

Effect of Single Oral Doses of Sitagliptin, a Dipeptidyl Peptidase-4 Inhibitor, on Incretin and Plasma Glucose Levels after an Oral Glucose Tolerance Test in Patients with Type 2 Diabetes

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Context: In response to a meal, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP) are released and modulate glycemic control. Normally these incretins are rapidly degraded by dipeptidyl peptidase-4 (DPP-4). DPP-4 inhibitors are a novel class of oral antihyperglycemic agents in development for the treatment of type 2 diabetes. The degree of DPP-4 inhibition and the level of active incretin augmentation required for glucose lowering efficacy after an oral glucose tolerance test (OGTT) were evaluated.

Objective: The objective of the study was to examine the pharmacodynamics, pharmacokinetics, and tolerability of sitagliptin.

Design: This was a randomized, double-blind, placebo-controlled, three-period, single-dose crossover study.

Setting: The study was conducted at six investigational sites.

Patients: The study population consisted of 58 patients with type 2 diabetes who were not on antihyperglycemic agents.

Interventions: Interventions included sitagliptin 25 mg, sitagliptin 200 mg, or placebo.

Main Outcome Measures: Measurements included plasma DPP-4 activity; post-OGTT glucose excursion; active and total incretin GIP levels; insulin, C-peptide, and glucagon concentrations; and sitagliptin pharmacokinetics.

Results: Sitagliptin dose-dependently inhibited plasma DPP-4 activity over 24 h, enhanced active GLP-1 and GIP levels, increased insulin/C-peptide, decreased glucagon, and reduced glycemic excursion after OGTTs administered at 2 and 24 h after single oral 25- or 200-mg doses of sitagliptin. Sitagliptin was generally well tolerated, with no hypoglycemic events.

Conclusions: In this study in patients with type 2 diabetes, near maximal glucose-lowering efficacy of sitagliptin after single oral doses was associated with inhibition of plasma DPP-4 activity of 80% or greater, corresponding to a plasma sitagliptin concentration of 100 nM or greater, and an augmentation of active GLP-1 and GIP levels of 2-fold or higher after an OGTT. (*J Clin Endocrinol Metab* 91: 4612–4619, 2006)

DIPEPTIDYL PEPTIDASE-4 (DPP-4) inhibitors represent a new therapeutic approach for the treatment of type 2 diabetes (1). These agents work by inhibiting the DPP-4 enzyme that degrades incretin hormones such as glucagon-like peptide (GLP)-1 and glucose-dependent insulinotropic

polypeptide (GIP), thereby stabilizing the intact (active) form of the hormones (2). Active GLP-1 and GIP stimulate glucose-dependent insulin biosynthesis and release, and GLP-1 also suppresses glucagon release, delays gastric emptying, and increases satiety (3). DPP-4 inhibitors improved glycemic control, insulin secretion, and β -cell function in rodents (4–6). In patients with type 2 diabetes, chronic treatment with DPP-4 inhibitors decreased postprandial glucose excursion, fasting plasma glucose (FPG), and hemoglobin A_{1c} (HbA_{1c}) and was well tolerated with neutral weight effects and a low incidence of hypoglycemia and gastrointestinal adverse events relative to placebo (7–9).

Sitagliptin is an oral and highly selective DPP-4 inhibitor (10). In sitagliptin-treated lean rodents, approximately 80% inhibition of plasma DPP-4 activity and 2- to 3-fold elevation

First Published Online August 23, 2006

Abbreviations: AUC, Area under the curve; DPP-4, dipeptidyl peptidase-4; E_{max}, maximum response; FPG, fasting plasma glucose; GIP, glucose-dependent insulinotropic peptide; GLP, glucagon-like peptide; GMR, geometric mean ratio; HbA_{1c}, hemoglobin A_{1c}; LS, least squares; OGTT, oral glucose tolerance test; PD, pharmacodynamic; PK, pharmacokinetic; WAA, weighted average augmentation.

JCEM is published monthly by The Endocrine Society (<http://www.endo-society.org>), the foremost professional society serving the endocrine community.

in active GLP-1 levels were associated with near maximal reduction in glucose excursion after an oral glucose tolerance test (OGTT) (10). In healthy men with normal glucose concentrations, single doses of sitagliptin up to 600 mg were well tolerated without increased incidence of hypoglycemia or gastrointestinal adverse experiences, compared with placebo (11). Additionally, sitagliptin inhibited plasma DPP-4 enzyme activity and augmented active GLP-1 levels in a dose-dependent manner and had a pharmacokinetic (PK) profile consistent with once-daily dosing (11).

The degree of DPP-4 inhibition and level of active incretin augmentation required for near-maximal glucose lowering efficacy with a DPP4 inhibitor are not known. Therefore, the pharmacodynamics (PD), PK, and tolerability of sitagliptin were examined after administration of single oral doses of sitagliptin (25 or 200 mg) and OGTTs in patients with type 2 diabetes.

Patients and Methods

Patients

The protocol was reviewed and approved by each study center. All patients provided written informed consent before enrollment. Eligible patients (age 21–60 yr) had type 2 diabetes and met all of the following criteria at the screening visit: FPG 126 mg/dl or greater and 250 mg/dl or less after an overnight fast of 8 h or longer; HbA_{1c} 6.5–11.0% inclusive; C-peptide greater than 0.8 ng/ml; and not on an antihyperglycemic agent within 3 months of screening visit.

Patients were excluded from participation for any of the following: history of type 1 diabetes; body mass index greater than 40 kg/m²; estimated creatinine clearance 60 ml/min or less; or history of symptomatic orthostatic hypotension.

Study design

This was a multicenter, randomized, double-blind, placebo-controlled, three-period, crossover study. At the screening visit, eligible patients were counseled to follow a dietary program for approximately 2 wk before randomization and throughout the study. This diet was based on American Diabetes Association recommendations (55–60% carbohydrate, 10–20% protein, and not more than 30% fat by kilocalorie). Patients were randomized using a computer-generated allocation schedule to one of six possible sequences during which they received single oral doses of sitagliptin 25 or 200 mg or placebo, separated by at least a 7-d washout interval. For each treatment period, patients were instructed to consume more than 150 g of carbohydrates/d for the 3 d before their visit. On the treatment day, patients who fasted overnight for at least 10 h were administered study drug and underwent an OGTT at 2 h postdose. At 24 h postdose, patients received either an OGTT or a standardized meal challenge. For the OGTT, a 75-g glucose solution was consumed within 5 min, and for the meal challenges, a mixed meal consisting of 578 kcal (70 g carbohydrate, 21 g fat, and 27 g protein) was ingested with 240 ml water within 10 min. Standardized meals at 6 and 12 h postdose and snacks at 8 and 15.5 h postdose were also provided during each treatment period.

Efficacy and tolerability parameters

The primary efficacy end point was the glucose excursion above the pre-OGTT glucose level over a 4-h interval [incremental area under the curve (AUC)_{0–240 min}] after administration of an OGTT at 2 h postdose. Secondary efficacy end points included incremental glucose AUC_{0–120 min} postadministration of either an OGTT or standardized meal at 24 h postdose and plasma sitagliptin PK data. Plasma DPP-4 activity was assessed over the 24-h period. Insulin, C-peptide, and glucagon levels were determined only for the 2 h after the OGTT at 2 h postdose. Active and total incretin levels were measured after the OGTT at 2 and 24 h postdose and the standardized meal at 6 and 24 h postdose.

Tolerability was assessed by clinical and statistical review of adverse

experiences, laboratory values, vital signs, electrocardiograms, and physical exams. All adverse experiences were rated by the investigator for intensity and relationship to study drug. Specific adverse experiences of clinical interest included hypoglycemia and gastrointestinal-related adverse experiences.

Assay procedures

PK. Plasma sitagliptin concentration was determined predose and 1, 2, 4, 8, 12, and 24 h postdose. In addition, a subset of patients had additional sampling at 48 and 72 h postdose ($n = 7$) to determine apparent terminal half-life for sitagliptin. Details on PK assays and calculations were previously described (11).

PD. Plasma DPP-4 activity was measured as previously described (11) with blood collected at 0, 1, 2, 4, 8, 12, and 24 h postdose.

For the OGTT at 2 h postdose, blood was collected at 0, 0.5, 1, 2, and 4 h after glucose ingestion and used to measure plasma glucose, insulin, C-peptide, and glucagon levels. For meals or OGTTs at other time points, blood samples were obtained at 0, 0.5, 1, and 2 h, and only plasma glucose was determined. Glucose was measured with a glucose/hexokinase assay kit (Roche Molecular Biochemicals Corp., Indianapolis, IN). Insulin was assayed by the Elecsys insulin system (Roche Diagnostics, Indianapolis, IN). Glucagon was determined using a RIA kit using double-antibody methodology (Diagnostic Products Corp., Los Angeles, CA). C-peptide was assayed using an ELISA kit (American Laboratory Products, Co., Windham, NH).

For plasma active and total GLP-1 and GIP and glucose concentrations, blood was collected at 0, 2 (before OGTT), 2.5, 3, 4, 6, 6.5, 7, 8, 24 (before OGTT or standardized meal), 24.5, 25, and 26 h postdose. Active and total GLP-1 levels were measured with specific commercially available ELISA kits (Linco Research, Inc., St. Charles, MO) and as previously described (11). Active and total GIP levels were measured as previously described (12–14).

Statistical methods

The incremental glucose AUC_{0–240 min} after the OGTT at 2 h postdose was analyzed in the log scale using an appropriate ANOVA model for a complete three-period, crossover design. The ANOVA model included factors for subject, period, and treatment. Backtransformed summary statistics including geometric least-squares (LS) means, geometric mean ratio (GMR) for between-treatment comparisons (with 95% confidence interval), and P values for the between-treatment comparisons were provided. Similar analyses were performed on the incremental glucose AUC_{0–120 min} after an OGTT at 24 h postdose and other metabolic parameters.

An ANOVA was used to compare the effect of sitagliptin *vs.* placebo on the weighted average inhibition of DPP-4 activity through 24 h relative to the baseline activity (area under DPP-4 inhibition time curve divided by 24 h). Predose measurements at each period were used as baseline. All analyses were carried out in the log-percent scale, and final results were reported as percent inhibition.

The active and total incretin concentrations and the active to total ratios after the OGTTs [*i.e.* area under the weighted average augmentation (WAA) of GLP-1 or GIP concentration time curve divided by 2 or 4 h] were compared between sitagliptin and placebo in the same manner as the weighted average inhibition in DPP-4 activity. All tests of significance were performed at $\alpha = 0.05$, two-tailed.

PK/PD assessment

The relationship between PD parameters and plasma sitagliptin concentration was also explored. Individual values for plasma DPP-4 inhibition were pooled over all of the patients and doses and were plotted *vs.* plasma sitagliptin concentrations. A simple maximum response (E_{max}) model was used to describe the inhibition of plasma DPP-4 activity relative to plasma sitagliptin concentrations, in which E_{max} was set at 100%. EC_{50} (the concentration needed for 50% plasma DPP-4 inhibition) was estimated using the Gauss-Newton method (15). Because 80% or more inhibition of plasma DPP-4 activity was associated with maximal reductions in glycemic excursion after an OGTT in mice (10), EC_{80} was also estimated.

In a subset of patients who completed an OGTT at both 2 and 24 h postdose ($n = 19$), the relationship between plasma sitagliptin concentrations and the reduction of post-OGTT incremental glucose (*i.e.* GMR for incremental glucose $AUC_{0-120 \text{ min}}$ after sitagliptin/incremental glucose $AUC_{0-120 \text{ min}}$ after placebo) was explored, assuming that an empirical inhibitory E_{max} PK/PD model could describe the relationship. Use of the GMR provided a control for placebo effects. Similar analyses were conducted to explore treatment-related increases in the WAA GLP-1 and GIP GMR using E_{max} PK/PD models. In this model, E_{max} represents the maximal drug effect, and E_0 represents the baseline effect. EC_{50} was calculated and represents the plasma concentration required to achieve 50% of the maximal effect. The predicted plasma concentration where 75% of the maximal effect would be observed (EC_{75}) was also estimated to represent near-maximal effects.

Results

Patients

Sixty-one patients were enrolled in the study, and 60 completed all three periods. Five patients were not included in the efficacy analysis: one patient was lost to follow-up after period 1; four patients were considered protocol violators (including three patients who were enrolled twice at different study centers). All patients were included in the safety assessment ($n = 61$). Of the 58 patients (excluding the three patients who enrolled twice), there were 42 men and 16 women with an average age of 50.0 yr (range 33–60 yr), weight of 87.6 kg (range 60.4–134.7 kg), and body mass index of 29.5 kg/m^2 (range 26.0–38.1 kg/m^2). The racial breakdown was 35 Caucasians, 2 blacks, and 21 Hispanics. At baseline, mean FPG was 182.2 mg/dl (range 116–291 mg/dl), and HbA_{1c} was 8.3% (range 6.5–11.7%) in these patients.

PD

Mean percent inhibition of plasma DPP-4 activity from baseline (*i.e.* predose) to 24 h is shown in Fig. 1A. Sitagliptin dose-dependently inhibited plasma DPP-4 activity. Plasma DPP-4 activity was inhibited by approximately 80 and 96% at 2 h postdose and 47 and 80% at 24 h postdose with sitagliptin 25 and 200 mg, respectively (Fig. 1A). The mean percent inhibition of DPP-4 activity over 24 h was significantly ($P < 0.001$) greater with both doses of sitagliptin [25 mg: 68.1% (95% confidence interval 66.6, 69.6) and 200 mg: 91.4% (90.9, 91.8)], compared with placebo [2.1% (–2.8, 6.7)], and the difference between sitagliptin doses was significant. For inhibition of plasma DPP-4 activity *vs.* plasma sitagliptin concentrations (Fig. 1B), approximately 50% inhibition was observed with a plasma sitagliptin concentration of 25.3 (0.5) nM [fitted EC_{50} value; mean (SE)], whereas EC_{80} was approximately 100 nM (EC_{80}).

The active and total GLP-1 profiles are shown in Fig. 2, A and B. After an OGTT at 2 h postdose, both sitagliptin doses significantly ($P < 0.001$) increased WAA active GLP-1 levels by approximately 2-fold relative to placebo (Fig. 2C). After an OGTT at 24 h postdose, sitagliptin 25 and 200 mg significantly ($P < 0.001$) increased WAA active GLP-1 levels by approximately 1.3- and 1.9-fold, respectively, compared with placebo; the difference between doses was also significant (Fig. 2C). Weighted average total GLP-1 was significantly reduced by 10–17% with sitagliptin, compared with placebo, after the OGTTs at 2 and 24 h postdose (Fig. 2C). The ratios of active to total GLP-1 levels, compared with placebo, were

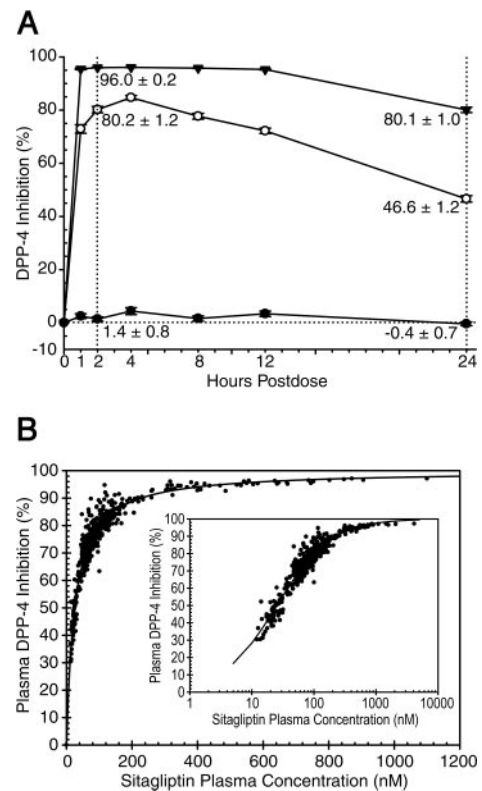


FIG. 1. A, Percent inhibition of plasma DPP-4 activity after administration of single oral doses of sitagliptin 25 (white circles) or 200 mg (black triangles) or placebo (black circles) from predose to 24 h postdose. Values presented next to vertical dotted lines represent percent plasma DPP-4 inhibition at the specific time point for each treatment. All data are expressed as geometric mean \pm SE. B, Inhibition of plasma DPP-4 activity *vs.* sitagliptin plasma concentrations (individual observations and model-fitted regression line). Inset represents plot on a semilogarithmic scale.

increased to a similar extent as the active GLP-1 results (data not shown).

Active and total GIP profiles are displayed in Fig. 3, A and B. Relative to placebo, WAA active GIP levels were significantly ($P < 0.001$) increased by approximately 2-fold with both sitagliptin doses after the OGTT at 2 h postdose and 1.4-fold with 25 mg and 2-fold with 200 mg after the OGTT at 24 h postdose (Fig. 3C). The difference between sitagliptin doses was also significant ($P < 0.001$) at 24 h postdose. After the OGTT at 2 and 24 h postdose, weighted average total GIP levels were significantly reduced by 16–29% after treatment with sitagliptin, compared with placebo (Fig. 3C). The ratio of active to total GIP levels was increased by approximately 1.7-fold with both doses after the OGTT at 2 h and approximately 1.5-fold with 25 mg and 2-fold with 200 mg after the OGTT at 24 h relative to placebo. Comparable effects on active and total GLP-1 and GIP levels were observed after meals administered at 6 and 24 h postdose (data not shown).

During the OGTT at 2 h postdose, sitagliptin 25 and 200 mg significantly ($P \leq 0.001$) reduced mean incremental glucose $AUC_{0-240 \text{ min}}$ by 22 and 26%, respectively, compared with placebo (Fig. 4A and Table 1). Both sitagliptin doses significantly increased insulin (21–22%) and C-peptide concentrations (13–21%) and decreased glucagon concentrations (7–

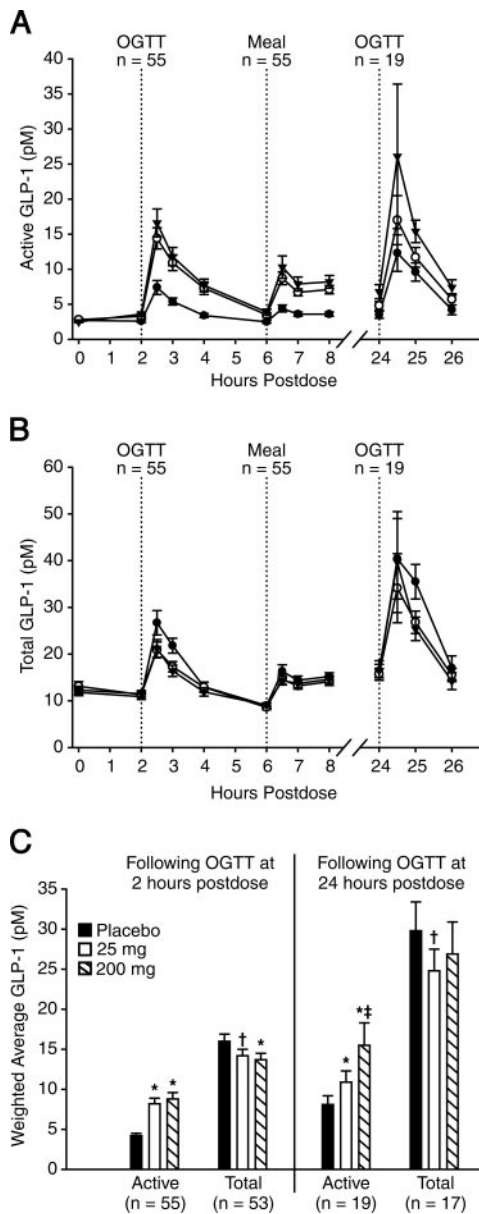


FIG. 2. Plasma profiles of active GLP-1 (A) and total GLP-1 (B) concentrations after administration of single oral doses of sitagliptin 25 (white circles) or 200 mg (black triangles) or placebo (black circles) and OGTTs at 2 and 24 h postdose and a standardized meal at 6 h postdose. Data are expressed as geometric mean \pm SE. C, Weighted average of plasma active and total GLP-1 concentrations after single-dose administration of sitagliptin or placebo and OGTTs at 2 or 24 h postdose. Weighted averages over 4 h were calculated after an OGTT at 2 h postdose, and weighted averages over 2 h were calculated after an OGTT at 24 h postdose. Data are expressed as geometric LS mean \pm SE. *, $P < 0.001$ sitagliptin vs. placebo; †, $P < 0.05$ for sitagliptin vs. placebo; ‡, $P < 0.001$ for sitagliptin 200 vs. 25 mg.

14%) after the OGTT at 2 h postdose relative to placebo (Fig. 4, B–D, and Table 1).

For patients administered an OGTT at 24 h postdose ($n = 19$), incremental glucose $AUC_{0-120 \text{ min}}$ was significantly ($P \leq 0.001$) reduced by 18% with the 200-mg dose and was numerically but not significantly lower by 9% with the 25-mg dose, compared with placebo (Table 1). In the subset of patients administered a standardized meal at 24 h postdose,

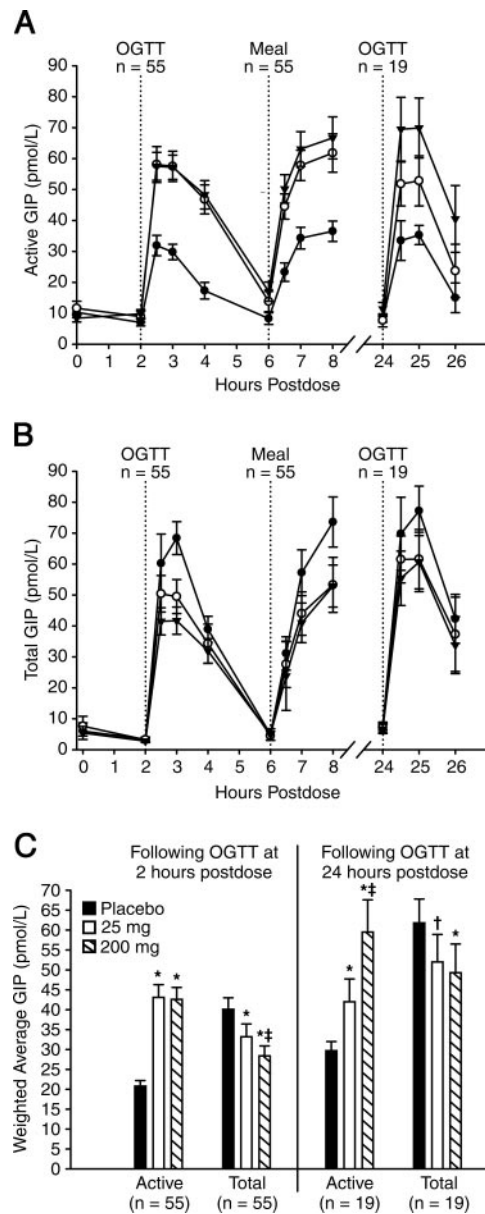


FIG. 3. Plasma profiles of active GIP (A) and total GIP (B) concentrations after administration of single oral doses of sitagliptin 25 (white circles) or 200 mg (black triangles) or placebo (black circles) and OGTTs at 2 and 24 h postdose and a standardized meal at 6 h postdose. Data are expressed as geometric mean \pm SE. C, Weighted average of plasma active and total GIP concentrations after single-dose administration of sitagliptin or placebo and OGTTs at 2 or 24 h postdose. Weighted averages over 4 h were calculated after an OGTT at 2 h postdose, and weighted averages over 2 h were calculated after an OGTT at 24 h postdose. Data are expressed as geometric LS mean \pm SE. *, $P < 0.001$ sitagliptin vs. placebo; †, $P < 0.05$ for sitagliptin vs. placebo; ‡, $P < 0.001$ for sitagliptin 200 vs. 25 mg.

sitagliptin did not significantly reduce postmeal glucose, although the level of glucose excursion after placebo administration was modest.

PK

Plasma sitagliptin $AUC_{0-24 \text{ h}}$ [geometric LS mean (SD) = 1.55 (0.29) and 14.10 (3.13) $\mu\text{M}\cdot\text{h}$], C_{max} [140.0 (35.1) and 1923

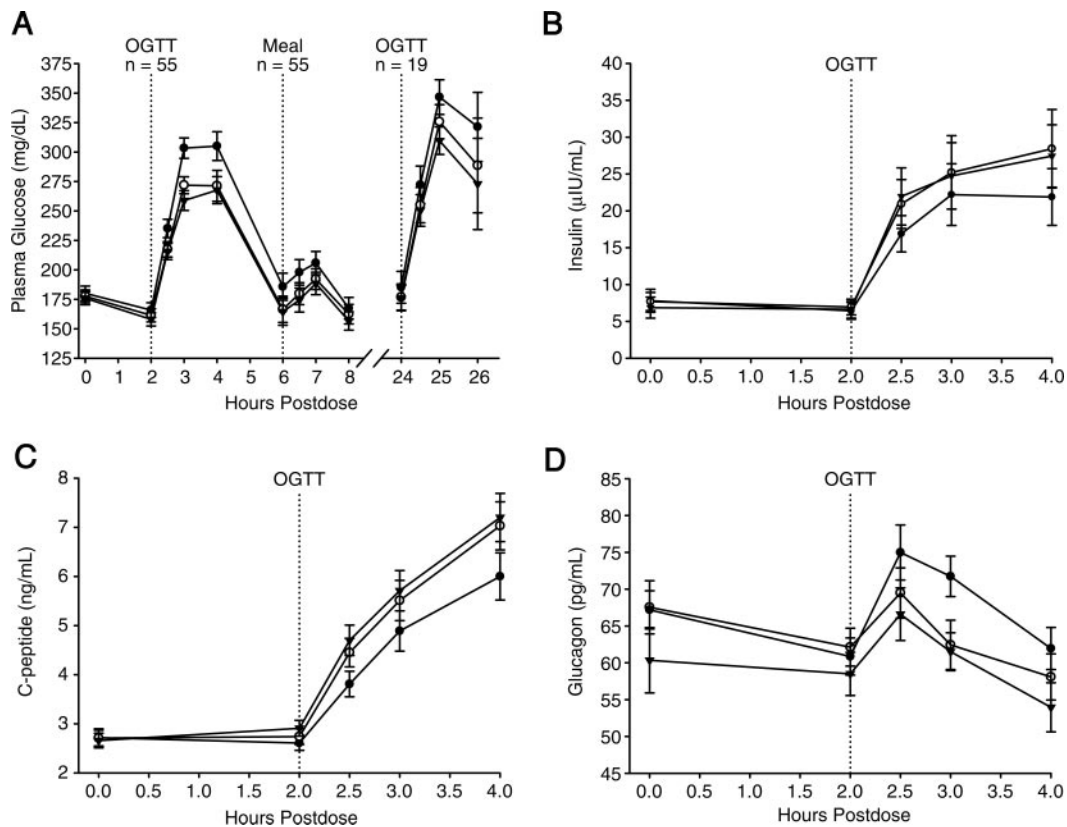


FIG. 4. Plasma glucose (A), serum insulin (B), serum C-peptide (C), and plasma glucagon (D) concentrations after administration of single oral doses of sitagliptin 25 (white circles) or 200 mg (black triangles) or placebo (black circles) and an OGTT at 2 h postdose. Plasma glucose concentrations are also displayed for the 2 h after a standardized meal at 6 h postdose and an OGTT at 24 h postdose. Data are expressed as geometric mean \pm SE.

(661.0) nM], C_{2h} [106 (43) and 1627 (791) nM], and C_{24h} [22.2 (7.5) and 96.3 (41.3) nM] were increased dose dependently, and median T_{max} was 4 and 2 h with sitagliptin 25 and 200 mg, respectively. In a subset of patients with plasma sampling up to 72 h postdose ($n = 7$), the apparent terminal half-life [harmonic mean (jackknife SD)] for sitagliptin averaged 13.1 (2.6) h for 25 mg and 11.0 (1.8) h for 200 mg.

PK/PD relationships

Sitagliptin PK/PD data were modeled in a subset of patients ($n = 19$) who completed an OGTT at both 2 and 24 h postdose. In this subset of patients, both doses of sitagliptin were associated with incretin effects and glucose-lowering after the OGTT at 2 h postdose that were similar to the results shown in Figs. 3C and 4C and Table 1 for the entire cohort. The relationship between plasma sitagliptin concentrations and the change in post-OGTT incremental glucose or active GLP-1 and GIP concentrations was explored with an empirical inhibitory E_{max} PK/PD model. For the purpose of this analysis, near-maximal effects were arbitrarily defined as 75% of the theoretical maximal effect (EC_{75}). In Table 2, the results are provided for the model that fit sitagliptin concentration *vs.* post-OGTT incremental glucose $AUC_{0-120\ min}$ GMR (sitagliptin/placebo) and the WAA active GLP-1 and GIP GMR (sitagliptin/placebo) along with EC_{75} results. The predicted EC_{75} values for reduction of post-OGTT incremental glucose and increases in active GLP-1 levels were both

approximately 100 nM, which was similar to the EC_{80} described above for inhibition of plasma DPP-4 activity. The predicted EC_{75} for effects on active GIP levels was lower at approximately 65 nM.

Tolerability

Sitagliptin doses were well tolerated, with an adverse experience profile that was generally similar to that observed with placebo. There were no serious adverse experiences during the sitagliptin treatment periods. One patient experienced a myocardial infarction 24 h postdose after receiving placebo, and the investigator did not consider this serious adverse experience to be related to study drug. No clinical or laboratory adverse experiences of hypoglycemia were reported. The incidence of gastrointestinal-related adverse experiences including nausea and vomiting was similar across the three treatments. No clinically significant, treatment-related changes from baseline were noted in routine blood and urine chemistry panels, complete blood count, electrocardiogram, vital signs, and physical examinations.

Discussion

The present study was the first clinical evaluation of the PK, PD, and tolerability of sitagliptin in patients with type 2 diabetes. The single dose PK profile of sitagliptin in these patients was similar to that previously observed in healthy

TABLE 1. Comparison of concentration-time AUCs for plasma glucose, insulin, C-peptide, and glucagon after single-dose administration of sitagliptin 25 or 200 mg or placebo and an OGTT at 2 or 24 h postdose

	OGTT at 2 h postdose			OGTT at 24 h postdose		
	Placebo (n = 54–55)	Sitagliptin, 25 mg (n = 54–55)	Sitagliptin, 200 mg (n = 54–55)	Placebo (n = 19)	Sitagliptin, 25 mg (n = 19)	Sitagliptin, 200 mg (n = 19)
Incremental glucose AUC (mg·h/dl) ^a	368.1	286.6	272.5	225.4	204.0	185.1
GMR (95% CI) ^b		0.78 (0.71, 0.85) ^c	0.74 (0.68, 0.81) ^c		0.91 (0.81, 1.02)	0.82 (0.73, 0.92) ^c
GMR (95% CI) ^d			0.95 (0.87, 1.04)			0.91 (0.81, 1.02)
Insulin AUC _(0–120 min) (μIU·h/ml)	38.0	46.3	46.1			
GMR (95% CI) ^b		1.22 (1.12, 1.33) ^c	1.21 (1.11, 1.32) ^c			
GMR (95% CI) ^d			0.99 (0.91, 1.08)	—	—	—
C-peptide AUC _(0–120 min) (ng·h/ml)	9.3	10.5	11.2			
GMR (95% CI) ^b		1.13 (1.07, 1.20) ^c	1.21 (1.13, 1.28) ^c			
GMR (95% CI) ^d			1.06 (1.00, 1.13) ^c	—	—	—
Glucagon AUC _(0–120 min) (pg·h/ml)	139.5	129.3	120.1			
GMR (95% CI) ^b		0.93 (0.87, 0.99) ^c	0.86 (0.81, 0.92) ^c			
GMR (95% CI) ^d			0.93 (0.87, 0.99) ^c	—	—	—

Data are expressed as geometric LS mean or geometric LS mean ratio (GMR) and 95% confidence interval (CI). —, Not measured at this time point.

^a Incremental glucose AUC_(0–240 min) for OGTT at 2 h postdose or AUC_(0–120 min) for OGTT at 24 h postdose.

^b GMR for sitagliptin/placebo.

^c $P \leq 0.001$.

^d GMR for sitagliptin 200 mg/sitagliptin 25 mg.

^e $P < 0.05$.

subjects (11). Plasma sitagliptin AUC increased in a dose-dependent manner. Sitagliptin dose-dependently inhibited plasma DPP-4 activity over 24 h. A simple E_{\max} model predicted an EC_{50} for plasma DPP-4 inhibition of approximately 25 nM and an EC_{80} of approximately 100 nM in these patients, which was nearly identical with the values estimated in healthy subjects (11).

In the present study, sitagliptin produced approximately 2-fold elevations in weighted average active GLP-1 and GIP levels during an OGTT 2 h postdose. At 24 h postdose, the 2-fold increases in active GLP-1 and GIP were still present for the 200-mg dose during either an OGTT or meal tolerance test. In agreement, vildagliptin 100 mg twice a day increased the mean GLP-1 and GIP levels (*i.e.* AUC/time) over the 13.5-h period after dosing by approximately 2-fold relative to placebo after 1 d of treatment (16). Sitagliptin enhanced incretin levels by inhibiting degradation of the active form rather than increasing secretion because the ratios of active to total GLP-1 and GIP were increased, and total GLP-1 or GIP levels were reduced. Augmentation in active GLP-1 and GIP levels and reduction in total GLP-1 and GIP levels were

observed in dogs with the DPP-4 inhibitor, NVP-DPP728, suggesting a possible feedback mechanism with increased secretion of intact peptides (17).

Treatment with DPP-4 inhibitors in the double incretin (GLP-1 and GIP) receptor knockout mice did not lower plasma glucose levels after an OGTT (18), suggesting that GLP-1 and GIP are major mediators of the glucoregulatory effects of DPP-4 inhibitors. These incretins lower glucose via multiple mechanisms including glucose-dependent increases in insulin (GLP-1 and GIP) and decreases in glucagon (GLP-1) release (19). In the present study, both insulin and C-peptide levels were significantly increased, and glucagon levels were significantly reduced with sitagliptin during an OGTT at 2 h postdose. These changes in glucoregulatory hormones were associated with 22–26% reduction in glucose excursion with sitagliptin treatment after an OGTT at 2 h postdose. The effectiveness of sitagliptin 200 mg was observed 24 h postdose with an 18% reduction in glucose observed during an OGTT. Effects on post-OGTT glucose were less apparent at 24 h postdose after the 25-mg dose. Treatment with other DPP-4 inhibitors has demonstrated similar

TABLE 2. Fitted PK/PD model parameters and predicted EC_{75} values for sitagliptin plasma concentration *vs.* GMRs for post-OGTT incremental glucose AUC_{0–120 min}, weighted average active GLP-1, and weighted average active GIP after single oral doses of sitagliptin and OGTTs

Model parameter	PD parameter		
	Post-OGTT incremental glucose AUC _{0–120 min} GMR ^a	Weighted average active GLP-1 GMR ^b	Weighted average active GIP GMR ^b
E_0	0.77 ± 0.02	0.99 ± 0.10	0.99 ± 0.08
E_{\max}	1.00 ± 0.02	2.47 ± 0.12	2.24 ± 0.09
EC_{50}	32 ± 16	37 ± 16	21 ± 10
Predicted EC_{75}	~100 nM	~110 nM	~65 nM

All parameters are fitted value ± SE. EC_{75} , 75% of a maximal effect (*e.g.* near-maximal).

^a GMR of sitagliptin/placebo: inhibitory sigmoid E_{\max} model.

^b GMR of sitagliptin/placebo: sigmoid E_{\max} model.

effects of postprandial glucose, insulin, and/or glucagon concentrations after a meal (7, 8, 16).

The levels of DPP-4 inhibition and active GLP-1 and GIP augmentation required for maximal postprandial glucose lowering in humans has not been completely elucidated with DPP-4 inhibitors. In contrast to therapy with GLP-1 analogs, DPP-4 inhibitor-related effects on incretin levels are episodic and meal related, making extrapolations from results with exogenously administered GLP-1 analogs problematic. Therefore, sitagliptin PK and PD data were evaluated in the 19 patients who completed an OGTT at both 2 and 24 h postdose. Before the OGTT at 2 h postdose, plasma sitagliptin levels were greater than 100 nM, and inhibition of plasma DPP-4 activity was greater than 80% with both sitagliptin doses. In response to an OGTT at 2 h postdose, active GLP-1 and GIP levels increased greater than 2-fold with both doses. At 24 h postdose, plasma sitagliptin concentration was approximately 100 nM with the 200-mg dose, providing about 80% plasma DPP-4 inhibition and was associated with nearly 2-fold augmentation of active GLP-1 and GIP levels after an OGTT. In contrast, at 24-h after the 25-mg dose, plasma sitagliptin concentration was less than 25 nM, DPP-4 inhibition was less than 50%, and 1.4-fold or less increases in active GLP-1 and GIP levels were observed after the OGTT. Based on these observations, near-maximal post-OGTT glucose-lowering effect with DPP-4 inhibition was associated with plasma sitagliptin concentrations of 100 nM or greater, inhibition of plasma DPP-4 activity of 80% or greater, and 2-fold or greater enhancement of active GLP-1 and GIP levels. Similarly, in sitagliptin-treated lean C57BL/6N mice, near-maximal glucose lowering efficacy during an OGTT was observed with approximately 80% DPP-4 inhibition and 2- to 3-fold increases in active GLP-1 levels (10).

These observations were corroborated by examining the relationship between sitagliptin plasma concentrations and stabilization of active GLP-1 and GIP levels or post-OGTT glucose lowering using E_{max} models. Based on this modeling, 80% inhibition of plasma DPP-4 activity (EC_{80}) was predicted to be associated with a plasma sitagliptin concentration of approximately 100 nM. Similarly, the predicted plasma concentration was approximately 100 nM for 75% of the maximal effects (EC_{75}) on reduction in post-OGTT incremental glucose levels, whereas the EC_{75} was approximately 110 nM and 65 nM for active GLP-1 and GIP levels, respectively.

Treatment with single doses of sitagliptin was generally well tolerated in this study. There were no treatment-related clinically relevant changes in vital signs and laboratory measurements with any treatment. Due to the effect of DPP-4 on the gut peptides, GLP-1 and GIP (20, 21), gastrointestinal-related adverse experiences including nausea and vomiting were of special clinical interest, and the incidence was similar among treatments in this study. Hypoglycemia was not observed, either by clinical or laboratory assessment. This result was not unexpected considering that glucose-lowering effects of incretins are glucose dependent (22).

In the present study in patients with type 2 diabetes, the PK/PD results predicted that sitagliptin doses that produced plasma sitagliptin concentrations of 100 nM or greater over 24 h would provide the necessary inhibition of plasma DPP-4 activity (80% or greater) and enhancement of active GLP-1

and GIP (2-fold or greater) to receive the near-maximal post-OGTT glucose lowering effect with DPP-4 inhibition. In healthy adults, single doses of sitagliptin of 100 mg or more were associated with plasma sitagliptin concentrations of at least 100 nM over 24 h (11). Longer-term studies are underway to assess the chronic efficacy of once-daily sitagliptin.

Acknowledgments

Received May 10, 2006. Accepted August 3, 2006.

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This work was supported by Merck & Co. (Whitehouse Station, NJ).

Summary of disclosure information: G.A.H., A.B., C.S., D.H., M.T., C.C., M.D.S., I.d.L., K.V.D., A.Q.W., W.Z., M.J.D., W.T., K.M.G., and J.A.W. are Merck & Co., Inc. or MSD employees and hold equity interests in the company. P.K., B.Y., and P.Z. were previously employed by Merck & Co., Inc. B.D., G.G., A.S., B.K., K.C.L., and M.S.K. have nothing to declare. J.J.H. consults for Merck & Co., Inc. and has received lecture fees from Merck & Co., Inc. C.F.D. consults for Novartis, Bristol Myers Squibb, and Takeda and has received lecture fees from Merck & Co., Inc.

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