg daily to a regimen that included indinavir 800 mg every 8 h 1 month before developing rhabdomyolysis. These case reports highlight the potential dangers of using statins in patients taking PI.

There are several important limitations to this study. It was not feasible to study all combinations of statins and PI. This study was designed and conducted prior to the approval of lopinavir/RTV or the widespread use of low dose RTV to pharmacologically enhance antiretroviral therapy. Whether the results will be similar for other PI at varying doses is not known. Several recent reports suggest that our results may be generalized to other PI [40,41]. Carr reported a fivefold increase in atorvastatin concentrations and a large decrease in the formation of the active metabolite in combination with lopinavir/RTV [40]. They also noted a non-significant increase in pravastatin concentrations with the addition of lopinavir/RTV (33%; 90% confidence interval, –9% to 94%). The different doses of RTV used in the study by Carr (100 mg twice daily) and our study (400 mg twice daily) may account for the disparate findings for pravastatin. Similarly, Hsyu reported that NFV increased the AUC of active simvastatin by 506% and the AUC of active atorvastatin by 74% similar to our results [41].

This study was not designed primarily to determine the effect of statins on RTV and SQV concentrations. This was a secondary objective and, as such, the sample size was not sufficient to exclude a clinically important drug interaction. However, our observations on the relative lack of effect of statins on the concentrations of PI are consistent with the findings of Carr and Hsyu for lopinavir and NFV/M8 metabolite concentrations, respectively [40±41].

The relationship between parent and metabolite drug concentrations and the overall effect on HMG-CoA reductase activity is also an important issue. Both atorvastatin and simvastatin have active metabolites that contribute to the drugs' lipid-lowering activity and probably their toxicity. These active metabolites are generated via CYP3A4. Consequently, inhibition of CYP3A4 would be associated with decreased generation of these active metabolites, combined with greater exposure to the parent compound. For atorvastatin, we clearly demonstrated decreased generation of these active metabolites with the use of RTV/SQV_{sgc}. Thus, the actual increase in total atorvastatin activity, which is the sum of the parent compound and active metabolites, was much less than the increase in exposure to the parent, atorvastatin itself. We did not measure the active metabolites of simvastatin acid. As a result, it is quite likely that we have over-estimated the true effect of RTV/SQV_{sgc} on the increase in total simvastatin activity as an inhibitor of HMG-CoA reductase activi-

### Table 4. Adverse clinical and laboratory events [n (%)].

<table>
<thead>
<tr>
<th>Events of grade 2 and higher</th>
<th>Total (n = 56)</th>
<th>Arm 1 (pravastatin; n = 14)</th>
<th>Arm 2 (atorvastatin; n = 14)</th>
<th>Arm 3 (simvastatin; n = 14)</th>
<th>Arm 4 (nelfinavir; n = 14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any laboratory event</td>
<td>6 (11)</td>
<td>2 (14)</td>
<td>1 (7)</td>
<td>2 (14)</td>
<td>1 (7)</td>
</tr>
<tr>
<td>Elevated CPK</td>
<td>1</td>
<td>1 (7)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Elevated AST/ALT</td>
<td>1</td>
<td>1 (7)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Elevated TG</td>
<td>4</td>
<td>0</td>
<td>1 (7)</td>
<td>2 (14)</td>
<td>1 (7)</td>
</tr>
<tr>
<td>Any signs/symptoms</td>
<td>8 (14)</td>
<td>1 (7)</td>
<td>1 (7)</td>
<td>4 (29)</td>
<td>2 (14)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>1 (7)</td>
<td>2 (14)</td>
</tr>
<tr>
<td>Diarrhea and anorexia</td>
<td>1</td>
<td>1 (7)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Shoulder and back pain</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1 (7)</td>
<td>0</td>
</tr>
<tr>
<td>Skin rash</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1 (7)</td>
<td>0</td>
</tr>
<tr>
<td>Psychological reaction</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1 (7)</td>
<td>0</td>
</tr>
<tr>
<td>Fatigue and dysguesia</td>
<td>1</td>
<td>0</td>
<td>1 (7)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

CPK, creatine phosphokinase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; TG, triacylglycerols.
ity. Nonetheless the effect of RTV/SQV<sub>ge</sub> was much greater for simvastatin than for atorvastatin, making the former drug–drug interaction likely to be more clinically significant. In future pharmacokinetic studies with simvastatin total HMG-CoA reductase activity should be measured to evaluate the overall pharmacokinetic effects.

In conclusion, this study demonstrates significant drug–drug interactions with RTV/SQV<sub>ge</sub> and selected statins. Simvastatin (and lovastatin) should be avoided in persons using PI. Atorvastatin may be used with caution in persons using PI, and dose escalation should be based on clinical indications with careful monitoring for toxicity. Pravastatin appears relatively safe for use in persons taking PI but the clinical effect of a 50% reduction in exposure secondary to RTV/SQV<sub>ge</sub> is unknown. Simvastatin, atorvastatin, and pravastatin do not appear to significantly affect the concentrations of RTV or SQV. Pravastatin does not significantly affect the concentrations of NFV or its M8 metabolite. Further research is required to determine the long-term clinical safety and efficacy of statins in persons taking PI.

Acknowledgements

The ACTG A5047 team would like to thank the 67 volunteers for graciously donating their time to this study. We would also like to thank all the study coordinators and sites for their hard work. Special thanks to K. Lamb, W. J. Burning, P. Lizak, E. Ferguson, H. Edmondson–Melancon, S. Brobst, N. Pugh, P. Clax, A. Japour, J. Staggers, K. Jorga, J. Wiebe, A. Jayewardene, and M. Royal for their invaluable assistance. Participating ACTG A5047 sites and investigators: LAC/USC Medical Center, F. R. Satter, D. Johnson, H. Edmondson-Melancon; Tulane University, J. J. L. Lertora, D. M. Mushatt; University of Hawaii/Manoa; Leahi Hospital, L. Oshita, D. Ogata-Arakaki, S. Souza; San Francisco General Hospital, M. Simmons, C. Arri, P. Barnett; University of Colorado HSC, S. Canmann, S. Fiorillo; Indiana University, J. Richardson, D. Heise; Washington University School of Medicine, L. Stiffler, M. Klebert; Johns Hopkins University, D. Jones, D. Wright, V. Williams; Stanford University, S. Valle, D. Slamonowitz, S. Stoudt; University of Miami, M. A. Fischl, J. Castro, R. Monroig; University of Washington, T. M. Hooton, L. Houseworth, M. Schwartz.

Sponsorship: Supported by the National Institute of Allergy and Infectious Diseases, AI-38858 to the AIDS Clinical Trials Group, AI-25897 to the University of Cincinnati, AI-25903 to Washington University, AI-27666 to Stanford University, and AI-38855 to the SDAC/Harvard School of Public Health. Additional support provided by Stanford University GCRC grant 5-M01-RR00070-38, University of California at San Francisco GCRC grant 5-M01-RR-00083-37 and the University of Colorado Health Sciences College GCRC grant 5-M01-RR-00051-37. Funded in part by Bristol-Myers Squibb, Inc. and Abbott Laboratories, Inc.

References

Statin and protease inhibitor drug interactions Fichtenbaum et al.


