Differential Effects of Tipranavir plus Ritonavir on Atorvastatin or Rosuvastatin Pharmacokinetics in Healthy Volunteers

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Running title: PK of Rosuvastatin and Atorvastatin with Tipranavir/r

Abstract

To identify pharmacokinetic drug-drug interactions between tipranavir/ritonavir (TPV/r) and, rosuvastatin and atorvastatin, we conducted two prospective, open-label, single arm,
two-period studies. The geometric mean ratio (GMR) was 1.37 (90% confidence interval, 1.15 to 1.62) for rosuvastatin AUC and 2.23 (90% confidence interval, 1.83 to 2.72) for rosuvastatin $C_{\text{max}}$ with TPV/r at steady-state versus alone. The GMR was 9.36 (90% confidence interval, 8.02 to 10.94) for atorvastatin AUC and 8.61 (90% confidence interval, 7.25 to 10.21) for atorvastatin $C_{\text{max}}$ with TPV/r at steady-state versus alone.

Tipranavir pharmacokinetic parameters were not affected by single-dose rosuvastatin or atorvastatin. Mild gastrointestinal intolerance, headache, and mild reversible liver enzyme elevations (grade 1 and 2) were the most commonly reported adverse drug reactions. Based on these interactions, we recommend low initial doses of rosuvastatin (5 mg) and atorvastatin (10 mg) with careful clinical monitoring of rosuvastatin- or atorvastatin-related adverse events when combined with TPV/r.
Tipranavir coadministered with low-dose ritonavir (TPV/r) is an effective treatment option in treatment-experienced HIV-infected patients with resistance to more than one protease inhibitor (PI) (10). TPV/r is associated with adverse effects (AEs) that include increased triglycerides and cholesterol. NIAID Division of AIDS (DAIDS) grade 3 to 4 cholesterol (>400 mg/dl) and grade 3–4 triglyceride (>750 mg/dl) elevations were higher in the TPV/r-treated patients compared with the comparator boosted PI-treated patients in phase III studies (10). Grade 3 to 4 cholesterol elevation was 4.3 versus 0.6/100 patient-exposure years and triglyceride elevation was 27.8 versus 21.6/100 patient-exposure years in the TPV/r versus comparator boosted PI-treated patients. The D:A:D study (N. Friis-Moller, P. Reiss, W. El-Sadr, A. D'Arminio Monforte, R. Thiébaut, R. De Wit, S. Weber, E. Fontas, M. Law, A. Phillips, and D. A. D. S. Group, presented at the 13th Conference on Retroviruses and Opportunistic Infections, Denver, CO, 5 to 8 February 2006) found a 16% increase in relative risk of myocardial infarction in PI-treated patients. This association is possibly explained by dyslipidemia. Since the HIV patient population is getting older, it is critical to control hyperlipidemia in PI-treated patients in order to reduce the risk of long-term cardiovascular complications. Potent 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase inhibitors (e.g., atorvastatin and rosuvastatin) are recommended for the treatment of hypercholesterolemia (2); however, their use in HIV-infected patients may be limited by clinically significant drug-drug interactions with PIs (6). Atorvastatin is metabolized extensively by cytochrome P450 (CYP)3A4 to metabolites that have in vitro inhibitory activity for HMG-CoA reductase similar to atorvastatin. Approximately 70% of the circulating inhibitory activity for HMG-CoA reductase has been attributed to these active metabolites (16). Since TPV/r
has a net inhibitory effect on CYP3A4 (M. Vourvahis, J. Dumond, K. Patterson, N. Rezk, N. White, S. Jennings, H. Tien, J. Sabo, T. MacGregor, and A. Kashuba, presented at the 14th Congress on Retroviruses and Opportunistic Infections, Los Angeles, CA; 25 to 28 February 2007), it has the potential to significantly increase circulating atorvastatin concentrations.

However, rosuvastatin is unlikely to interact with TPV/r since it is not a CYP3A4 substrate and it is not extensively metabolized (1). In order to provide guidance for clinical use, pharmacokinetic studies in healthy volunteers evaluated the drug-drug interactions between steady-state TPV/r and single-dose rosuvastatin (Rosuvastatin Study) and atorvastatin (Atorvastatin Study).

**MATERIALS AND METHODS**

**Subjects.**

Each study protocol was approved by the local institutional review board, and written informed consent was obtained from all volunteers before enrollment in these studies.

**Rosuvastatin Study**

HIV-negative healthy men and non-pregnant women volunteers aged 18 to 65 years with a body mass index (BMI) of 18 to 30 kg/m² were enrolled in this study. Exclusion criteria included use of any medications, herbal therapies, or grapefruit juice within 14 d before study entry or alcohol intake within 48 h before pharmacokinetic sampling days; active hepatitis B or C infection; and significant laboratory abnormalities. All women were
required to have a negative pregnancy test result at study entry and were instructed to use a barrier contraceptive method during the study period.

Atorvastatin Study

Healthy men and women volunteers aged 18 to 60 years, with a BMI 18 to 29 kg/m², were eligible for this study. Subjects were considered to be healthy based on their medical histories, physical examination, electrocardiogram, urinalysis, routine tests of biochemistry and hematology, and hepatitis B, C, and HIV status. Throughout the study period, subjects were instructed to abstain from alcohol, grapefruit/grapefruit juice (starting 10 d prior to the first study day), Seville oranges, and over-the-counter herbal medications (garlic supplements, St. John’s wort, milk thistle) starting 5 d prior to the first study day. Methylxanthine-containing foods or drinks were not allowed within 72 h prior to and during pharmacokinetic sampling days.

Study design and procedures.

Rosuvastatin Study

This was a prospective, open-label, single-arm, inpatient steady-state pharmacokinetic study. Subjects received a single 10 mg dose of rosuvastatin (Crestor; AstraZeneca Pharmaceuticals LP; Wilmington, DE) on day 1, followed by tipranavir 500 mg/ritonavir 200 mg twice daily for 11 days (days 3 to 13), with a single 10 mg dose of rosuvastatin given on day 12. All study drug doses were given under direct observation by the nursing staff of the Johns Hopkins University General Clinical Research Center. All subjects received 100% of study medications. Intensive pharmacokinetic sampling was performed...
on days 1 to 3 (before the addition of TPV/r) and days 12 to 14 (with TPV/r at steady state) for rosuvastatin, and days 12 and 13 for tipranavir and ritonavir. A standard 670 Kcal (33% fat) breakfast was served to subjects within 30 min before morning medication administration. No caffeine or food was allowed until 5 h post-dose. No grapefruit juice was allowed on pharmacokinetic sampling days. Blood samples were collected at the following time points for tipranavir and ritonavir: 0 (pre-dose), 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, and 12 h post-dose; and 0 (pre-dose), 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 24, and 48 h post-dose for rosuvastatin. Pharmacokinetic comparison of single-dose rosuvastatin with single-dose rosuvastatin when coadministered with TPV/r 500 mg/200 mg twice daily at steady state was undertaken. Short-term safety and steady-state pharmacokinetics of tipranavir and ritonavir when coadministered with single-dose rosuvastatin (10 mg) also were evaluated.

Atorvastatin Study

The single-dose pharmacokinetics of atorvastatin, ortho-hydroxy-atorvastatin, and para-hydroxy-atorvastatin were assessed on day 1 after ingestion of 40 mg atorvastatin (Lipitor; Pfizer Inc; New York, NY) alone. From day 14 to 21 subjects received TPV/r 500 mg/200 mg twice daily TPV/r dosages from Day 13 evening to Day 18 morning were taken at home. All dosages before and after this period were DOT. Subjects were asked to log their adherence to study medications. All subjects had 100% adherence with study medication. The steady-state pharmacokinetics of tipranavir were assessed on day 19 after ingestion of TPV/r alone, and on day 20 after administration of TPV/r 500 mg/200 mg twice daily plus a single dose of atorvastatin 10 mg, as an increase in atorvastatin
concentrations was expected in combination with steady-state TPV/r 500 mg/200 mg twice daily. Blood samples for analysis of atorvastatin and hydroxy-metabolites were collected on days 1 and 20 in ethylenediaminetetraacetic acid (EDTA)-containing tubes by an indwelling catheter or venipuncture of a forearm vein just before, and at 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 24, and 48 h after, ingestion of atorvastatin (14 samples).

Blood samples for analysis of tipranavir concentrations were collected in heparinized tubes on days 19 and 20 by an indwelling catheter or venipuncture of a forearm vein just before, and at 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, and 12 h after, ingestion of TPV/r (12 samples). Ritonavir plasma concentrations were not measured in this study.

On pharmacokinetic sampling days, atorvastatin and TPV/r were administered orally at 8 AM with 240 ml of water after an overnight fast. A light snack (i.e., 240 ml of low-fat milk/dry bread) was allowed at no less than 1 h prior to dosing or 2 h after dosing to minimize nausea and vomiting if necessary. Subjects were kept in an upright position for the first 2 h after drug administration. A standardized lunch and dinner were served 4 and 10 h after the morning dose, respectively (500 to 682 Kcal, 23% to 25% from fat).

Total daily fluid intake was restricted to a maximum of 3 L.

Subject safety was monitored by assessment of all AEs at each visit, in addition to laboratory assessment of safety parameters.

Bioanalysis.

Rosuvastatin assay

A bioanalytic method has been developed and validated by PPD (Richmond, VA) for the analysis of rosvastatin in human plasma containing dipotassium EDTA. A 200-µl
sample aliquot is fortified with 200 µl of internal standard (IS) working solution. Analytes were isolated by protein precipitation with 400 µl of acetonitrile. Sample extraction steps were controlled and automated using a Tomtec (Hamden, CT) Quadra 96 Model 320. The supernatant was transferred to another plate and evaporated to dryness under a nitrogen stream at 45°C to 50°C. The remaining residue was reconstituted with 150 µl of 20:80:0.1 acetonitrile/water/formic acid, v/v/v. The final extract was analyzed via high-performance liquid chromatography, tandem mass spectrometry (HPLC/MS/MS) detection.

Eight calibration standards were analyzed in duplicate over the nominal concentration range of 0.100 to 100 ng/ml. For this validation, the lower limit of quantitation was nominally 0.100 ng/ml for rosuvastatin.

Atorvastatin assay

Plasma concentrations of atorvastatin and its hydroxy-metabolites were measured by validated liquid chromatography (LC)/MS/MS at MDS Pharma Services (Saint-Laurent, Quebec, Canada). The respective deuterated analogs of the analytes were used as ISs. Briefly, the analytes were extracted from 200 µl EDTA plasma by solid-phase extraction using C₈ cartridges. After isolation and evaporation to dryness, the analytes were reconstituted and 200 µl was injected for LC/MS/MS analysis. The analytes were separated on a Phenomenex (Torrance, CA) Luna C₈ column and quantified by mass spectrometry under multiple reaction monitoring mode. The lower limit of quantitation was 0.225 ng/ml for atorvastatin, 0.250 ng/ml for parahydroxy-atorvastatin, and 0.175 ng/ml for orthohydroxy-atorvastatin using a 200 µl sample.
Tipranavir assay

A validated HPLC/MS/MS method was used to measure plasma drug concentrations of tipranavir and ritonavir (17). In general terms, ritonavir, tipranavir, and an IS are extracted from EDTA human plasma by a two-step liquid/liquid extraction using an ethyl acetate/hexane mixture followed by a hexane wash. The analytes are separated and detected by an LC/MS/MS system utilizing a 2.0 X 30 mm Synergi Polar RP (Phenomenex) column with a formic acid/acetic acid/acetonitrile mobile phase. For the atorvastatin study, tipranavir and IS (PNU-109011) were extracted from 50 µl heparinised plasma by liquid/liquid extraction using 600 µl of a mixture of ethyl acetate/hexane (9/1 v/v) after addition of 100 µl borate buffer (pH 9).

High and low calibration ranges were used to predict unknown concentrations. The high calibration curve ranged from 20,000 ng/ml to 1,000 ng/ml. The low calibration curve ranged from 2,000 ng/ml to 25.0 ng/ml.

Data analysis and statistical methods.

Pharmacokinetic analysis

For both studies, noncompartmental methods were used for pharmacokinetic analysis (WinNonlin versions 5.01 and 4.0, Pharsight Corporation, Mountain View, CA). The highest observed plasma concentration was defined as $C_{\text{max}}$, with the corresponding sampling time as $t_{\text{max}}$. The elimination rate constant ($\lambda z$) was determined by least squares linear regression analysis (log C versus t) of the last data points ($n \geq 3$). The $t_{1/2}$ was calculated by the equation $t_{1/2} = \ln 2/\lambda z$. After single-dose administration, the AUC$_{0-\infty}$ was
calculated using the linear-log trapezoidal rule (linear up/log down) from zero to the last measured concentration (AUC$_{last}$), with extrapolation to infinity by dividing the last measured concentration by $\lambda z$ (C$_{last}$/\lambda z). AUC$_{0-12h}$ was estimated using the linear-log trapezoidal rule (linear up/log down). The concentration at 12 h post-dose was defined as $C_{12h}$. The $Cl/F$, where $F$ represents the oral bioavailability, was calculated as dose/AUC, and the volume of distribution (V) was calculated as $(Cl/F)/\lambda z$.

Statistical analysis

Statistical analysis was performed with SAS (release 8.01; SAS Institute Inc., Cary, NC) and Stata (version 8; Stata Corp, College Park, TX). Statistical comparisons were performed after logarithmic transformation. Summary statistics for each of the pharmacokinetic parameters were tabulated by regimen and study day. GM ratios and corresponding 90% confidence intervals of pharmacokinetic parameters were calculated. Lack of a clinically relevant interaction was declared if the 90% confidence interval of the GM ratio was completely contained in the acceptance range of 0.80 to 1.25.

Prior to statistical analysis of the effect of TPV/r on atorvastatin (day 1 versus day 20), the atorvastatin AUC$_{0-\infty}$ and $C_{max}$ were multiplied by four to adjust for the difference in atorvastatin dose between day 1 (40 mg) and day 20 (10 mg). No dose adjustments were made for the analysis of the hydroxy-metabolite concentrations.

In addition to the analysis of atorvastatin and its hydroxy-metabolites individually, the effect on total HMG-CoA reductase inhibitory activity was assessed. The total HMG-CoA reductase inhibitory activity was calculated as the sum of the time-
specific molar concentrations of atorvastatin (normalized for 40 mg dose), orthohydroxy-atarvastatin, and parahydroxy-atorvastatin.

Nonparametric tests were used for comparisons between pharmacokinetic parameters (Mann-Whitney U test or Wilcoxon signed ranks test as appropriate). Spearman’s correlation was used to test for associations between continuous variables. A $P$ value $< 0.05$ was considered statistically significant for all comparisons. The within-subject variability in the steady-state tipranavir $AUC_{0-12h}$ was calculated as the percentage difference between the individual tipranavir $AUC_{0-12h}$ on day 19 and day 20.

For sample size calculations in both studies it was assumed that the coefficient of variation would range from 20% to 40% for rosuvastatin and atorvastatin $AUC$. Therefore, a sample size of 20 would provide $>90\%$ power to detect a $\geq 25\%$ change in rosuvastatin and atorvastatin $AUC$.

RESULTS

Rosuvastatin study.

Subjects

Of 29 subjects (five women, 24 men), 16 evaluable subjects completed the study. The population was mostly African American (76%), with a median age (range) of 42 (18 to 64) years, a median weight of 78 (51 to 96) kg, and a median height of 175 (162 to 196) cm. No Asian Americans participated in the study.

Interaction between TPV/r and rosuvastatin
With TPV/r coadministration, the rosuvastatin geometric mean (GM) area under the curve (AUC) was 38.6 ng·hr/ml, a 37% increase compared with rosuvastatin alone ($P = 0.0006$) (Figure 1, Figure 2). The rosuvastatin GM maximum plasma concentration ($C_{\text{max}}$) was 5.78 ng/ml with TPV/r coadministration, a 123% increase compared with rosuvastatin alone ($P < 0.001$) (Table 1). Rosuvastatin clearance also was decreased by 27% with TPV/r coadministration; this resulted in a significant increase in plasma half-life ($t_{1/2}$; 20.6 h versus 9.01 h; $P < 0.001$). Tipranavir and ritonavir pharmacokinetic parameters were not affected by single-dose rosuvastatin (Table 3).

**Safety**

The most common AEs associated with study drug administration were diarrhea (10.3%), nausea (13.8%), abdominal cramp (10.3%), flatulence (10.3%), headache (13.8%), and grade 1 liver enzyme elevations (34.5%) not resulting in study drug discontinuation. With the exception of one grade 2 nausea, one grade 2 rash, and eight grade 2 to 3 liver enzyme elevations, all AEs were of mild intensity (grade 1). Of 29 subjects enrolled, 13 subjects did not complete the study. Eight subjects discontinued because of grade 2 to 3 liver enzyme elevations, which were reversible upon study drug discontinuation. None of the subjects who had an increase in liver enzyme levels developed signs or symptoms of clinical hepatitis. One subject withdrew because of a grade 3 hypersensitivity reaction (mild shortness of breath, diffuse rash, and liver enzyme elevations). Three subjects withdrew consent, and one subject was administratively discharged due to a positive drug screen. The frequency and severity of these AEs are consistent with those observed in clinical trials involving HIV-infected patients treated with TPV/r. The rate of
discontinuation in this study was high due to a lower threshold for study drug discontinuation (10). The potential for increased risk of hepatotoxicity with the coadministration of TPV/r plus rosuvastatin could not be assessed since all liver enzyme elevations occurred before coadministration began (day 8). All AEs resolved with study drug discontinuation.

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Atorvastatin study.

Subjects

Twenty-three subjects (11 men, 12 women) were recruited into this study. The population was mostly white (95.7%), with a median (range) age of 32 (18 to 55) years, a median weight of 72 (55 to 99) kg, and a median height of 168 (158 to 189) cm. The atorvastatin pharmacokinetic results from one subject, and the steady-state tipranavir pharmacokinetic results from another subject, were excluded from all statistical analyses because of nonphysiologic results (> five-fold increase in drug concentration at the end of the dosing interval). However, the outcome of the study was not different with or without the data from these subjects (data not shown).

Interaction between TPV/r and atorvastatin

TPV/r increased the dose-adjusted atorvastatin AUC from zero to infinity ($\text{AUC}_{0-\infty}$) and $C_{\text{max}}$ by approximately nine-fold (Table 2, Figures 3 and 4). As both apparent oral clearance (Cl/F) and apparent volume of distribution (V/F) were decreased almost proportionally, there was no effect on the $t_{1/2}$ of atorvastatin. TPV/r inhibited the formation of ortho- and parahydroxy-atorvastatin, and reduced the $\text{AUC}_{0-\infty}$ by 89% ($P =$ 251
0.002) and 82% (P = 0.001), respectively. Total HMG-CoA reductase inhibitory activity increased by approximately four-fold in the presence of TPV/r. The GM ratios (90% confidence intervals) for the \( \text{AUC}_{0-\infty} \) and \( \text{C}_{\text{max}} \) of the total HMG-CoA reductase inhibitory activity were, respectively, 3.87 (3.13 to 4.33); \( P < 0.001 \) and 4.97 (3.71 to 5.51); \( P < 0.001 \).

Single-dose atorvastatin did not affect the steady-state pharmacokinetics of tipranavir (Table 3).

Safety

In general, treatment with TPV/r 500 mg/200 mg twice daily alone and in the presence of single-dose atorvastatin was well tolerated in this study. There were no study discontinuations due to AEs or for any other reason. One serious AE was reported in this study (sprained ankle due to exercise), which was unrelated to the study drugs.

The most frequently reported AEs during all treatment phases were related to the gastrointestinal tract (\( n = 22 \) [95.7%]), followed by the nervous system (\( n = 16 \) [69.6%]). During treatment with TPV/r alone, 17 (73.9%) subjects reported diarrhea, 11 (47.8%) reported nausea, and nine (39.1%) reported abdominal pain. Flatulence, loose stools, and dyspepsia occurred in six (26.1%), five (21.7%), and three (13%) subjects, respectively, during treatment with TPV/r. With the exception of one case of moderate nausea, all gastrointestinal events were of mild intensity and rarely required treatment intervention. Headache and dysgeusia were reported by eight (34.8%) and four (17.4%) subjects, respectively, during treatment with TPV/r.
With the exception of one subject with an asymptomatic DAIDS grade 3 elevation in alanine aminotransferase, there were no clinically relevant changes (grade 3 or higher) in any of the laboratory tests. The largest deviations from median baseline were observed for triglycerides and alanine aminotransferase levels, which increased by 1.7-fold and 3.1-fold, respectively, relative to baseline.

**DISCUSSION**

A pharmacokinetic drug interaction was predicted to be unlikely to occur between rosuvastatin and TPV/r since rosuvastatin is not a known substrate, inhibitor, or inducer of CYP3A4. Furthermore, rosuvastatin is not extensively metabolized, with approximately 10% of a radio-labeled dose recovered as N-desmethyl rosuvastatin. This major metabolite is formed principally by CYP2C9 (1). Although in vitro studies indicate that tipranavir is an inhibitor of CYP2C9, an in vivo cocktail study determined the steady-state levels of tipranavir (500 mg) plus ritonavir 200 mg twice daily did not affect plasma (S)-warfarin concentrations, a CYP2C9 substrate (M. Vourvahis, presented at the 8th International Workshop on Clinical Pharmacology of HIV Therapy, Budapest, Hungary, 16 to 18 April 2007). When TPV/r was coadministered with rosuvastatin at steady state, rosuvastatin AUC$_{0-\infty}$ and C$_{\text{max}}$ were increased by 37% and 123%, respectively, after single-dose rosuvastatin. Similarly, concomitant administration of steady-state TPV/r significantly increased atorvastatin AUC$_{0-\infty}$. However, atorvastatin AUC fold-increase was significantly higher. The differential effect of TPV/r on the pharmacokinetics of rosuvastatin and atorvastatin may be due to the difference in the mechanism of interaction.
Rosuvastatin is not a P-glycoprotein (Pgp) substrate, but is a substrate of organic anion transporting polypeptide 1B1 (OATP1B1) and breast cancer resistance protein (BCRP), also known as ATP-binding cassette transporter G2 (ABCG2) (13). OATP1B1 is located at the basolateral membrane of hepatocytes and functions as an influx transporter to facilitate entry of drugs into hepatocytes. These types of substrates include several statins (i.e., pravastatin, cerivastatin, atorvastatin and rosuvastatin) (8, 11). BCRP is expressed in many tissue barriers throughout the body, including the epithelium of the small intestine, liver canalicular membrane ducts, placental syncytiotrophobasts, and lobules of the breast (5, 18). In the gastrointestinal tract, BCRP is localized in the apical membrane of the intestinal epithelia, with the highest BCRP mRNA expression in the duodenum (7). Unlike OATP1B1, BCRP generally functions as an efflux transporter that facilitates hepatobiliary excretion and decreases intestinal absorption of BCRP substrates. The marked effect on the oral bioavailability of BCRP substrates was demonstrated in a BCRP 1 knockout murine model. The plasma concentration of nitrofurantoin, a substrate of BCRP, was increased by four-fold and two-fold compared with wild-type mice after oral and intravenous administration, respectively (21). Similarly, BCRP (ABCG2) polymorphism (c.421AA genotype) in healthy volunteers was associated with a significant increased atorvastatin and rosuvastatin AUC by 72% and 100%, respectively (14). Cyclosporine, an OATP1B1 and BCRP inhibitor, significantly increases rosuvastatin and atorvastatin serum concentrations by 11-fold and eight-fold, respectively (9, 25). Tipranavir, ritonavir, and lopinavir are also BCRP inhibitors in vitro (20, 28). Concentration-dependent inhibition of [3H]estradiol-17β-D-glucuronide uptake by ritonavir is also observed in OATP1B1 transfected HeLa cells and OATP-mediated
The potential mechanism of interaction in this study may be the result of dual inhibition of OATP1B1 and BCRP transporters by ritonavir and tipranavir. Inhibition of OATP1B1 may lead to a decrease in hepatocyte uptake of rosuvastatin and atorvastatin, whereas inhibition of BCRP decreases hepatobiliary excretion and increases rosuvastatin and atorvastatin absorption.

In addition to OATP1B1 and BCRP transporter mediated interaction, inhibition of first-pass metabolism of atorvastatin, rather than inhibition of its systemic metabolism may explain the 8.6-fold increase in atorvastatin $C_{\text{max}}$ (8.6-fold increase) without an effect on the $t_{1/2}$. The absolute oral bioavailability of atorvastatin is low (14%), which is likely a result of the combined effects of intestinal metabolism by CYP3A4 and transport into the gut lumen by the multidrug efflux transporter Pgp (5). Assuming linear pharmacokinetics of atorvastatin (24), coincidentally the formation of ortho- and parahydroxy-atorvastatin was inhibited. The $\text{AUC}_{0-\infty}$ of these metabolites was reduced by 89% and 82%, respectively, in the presence of TPV/r. Steady-state TPV/r has an inhibitory effect on CYP3A4/5 but minimal effects on Pgp (6), and may thus increase the atorvastatin bioavailability, as suggested in the current study. Previous studies have reported increased bioavailability of various CYP3A4 and/or Pgp substrates in the presence of ritonavir (e.g., digoxin, saquinavir) (23, 27). Similarly near-complete absorption of atorvastatin in the presence of TPV/r ($F \approx 100\%$) may explain the observed changes in the $\text{AUC}_{0-\infty}$ and $C_{\text{max}}$, the delayed time of $C_{\text{max}}$ ($T_{\text{max}}$), and the proportional reduction in both the CI/F and the V/F resulting in no change in the $t_{1/2}$. On the other hand, rosuvastatin $t_{1/2}$ was increased by approximately 2-fold. This finding suggests that alternative metabolic pathways for rosuvastatin may be present.
These findings are consistent with drug interaction studies evaluating the effect of another PI, lopinavir/ritonavir (LPV/r), when coadministered with rosuvastatin at steady state. A prospective study conducted in healthy volunteers found that rosuvastatin AUC$_{\infty}$ and C$_{max}$ were increased 2.1- and 4.7-fold, respectively, when coadministered with LPV/r (15). Similar to the current atorvastatin study, the t$_{1/2}$ was not affected; however, the magnitude of drug interaction observed between rosuvastatin and LPV/r was several-fold higher. This effect may be due to the higher inhibitory potency of BCRP observed with lopinavir in vitro (28). Coadministration of atazanavir/ritonavir with rosuvastatin was found to result in a 210% increase in rosuvastatin AUC; however, no significant interaction was observed with fosamprenavir/ritonavir (3). The lack of interaction observed with fosamprenavir/ritonavir may be due to its low BCRP and OATP1B1 inhibitory potential (Theil-Demby VE, Harmon K, Naqwe M, Humphreys J, Wire MB, Polli JW. OATP1B1, OATP1B3 and BCRP Transport Characterization for Fosamaprevir, Amprenavir, and Lopinavir. American Association of Pharmaceutical Scientists (AAPS), November 2008.) Increased exposure to atorvastatin has previously been observed in combination with other HIV PIs, which are all substrates/inhibitors of CYP3A4. Coadministration with lopinavir/ritonavir 400 mg/100 mg twice daily increased the atorvastatin (20 mg once daily) AUC$_{0-24h}$ and C$_{max}$ by 5.9- and 4.7-fold respectively, and increased the AUC$_{0-24h}$ and C$_{max}$ of the total HMG-CoA reductase inhibitory activity by 2.5- and 4.5-fold, respectively (R. A. Carr, A. K. Andre, and R. J. Bertz, presented at the 40th Interscience Conference on Antimicrobial Agents and Chemotherapy, Toronto, Ontario, Canada, 17 to 20 September 2000) (6). The combination of saquinavir/ritonavir 400 mg/400 mg twice daily increased the median atorvastatin (40 mg) AUC$_{0-24h}$ by 3.5-
fold, and the AUC\textsubscript{0-24h} of the total HMG-CoA reductase inhibitory activity by 1.8-fold (6). Nelfinavir, a moderately strong inhibitor of CYP3A4, increased the AUC\textsubscript{0-24h} and C\textsubscript{max} of the total HMG-CoA reductase inhibitory activity by 1.7- and 2.2-fold, respectively, after administration of atorvastatin 10 mg once daily for 14 days (12). The combination of darunavir/ritonavir 300 mg/100 mg twice daily with atorvastatin 10 mg daily resulted in an AUC\textsubscript{0-24h} that was 15% lower than the AUC\textsubscript{0-24h} of atorvastatin 40 mg daily alone, suggesting an almost four-fold increase in atorvastatin AUC\textsubscript{0-24h} (R. M. W. Hoetelmans, A. Lasure, A. Koester, M. de Pauw, B. van Baelen, M. Peeters, W. Parys, and E. Lefebvre, presented at the 44th Interscience Conference on Antimicrobial Agents and Chemotherapy, Washington, DC, 30 October to 2 November 2004).

One of the most serious AEs associated with HMG-CoA reductase inhibitors (statins) is rhabdomyolysis, which can result in significant morbidity and mortality (22). Although rhabdomyolysis may occur in 0.1% to 0.5% of the population when statins are prescribed as monotherapy, the risk is greatly increased when drugs that inhibit CYP3A4-mediated metabolism are used concomitantly (22). Several cases of atorvastatin-associated rhabdomyolysis have been reported in patients using atorvastatin in combination with CYP3A4 inhibitors such as cyclosporine or delavirdine (4, 19). Although the safety and efficacy of rosuvastatin and atorvastatin could not be assessed by these single-dose studies, to minimize the risk of rhabdomyolysis, careful monitoring for any signs or symptoms of toxicity is recommended. Alternatively, statins with a lower likelihood of interactions could be considered.

In conclusion, we observed a clinically relevant increase in rosuvastatin and atorvastatin concentrations during coadministration of TPV/r 500 mg/200 mg twice daily.
Based on these results, a low initial dose of rosuvastatin (5 mg) and atorvastatin (10 mg) is recommended when combined with TPV/r, with careful clinical monitoring of rosuvastatin—or atorvastatin-related AEs such as myopathy.
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REFERENCES


Figure Legends

FIG. 1. Plasma rosuvastatin concentration-time profile in the absence and presence of steady-state TPV/r.

FIG. 2. Effect of steady-state TPV/r on single-dose rosuvastatin Cmax, Cp24h, and AUC0-24h.

FIG. 3. Plasma drug concentration-time profile for atorvastatin (Panel A), ortho-hydroxy-atorvastatin (Panel B), and para-hydroxy-atorvastatin (Panel C). Open circles: 40 mg atorvastatin alone; closed circles: 10 mg atorvastatin + steady-state TPV/r; *: atorvastatin 10 mg profile corrected to a 40 mg dose (Panel A). Values are mean ng/ml ± SD.

FIG. 4. Effect of steady-state TPV/r on single-dose atorvastatin Cmax, Cp12h, and AUC0-∞.
TABLE 1. Pharmacokinetic parameters of rosuvastatin ± TPV/r and TPV/r ± rosuvastatin

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Rosuvastatin geometric mean (CV%)</th>
<th>Rosuvastatin (+TPV/r) geometric mean (CV%)</th>
<th>Geometric mean ratio (90% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C_{max} (ng/ml)</td>
<td>2.59 (41)</td>
<td>5.78 (55)</td>
<td>2.23 (1.83–2.72)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>T_{max} (h)^a</td>
<td>5 (1.48–6.02)^a</td>
<td>3 (2–4)^a</td>
<td>N/A</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>C_{24h} (ng/ml)</td>
<td>0.186 (46)</td>
<td>0.163 (21)</td>
<td>0.88 (0.73–1.06)</td>
<td>0.26</td>
</tr>
<tr>
<td>AUC_{0-∞} (ng·h/ml)</td>
<td>28.2 (59)</td>
<td>38.6 (38)</td>
<td>1.37 (1.15–1.62)</td>
<td>0.006</td>
</tr>
<tr>
<td>t_{1/2} (h)</td>
<td>9.01 (69)</td>
<td>20.6 (48)</td>
<td>2.29 (1.63–3.21)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cl/F (l/h)</td>
<td>355 (59)</td>
<td>259 (38)</td>
<td>0.73 (0.62–0.87)</td>
<td>0.006</td>
</tr>
</tbody>
</table>

^aT_{max} presented as median (range).

AUC_{all}, area under the plasma concentration versus time curve; AUC_{0-∞}, area under the plasma concentration versus time curve from zero to infinity; CI, confidence interval; C_{max}, maximum plasma concentration; C_{min}, minimum plasma concentration; Clearance_{ss}, steady-state plasma clearance; Cl/F, apparent oral plasma clearance; CV, coefficient of variation; t_{1/2}, plasma elimination half-life; T_{max}, time of C_{max}. TPV/r, tipranavir/ritonavir.
TABLE 2. Geometric mean (CV%) single-dose pharmacokinetic parameters of atorvastatin, orthohydroxy-atorvastatin, and parahydroxy-atorvastatin, after administration of atorvastatin 40 mg alone and after coadministration of atorvastatin 10 mg and steady-state tipranavir/ritonavir 500 mg/200 mg twice daily (TPV/r, n = 22)

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Pharmacokinetic parameter</th>
<th>Atorvastatin 40 mg alone (day 1)</th>
<th>Atorvastatin 10 mg + TPV/r (day 20)</th>
<th>Geometric mean ratio (90% CI)</th>
<th>P value&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Observed</td>
<td>Normalized&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atorvastatin</td>
<td>AU&lt;sub&gt;0-1&lt;/sub&gt; (h·ng/ml)</td>
<td>89.3 (38)</td>
<td>209 (41)</td>
<td>836 (41)</td>
<td>9.36 (8.02–10.94)</td>
</tr>
<tr>
<td></td>
<td>C&lt;sub&gt;max&lt;/sub&gt; (ng/ml)</td>
<td>17.6 (40)</td>
<td>37.8 (42)</td>
<td>151 (42)</td>
<td>8.61 (7.25–10.21)</td>
</tr>
<tr>
<td></td>
<td>T&lt;sub&gt;max&lt;/sub&gt; (h)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.5 (0.5–8.0)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3.0 (1.5–4.0)&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Cl/F (l/h)</td>
<td>448 (38)</td>
<td>47.8 (41)</td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>V/F (l)</td>
<td>4536 (43)</td>
<td>432 (54)</td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>t&lt;sub&gt;1/2&lt;/sub&gt; (h)</td>
<td>7.0 (32)</td>
<td>6.3 (36)</td>
<td></td>
<td>0.30</td>
</tr>
<tr>
<td>Orthohydroxy-</td>
<td>AU&lt;sub&gt;0-1&lt;/sub&gt; (h·ng/ml)</td>
<td>117 (21)</td>
<td>13.6 (124)</td>
<td></td>
<td>0.11 (0.08–0.17)</td>
</tr>
<tr>
<td>atorvastatin</td>
<td>C&lt;sub&gt;max&lt;/sub&gt; (ng/ml)</td>
<td>12.4 (41)</td>
<td>0.301 (35)</td>
<td></td>
<td>0.02 (0.02–0.03)</td>
</tr>
<tr>
<td></td>
<td>T&lt;sub&gt;max&lt;/sub&gt; (h)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.5 (0.5–8.0)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>4.0 (1.0–12.0)&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
<td>0.006</td>
</tr>
<tr>
<td></td>
<td>t&lt;sub&gt;1/2&lt;/sub&gt; (h)</td>
<td>8.6 (21)</td>
<td>27.7 (158)</td>
<td></td>
<td>0.010</td>
</tr>
</tbody>
</table>
Table 3. Effect of rosuvastatin and atorvastatin on the pharmacokinetics of tipranavir co-administered with ritonavir

<table>
<thead>
<tr>
<th>TPV PK Parameter</th>
<th>Effect of Rosuvastatin</th>
<th>Effect of Atorvastatin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(N = 16)</td>
<td>(N = 22)</td>
</tr>
<tr>
<td></td>
<td>TPV/r alone</td>
<td>TPV/r + rosuvastatin</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; (µM)</td>
<td>72.5 (22)</td>
<td>78.5 (24)</td>
</tr>
<tr>
<td>T&lt;sub&gt;max&lt;/sub&gt; (h)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.5 (2.0–5.0)</td>
<td>3.0 (1.6–5.0)</td>
</tr>
<tr>
<td>C&lt;sub&gt;12h&lt;/sub&gt; (µM)</td>
<td>15.4 (59)</td>
<td>15.2 (40)</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0-12h&lt;/sub&gt; (µM·h)</td>
<td>477 (30)</td>
<td>504 (27)</td>
</tr>
<tr>
<td>t&lt;sub&gt;1/2&lt;/sub&gt; (h)</td>
<td>3.94 (33)</td>
<td>3.77 (23)</td>
</tr>
<tr>
<td>Clearance&lt;sub&gt;ss&lt;/sub&gt; (L/h)</td>
<td>1.74 (38)</td>
<td>1.65 (38)</td>
</tr>
<tr>
<td>V&lt;sub&gt;z/F&lt;/sub&gt; (L)</td>
<td>9.9 (38)</td>
<td>9.0 (22)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Calculated as the ratio of TPV/r + statin to TPV/r alone.

<sup>b</sup>T<sub>max</sub> presented as median (range), otherwise geometric mean (CV%)

TABLE 3. (continued)

| Para-hydroxy- | AUC<sub>0-∞</sub> (h·ng/ml) | 19.5 (65) | 4.07 (35) | 0.18 (0.14–0.24) | 0.001 |

- 30 -
<table>
<thead>
<tr>
<th></th>
<th>$C_{\text{max}}$ (ng/ml)</th>
<th>0.586 (54)</th>
<th>0.610 (32)</th>
<th>1.04 (0.87–1.25)</th>
<th>0.795</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$T_{\text{max}}$ (h)$^a$</td>
<td>9.0 (0.5–24.0)$^b$</td>
<td>4.0 (2.0–5.0)$^b$</td>
<td></td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>$t_{1/2}$ (h)</td>
<td>21.0 (99)</td>
<td>3.17 (41)</td>
<td></td>
<td>0.001</td>
</tr>
<tr>
<td>Total HMG-CoA reductase</td>
<td>$\text{AUC}_{0-\infty}$ (hr·nM)</td>
<td>393 (27)</td>
<td>396 (43)</td>
<td>1519 (42)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>inhibitory activity</td>
<td>$C_{\text{max}}$ (nM)</td>
<td>54.8 (37)</td>
<td>69.4 (42)</td>
<td>272.6 (42)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

$^a$Dose-adjusted assuming linear pharmacokinetics for atorvastatin (normalized = observed value X 4).

$^b$Calculated as the ratio of atorvastatin + TPV/r to atorvastatin alone.

$^c$P value for difference between atorvastatin alone and atorvastatin + TPV/r using the Wilcoxon matched pairs signed ranks test.

$^d$T$_{\text{max}}$ presented as median (range).

AUC$_{0-\infty}$, area under the plasma concentration versus time curve from zero to infinity; CI, confidence interval; Cl/F, apparent oral plasma clearance; $C_{\text{max}}$, maximal plasma concentration; CV, coefficient of variation; HMG-CoA, 3-hydroxy-3-methyl-glutaryl-CoA; $T_{\text{max}}$, time of $C_{\text{max}}$; V/F, apparent volume of distribution; $t_{1/2}$, plasma elimination half-life.
Figure 1. Plasma rosuvastatin concentration-time profile in the absence and presence of steady-state TPV/r.
Figure 2. Effect of steady-state TPV/r on single-dose rosuvastatin C$_{max}$, C$_{p24}$, and AUC$_{0-24h}$. 

![Graph showing the effect of steady-state TPV/r on single-dose rosuvastatin C$_{max}$, C$_{p24}$, and AUC$_{0-24h}$](http://aac.asm.org/)
Figure 3. Plasma drug concentration-time profile for atorvastatin (Panel A), ortho-hydroxy-atorvastatin (Panel B) and para-hydroxy-atorvastatin (Panel C). Open circles: 40 mg atorvastatin alone; closed circles: 10 mg atorvastatin + steady-state TPV/r; *: atorvastatin 10 mg profile corrected to a 40 mg dose (Panel A). Values are mean ng/ml ± SD.
Figure 4. Effect of steady-state TPV/r on single-dose atorvastatin

Cmax, C_{12h} and AUC_{0-\infty}.