Effect of Sitagliptin on the Pharmacokinetics of Simvastatin

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Keywords: *dipeptidyl peptidase-4; incretin; statins; drug interactions*

Journal of Clinical Pharmacology, 2009;49:483-488

Patients with type 2 diabetes are at an increased risk for cardiovascular morbidity and mortality.[1] Treatment with HMG-CoA reductase inhibitors (statins) reduces the risk of cardiovascular events in patients with diabetes.[2,3] Treatment guidelines consider diabetes as a coronary heart disease risk equivalent and recommend statin therapy for patients with diabetes.[4]

Dipeptidyl peptidase-4 (DPP-4) inhibitors are a new class of oral antihyperglycemic agents.[5] Sitagliptin, a highly selective DPP-4 inhibitor, is available in many countries for the treatment of patients with type 2 diabetes.[6] Because sitagliptin and a statin may be commonly administered to patients with type 2 diabetes, the potential for sitagliptin to alter the pharmacokinetics of simvastatin, a CYP3A4 substrate,[7] was evaluated in this study. The metabolism of simvastatin is complex, resulting in the generation of multiple active and inactive metabolites.[7,8] Active HMG-CoA reductase inhibitors represent the total pool of pharmacologically active species in plasma following simvastatin administration (ie, simvastatin acid and other active metabolites).[9] Therefore, assessment of the $AUC_{0\text{-last}}$ for active HMG-CoA reductase inhibitors was the primary endpoint in this study. The pharmacokinetic parameters for simvastatin (lactone; pharmacologically inactive), simvastatin acid (active), and total HMG-CoA reductase inhibitors were secondary endpoints in this study.

METHODS

Patients

Subjects were healthy young male and female subjects, 18 to 45 years of age and within 30% of their ideal body weight. Women used appropriate double-barrier contraception methods throughout the study and, prior to dosing in each period, had a pregnancy test. Subjects agreed to refrain from strenuous exercise and were not allowed to be on any medications throughout the study. Subjects with a history of drug or alcohol abuse within the 2 years prior to the screening visit were excluded from the trial.

All subjects provided written informed consent for participation in this study. The study was approved by the ethical review committee for the investigational site (Metabolic Unit, Diabetes Center, Free University Brussels, and UZ Brussels, Belgium). The study was performed in accordance with the Declaration of Helsinki.

Study Design

This was a single-center, randomized, open-label, 2-period, crossover study. Subjects were randomized to the sequence of 2 treatments in periods 1 and 2

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DOI: 10.1177/0091270008330983

(eg, treatment A in period 1 and treatment B in period 2, or vice versa) using a computer-generated allocation schedule. For treatment A, subjects received a single open-label dose of simvastatin 20 mg. For treatment B, subjects received open-label, once-daily sitagliptin 200 mg (2 × 100-mg tablets) on days 1 through 5 and a single dose of simvastatin 20 mg on day 5. Simvastatin 20 mg is the recommended starting dose for patients with hypercholesterolemia and was selected for the present study to provide sufficiently high plasma drug levels to assess any potential interaction. Each dosing was witnessed by study site personnel. Treatment periods were separated by at least a 7-day washout period. All subjects had a poststudy visit or received a telephone call approximately 14 days after the last dose in period 2 to determine if any adverse experiences occurred following the final treatment period.

For treatment A (simvastatin 20 mg on day 1 only), subjects reported to the clinical research unit (CRU) in the evening prior to dosing. Subjects were required to fast from food and drink, except water, for at least 8 hours. After the overnight fast, a blood sample was obtained between 08:00 and 10:00 and prior to administration of simvastatin 20 mg. Following dose administration, additional blood samples were obtained at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 16, and 24 hours. Subjects were provided lunch, dinner, and a snack at 4, 10, and 13 hours following dosing, respectively. Subjects were discharged from the CRU after the 24-hour blood sampling.

For treatment B (single dose of simvastatin following 5 days of dosing with once-daily sitagliptin 200 mg), subjects reported to the CRU on the morning of days 1 through 4 to receive their sitagliptin dose. On the evening prior to day 5, subjects reported to the CRU and underwent similar fasting, blood sampling, and test procedures as described above. For treatment B, simvastatin 20 mg and sitagliptin 200 mg were coadministered following collection of the predose blood sample.

In this study, a 5-day dosing schedule was used for sitagliptin because steady-state concentration for sitagliptin occurs by day 3 with daily dosing.[10] Sitagliptin 200 mg once daily was used because it was selected at that time as the top dose for some of the phase 3 clinical trials[6] and it also augmented the sensitivity for detecting a potential interaction with simvastatin.

Bioanalytical and Pharmacokinetic Analysis

Plasma was analyzed for concentrations of active and total HMG-CoA reductase inhibitors, simvastatin, and simvastatin acid.

The inhibitory activity of the plasma samples was measured using an enzyme inhibition assay procedure with simvastatin acid as a standard, and thus, concentrations are reported in simvastatin acid equivalents.[9] HMG-CoA reductase inhibitors in the plasma were measured before and after base hydrolysis, with the corresponding plasma concentration values referred to as active and total HMG-CoA reductase inhibitor concentrations, respectively. The lower limit of quantification for both the active and total HMG-CoA reductase inhibitor assays is 0.4 ng-eq/mL, with a linear calibration range of 0.4 to 10 ng-eq/mL.

The basis of this assay procedure is the inhibition of HMG-CoA reductase by simvastatin acid and other active metabolites of simvastatin. In this assay, if no inhibitors are present, ^{14}C-HMG-CoA is converted to ^{14}C-mevalonic acid and then to ^{14}C-mevalonolactone after lactonization. An increase in the concentration of the inhibitory species will, therefore, result in a decrease in the amount of ^{14}C-mevalonolactone detected. The concentration of HMG-CoA reductase inhibitors in study samples can be determined by interpolation from a standard curve with the HMG-CoA reductase activity (in percentages) plotted against the log of the concentration of simvastatin acid. Reductase activity (measured in percentages) at each spiked inhibitor concentration was calculated as counts per minute (cpm) ^{14}C-mevalonolactone obtained at that concentration divided by the cpm obtained for a plasma blank.

Because the assay procedure involves many steps and no internal standard is used, acceptable assay precision is maintained by assaying each blank, standard, quality control (QC), and study sample in duplicate. When the deviation between the 2 values is smaller than 10%, the average of the 2 duplicate values was reported as the final concentration for the sample. If the deviation between the 2 duplicate values is greater than 10% for a particular sample, the assay result for this sample is considered not acceptable, and reanalysis of the sample is performed.

The assay specificity was evaluated to determine the impact of endogenous materials in the biomatrices on the reproducibility of the assay. Different sources (N = 5) of human plasma were assayed together with neat reconstitution solution as the blank reference. The scintillation counts of ^{14}C-mevalonolactone resulting from different biomatrices showed similar values and were similar to those derived from the blank, indicating that no intrinsic HMG-CoA reductase inhibitory activity exists in those biomatrices that may cause interference with the assay.

At least 2 sets of low, middle, high, and diluting (when applicable) QC samples were assayed along with the study samples in each active and total inhibitor 96-well assay plate. The overall interassay precision obtained from QC samples throughout the assay runs was ≤4.9% coefficient of variation (CV) for both active and total inhibitors QC sets. The overall interassay accuracy obtained from QC samples was in the range of 94.1% to 111.9% and 89.0% to 114.4% of nominal values for the active and total inhibitors QC sets, respectively.

Plasma concentrations for simvastatin lactone and simvastatin acid were determined simultaneously according to a liquid chromatography method coupled with a tandem mass spectrometry method (LC/MS/MS).[11] For the determination of simvastatin lactone and simvastatin acid in plasma by the LC/MS/MS, simvastatin lactone and simvastatin acid are simultaneously extracted from 0.32 mL of human plasma using solid-phase extraction on an Oasis HLB μElution SPE plate and were chromatographed through a Phenomonex Synergi Max-RP column (50 × 2 mm id, 4 μm) with a mobile phase that consisted of acetonitrile/methyl ammonium acetate (1 mM, pH 4.5; 80:20, v/v). Samples were analyzed by a PE SciexAPI 3000 mass spectrometer through a turbo ion spray interface. $^{13}CD_3$-labeled simvastatin lactone and simvastatin acid were used as internal standards for simvastatin lactone and simvastatin acid, respectively. The analytes and internal standards were detected by selected reaction monitoring (SRM) in negative ion detection mode (for simvastatin acid) for the first ~2 minutes and positive ion detection mode (for simvastatin lactone) for the rest of the analysis run. The precursor → product ions monitored in the SRM mode were: *m/z* 435.2 $(M\text{-}H)^-$ → 319.1 (for simvastatin acid), *m/z* 439.2 $(M\text{-}H)^-$ → 319.1 (for [$^{13}CD_3$]-simvastatin acid), *m/z* 450.3 $(M+CH_3NH_3)^+$ → 285.3 (for simvastatin lactone), and *m/z* 454.3 $(M+CH_3NH_3)^+$ → 285.3 (for [$^{13}CD_3$] –simvastatin lactone). The lower limit of quantification for both simvastatin lactone and simvastatin acid is 0.05 ng/mL, with a linear calibration range that is 0.05 to 50 ng/mL using 0.32 mL of plasma. No detectable interferences at the retention times of the analytes were found in control plasma blank samples of either assay.

Two sets of low, middle, and high QC samples were assayed along with the study samples in the 96-well assay plates for both simvastatin lactone and simvastatin acid. The overall interassay precision obtained from QC samples throughout the assay runs was ≤4.4% CV for both simvastatin lactone and simvastatin acid QC sets. The overall interassay accuracy obtained from QC samples was in the range of 94.5% to 98.5% and 92.5% to 99.0% of nominal values for the simvastatin lactone and simvastatin acid QC sets, respectively.

For each analyte, maximum observed plasma concentration (C_{max}) and time of occurrence of C_{max} (T_{max}) were determined by inspection of concentration-time data. $AUC_{0\text{-last}}$ was determined using the linear trapezoidal method up to the last time point with a plasma concentration above the assay limit of quantification.[12]

The apparent elimination half-life of total and active HMG-CoA reductase inhibitors was not calculated because these analytes represent multiple species, presumably with different half-lives. Simvastatin lactone is metabolized reversibly to simvastatin acid, and hence, half-life cannot be reliably estimated from the terminal phase. Thus, simvastatin lactone and simvastatin acid half-lives were not determined.

Statistical Analysis

The plasma pharmacokinetic parameters ($AUC_{0\text{-last}}$, C_{max}, and T_{max}) of active and total HMG-CoA reductase inhibitors and simvastatin lactone and simvastatin acid following a single oral 20-mg dose with or without multiple once-daily oral 200-mg doses of sitagliptin were compared using a linear mixed-effects model appropriate for a 2-period, crossover design. The model contained fixed effects for sequence, period, and treatment and a random effect for subject-within-sequence. A natural-log transformation was applied to the $AUC_{0\text{-last}}$ and C_{max} data prior to statistical analysis. T_{max} data were rank transformed and analyzed using the same mixed-effects model. Geometric means for $AUC_{0\text{-last}}$ and C_{max} were calculated with back-transformation from the log scale. Ninety-percent confidence intervals (CIs) were computed from the above model for the $AUC_{0\text{-last}}$ and C_{max} geometric mean ratios (GMRs) of simvastatin plus sitagliptin versus simvastatin alone. As prespecified, if the 90% CI for the $AUC_{0\text{-last}}$ GMR of active HMG-CoA reductase inhibitors was contained within the prespecified bounds of (0.50, 2.00), it was concluded that simvastatin pharmacokinetics were not clinically meaningfully altered by coadministered sitagliptin.

RESULTS

Subjects

The 12 subjects (9 men and 3 women) randomized in this study were Caucasian and had a mean age of 31 years (range, 21-40 years). All subjects completed

Table I Summary Statistics for the Pharmacokinetic Parameters of Active and Total Plasma HMG-CoA Reductase Inhibitors, Simvastatin Acid, and Simvastatin Lactone

Pharmacokinetic Parameter	Simvastatin + Sitagliptin (n = 12)	Simvastatin (n = 12)	GMR (90% CI)	*P Value*[a]
Active HMG-CoA reductase inhibitors				
$AUC_{0\text{-last}}$, ng-eq h/mL[b]	61.1 ± 37.8	57.9 ± 32.6	1.06 (0.88, 1.26)	.595
C_{max}, ng-eq/mL[b]	12.2 ± 10.7	13.1 ± 11.9	0.94 (0.66, 1.34)	.748
T_{max}, h[c]	1.8 ± 1.6	1.8 ± 1.1	—	.663
Total HMG-CoA reductase inhibitors				
$AUC_{0\text{-last}}$, ng-eq h/mL[b]	161.6 ± 80.5	159.6 ± 67.3	1.01 (0.80, 1.28)	.924
C_{max}, ng-eq/mL[b]	46.8 ± 39.1	53.1 ± 42.1	0.88 (0.59, 1.31)	.576
T_{max}, h[c]	1.8 ± 1.0	1.3 ± 0.9	—	.630
Simvastatin acid				
$AUC_{0\text{-last}}$, ng·h/mL[b]	9.1 ± 9.4	8.2 ± 8.4	1.12 (0.93, 1.35)	.307
C_{max}, ng/mL[b]	0.9 ± 0.9	0.8 ± 0.8	1.06 (0.86, 1.32)	.615
T_{max}, h[c]	4.0 ± 1.2	4.0 ± 2.9	—	.290
Simvastatin lactone				
$AUC_{0\text{-last}}$, ng·h/mL[b]	11.6 ± 9.0	13.5 ± 9.9	0.85 (0.60, 1.22)	.442
C_{max}, ng/mL[b]	2.9 ± 3.3	3.7 ± 4.6	0.80 (0.51, 1.26)	.396
T_{max}, h[c]	1.5 ± 0.8	1.0 ± 0.8	—	.639

Abbreviations: —, not determined; CI, confidence interval; GMR, geometric mean ratio for (simvastatin + sitagliptin)/simvastatin.
[a] For $AUC_{0\text{-last}}$ and C_{max}, *P* value is for testing if GMR = 1.00. *P* value <.050 indicates the true GMR is statistically different from 1.00.
[b] Geometric means ± standard deviation back-transformed from the log scale.
[c] Median ± standard deviation for median.

both treatment periods and were included in the pharmacokinetic and safety analyses.

Pharmacokinetics

As shown in Table I and Figure 1A, coadministration of sitagliptin and simvastatin had no meaningful effect on the pharmacokinetics for active HMG-CoA reductase inhibitors, with a GMR (90% CI) for $AUC_{0\text{-last}}$ and C_{max} of 1.06 (0.88, 1.26) and 0.94 (0.66, 1.34), respectively. There was also no statistically meaningful difference in T_{max} between treatments (Table I). Furthermore, the 90% CI of the GMR for the $AUC_{0\text{-last}}$ of the active inhibitors was contained within the protocol prespecified comparability bounds of (0.50, 2.00), indicating that this parameter was not altered in a clinically meaningful manner with coadministration of sitagliptin. In addition, there were no clinically or statistically meaningful differences in $AUC_{0\text{-last}}$, C_{max}, or T_{max} between treatments for the other 3 analytes evaluated (Table I and Figure 1B-D).

Safety and Tolerability

Both treatment regimens were generally well tolerated. Ten subjects reported a total of 29 adverse experiences (12 in the treatment A arm and 17 in the treatment B arm), none of which resulted in study discontinuation. Of these 29 adverse experiences, a total of 8 adverse experiences reported in 4 patients (all were reported in the treatment B coadministration arm) were considered by the investigators to be "possibly" related to study drug. The drug-related adverse experiences were headache (n = 5), stomachache/stomach heaviness (n = 2), and vomiting (n = 1), and each were rated by the investigator as mild to moderate in intensity. One female subject had a serious adverse experience (pneumonia requiring hospitalization), which occurred 12 days after the final treatment period and was rated as not drug related by the investigator.

DISCUSSION

As statins are a recommended preventative therapy for patients with type 2 diabetes,[4] it was important to assess potential drug interactions between simvastatin, a commonly prescribed statin, and the new oral antihyperglycemic agent, sitagliptin. In the present study in healthy subjects, multiple once-daily doses of sitagliptin 200 mg did not meaningfully alter active inhibitor, total inhibitor, simvastatin acid, or simvastatin plasma concentrations following a single simvastatin dose. Although not directly assessed in the present study, this lack of effect on simvastatin pharmacokinetic parameters would also be expected with the approved sitagliptin dose (100 mg once daily). Furthermore, these results demonstrate that sitagliptin is not an inhibitor of

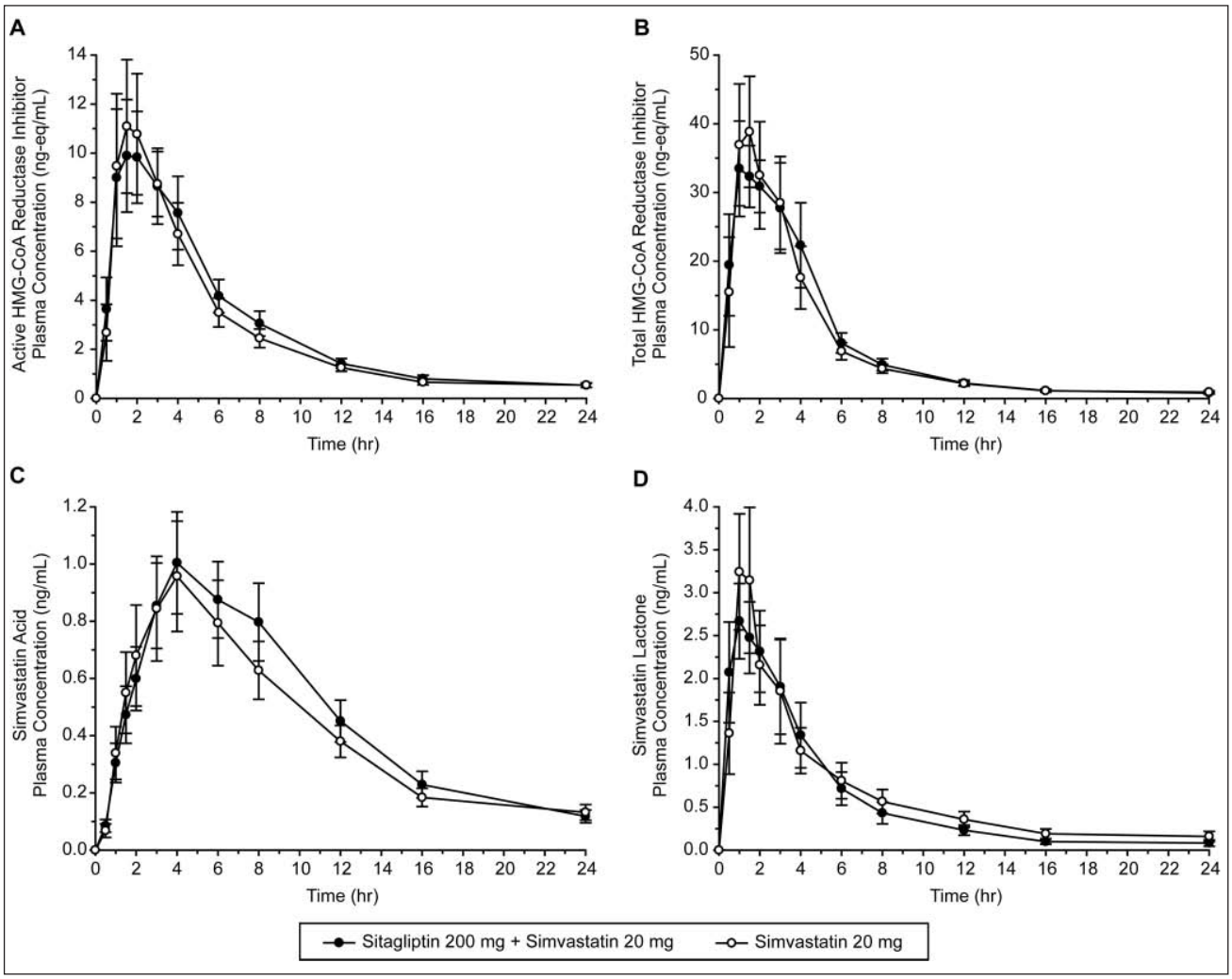

Figure 1. Mean plasma concentrations of (A) active HMG-CoA reductase inhibitor, (B) total HMG-CoA reductase inhibitor, (C) simvastatin acid, and (D) simvastatin lactone over 24 hours following a single dose of simvastatin 20 mg with or without multiple, once-daily, sitagliptin 200-mg doses in healthy subjects.

CYP3A4-mediated metabolism. Sitagliptin is mainly excreted (~80%) unchanged in urine and undergoes minimal metabolism primarily by CYP3A4 with minor contribution from CYP2C8.[13] These results are also consistent with the results of in vitro studies that have shown that sitagliptin is not a potent reversible inhibitor or time-dependent inhibitor of CYP3A4. In addition, in human hepatocyte cultures, sitagliptin had no effect on the expression levels of CYP3A4 mRNA or on CYP3A activity.[14] Thus, considering the wide therapeutic index associated with sitagliptin,[6] an effect of simvastatin on sitagliptin pharmacokinetics was not expected and not evaluated in the present study.

In conclusion, steady-state sitagliptin does not alter the pharmacokinetics of a single dose of simvastatin. Simvastatin and sitagliptin were generally well tolerated. Therefore, no dose adjustment for simvastatin is recommended when simvastatin is coadministered with sitagliptin.

Financial disclosure: This study was funded by Merck & Co, Inc, Whitehouse Station, New Jersey. A.B., J.C., A.M., B.A.R., L.S., R.J.V., A.Y., M.D., T.L., J.A.W., and G.A.H. are employed or were formerly employed (J.J.Z., M.J.D.) by Merck & Co, Inc, the manufacturer of sitagliptin and simvastatin, and may hold stocks or stock options in the company.

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