PHARMACODYNAMICS AND DRUG ACTION

Pharmacokinetics and pharmacodynamics of sitagliptin, an inhibitor of dipeptidyl peptidase IV, in healthy subjects: Results from two randomized, double-blind, placebo-controlled studies with single oral doses

Background: **Sitagliptin (MK-0431 [(2R)-4-oxo-4-(3-[trifluoromethyl]-5,6-dihydro[1,2,4]triazolo[4,3-a]-pyrazin-7[8H]-yl)-1-(2,4,5-trifluorophenyl)butan-2-amine]) is an orally active, potent, and selective inhibitor of dipeptidyl peptidase IV (DPP-IV) currently in phase III development for the treatment of type 2 diabetes.**
Methods: **Two double-blind, randomized, placebo-controlled, alternating-panel studies evaluated the safety, tolerability, pharmacokinetics, and pharmacodynamics of single oral doses of sitagliptin (1.5-600 mg) in healthy male volunteers.**
Results: **Sitagliptin was well absorbed (approximately 80% excreted unchanged in the urine) with an apparent terminal half-life ranging from 8 to 14 hours. Renal clearance of sitagliptin averaged 388 mL/min and was largely uninfluenced by the dose administered. The area under the plasma concentration–time curve for sitagliptin increased in an approximately dose-dependent manner and was not meaningfully influenced by food. Single doses of sitagliptin markedly and dose-dependently inhibited plasma DPP-IV activity, with approximately 80% or greater inhibition of DPP-IV activity occurring at 50 mg or greater over a 12-hour period and at 100 mg or greater over a 24-hour period. Compared with placebo, sitagliptin produced an approximately 2-fold increase in postmeal active glucagon-like peptide 1 levels. Sitagliptin was well tolerated and was not associated with hypoglycemia.**
Conclusions: **This study provides proof of pharmacologic characteristics for sitagliptin in humans. By inhibiting plasma DPP-IV activity, sitagliptin increases the postprandial rise in active glucagon-like peptide 1 concentrations without causing hypoglycemia in normoglycemic healthy male volunteers. Sitagliptin possesses pharmacokinetic and pharmacodynamic characteristics that support a once-daily dosing regimen. (Clin Pharmacol Ther 2005;78:675-88.)**

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The global burden of type 2 diabetes is an ever-increasing problem, with a projected doubling of the prevalence of diabetes from approximately 171 million persons in 2000 to 336 million individuals worldwide by 2030.[1] Antihyperglycemic therapy represents a cornerstone of the treatment strategy for any diabetic patient. However, many patients remain inadequately treated because existing therapies have a number of shortcomings, including safety and tolerability issues (eg, hypoglycemia, weight gain, and gastrointestinal intolerance) and inconvenience of dosing regimens.[2] In addition, current therapies typically become less effective over time as a result of progressive loss of β-cell function. As a result, more than 50% of patients with diabetes do not achieve current glycemic goals.[3] A medical need, therefore, exists for additional antihyperglycemic therapies that can be used as monotherapy or in combination, that have a distinct mechanism of action from currently available agents, and that possess improved and durable efficacy with excellent tolerability.

Dipeptidyl peptidase (DPP) IV inhibitors represent a new therapeutic approach to the treatment of type 2 diabetes.[4] DPP-IV inhibitors are incretin enhancers that act by inhibiting the inactivation of incretins, particularly glucagon-like peptide 1 (GLP-1), which stimulate postprandial insulin secretion.[4-7] GLP-1 is normally released by enteroendocrine L cells into the circulation after a meal to potentiate glucose clearance, but its effects are short-lived as a result of rapid inactivation by DPP-IV.[4,8,9] Inhibition of DPP-IV extends the half-life of GLP-1, thereby promoting glucose-dependent biosynthesis and release of insulin and glycemic control.[10] Long-term treatment with DPP-IV inhibitors has been shown to reduce glycosylated hemoglobin levels, fasting plasma glucose levels, and postprandial glucose excursion and to be well tolerated in patients with type 2 diabetes.[11-13]

DPP-IV inhibitors have many theoretic advantages over other antihyperglycemic agents. Compared with currently available insulin secretagogues, DPP-IV inhibitors, by enhancing active (intact) levels of GLP-1, increase insulin release and suppress glucagon release in a glucose-dependent manner.[14] As a result, DPP-IV inhibitors may pose less of a hypoglycemia risk than that observed with insulin, sulfonylureas, or meglitinides. Second, unlike the weight gain typically found with insulin, sulfonylurea, or thiazolidinedione therapy,[2] a 52-week treatment period with LAF-237, a DPP-IV inhibitor, produced no weight gain.[13] Finally, DPP-IV inhibitors may have long-term beneficial effects on β-cell function and mass as demonstrated with long-term treatment in mice and rats.[15,16] DPP-IV inhibitors may also have several potential advantages over long-acting GLP-1 analogs, which are also being investigated for the treatment of diabetes.[17] Unlike GLP-1 analogs, DPP-IV inhibitors can be administered orally and are not expected to produce side effects such as nausea and vomiting that were noted with pharmacologic GLP-1 levels.[18-20]

Sitagliptin ([2R]-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro(1,2,4)triazolo(4,3-a)pyrazin-7(8H)-yl]-1-[2,4,5-trifluorophenyl]butan-2-amine) is an orally active, potent, and selective inhibitor of DPP-IV currently in phase III development for the treatment of type 2 diabetes.[21] In particular, sitagliptin is highly selective and demonstrates at least a 2600-fold margin over activity against the closely related enzymes DPP8 and DPP9.[21] Inhibition of one or both of these closely related enzymes with selective DPP8/DPP9 inhibitors was associated with multiorgan toxicity in experimental animals[22] and with attenuation of T-cell function in vitro.[23] The clinical relevance of DPP8/DPP9 inhibition is unknown. Preclinical studies indicate that sitagliptin inhibited DPP-IV activity, elevated active GLP-1 levels, and markedly reduced glucose levels after an oral glucose load.[21] In general, inhibition of DPP-IV activity by 80% or greater or a 2-fold augmentation of postprandial, active GLP-1 levels (or both) was associated with a maximal or near-maximal short-term lowering of glucose levels in these animal studies. To date, there are limited published data on the pharmacokinetic and pharmacodynamic properties of DPP-IV inhibitors in humans. The purpose of this study, representing the first introduction of sitagliptin to humans, was to evaluate the pharmacokinetics, pharmacodynamics, and tolerability of single oral doses of sitagliptin in healthy normoglycemic male subjects.

METHODS

Subjects

A total of 34 healthy male volunteers were enrolled into 2 separate study protocols. All subjects were nonsmokers with a mean age of 32.7 years (range, 18-45 years), weighed within 15% of the ideal height/weight range with a mean body weight of 76.8 kg (range, 61.8-86.9 kg), had a creatinine clearance of at least 80 mL/min, and were normoglycemic. Subjects were in good general health according to routine medical history, physical examination, vital signs, and laboratory data. Subjects were excluded if they had any relevant history of renal, hepatic, cardiovascular, gastrointestinal, or neurologic disease or had diabetes or impaired glucose tolerance. Subjects were also excluded if they

had donated blood, participated in another clinical study within 4 weeks before study start, or anticipated needing any prescription or nonprescription drugs.

Every subject gave written informed consent. The protocols were approved by the institutional review board of the study center. The protocols were conducted in accordance with the guidelines on good clinical practice and with ethical standards for human experimentation established by the Declaration of Helsinki.

Study design

Two double-blind, randomized, placebo-controlled, alternating-panel studies (protocols 001 and 002) were conducted at a single study center to assess the pharmacokinetics, pharmacodynamics, and tolerability of sitagliptin in healthy male volunteers with normal glucose concentrations. When combined, the 2 studies covered a broad range of sitagliptin doses from 1.5 to 600 mg. Subjects reported to the study unit on the evening before study drug administration and remained in the unit for up to 26 hours after dosing. Except for a period specifically for assessing the effect of food on sitagliptin pharmacokinetics, each dose was administered after an overnight fast.

Study 001 consisted of 4 study periods for each of 2 panels (A and B). A total of 6 subjects in each panel received single oral doses of sitagliptin and 2 subjects in each panel received placebo in a randomized, balanced manner. Subjects who had fasted in panel A received placebo or sitagliptin at doses of 1.5, 12.5, 50, and 200 mg, and those in panel B received placebo or sitagliptin at doses of 5, 25, and 100 mg. In period 4 of panel B, subjects received a repeat dose of 25 mg sitagliptin after a standard, high-fat breakfast (2 eggs, 2 bacon strips, 2 pieces of toast with butter, 55-110 g fried potato, and 250 mL whole milk) to assess the effect of food on pharmacokinetic parameters.

Study 002 consisted of 2 study periods for each of 2 panels (C and D). Six subjects in each panel received single oral doses of sitagliptin and 3 subjects in each panel received placebo in a randomized, balanced manner. Subjects who had fasted in panel C received placebo or sitagliptin at doses of 200 and 600 mg, and those in panel D received placebo or sitagliptin at doses of 400 and 600 mg.

In each protocol subjects received a standardized meal at 4, 10, and 24 hours after dosing to assess the pharmacodynamic effects of sitagliptin with respect to GLP-1, glucose, glucagon, insulin, and C peptide. The meal (grilled chicken, pasta, olive oil, diced peaches, and 250 mL water) totaled 2428 kJ, with a nutrient breakdown of 60% carbohydrate, 22% protein, and 18% fat.

Pharmacokinetic assessments

Blood (5 mL) was drawn via an indwelling intravenous catheter in a forearm vein and processed by centrifugation for determination of plasma sitagliptin concentrations before dosing; at 0.5, 1, 2, 4, 6, 8, 12, 16, and 24 hours after dosing; and, depending on the period and dose, at additional time points at 36 and 48 hours after dosing. Urine was collected from each subject administered doses of sitagliptin greater than 12.5 mg. Urine collections occurred before dosing and at the following intervals: 0 to 2 hours, 2 to 4 hours, 4 to 8 hours, 8 to 12 hours, 12 to 24 hours, 24 to 36 hours, and 36 to 48 hours after dosing. Urine was kept on ice during collection, with the volume being recorded to the nearest 5 mL, and a 4-mL aliquot was saved for analysis. Plasma and urine samples were stored at −70°C until assayed.

Sitagliptin was assayed in plasma and urine by use of a high-turbulence liquid chromatography online extraction method.[24,25] Sitagliptin and internal standard were detected by mass spectroscopy by use of selected reaction monitoring with turbo ion spray interface in the positive ion mode. The assay for sitagliptin in plasma was linear over the range of concentrations from 1.23 to 2350 nmol/L and had a lower limit of quantitation of 1.23 nmol/L (corresponding values for sitagliptin in urine were 0.246 to 123 μmol/L and 0.246 μmol/L, respectively).

Plasma concentrations were used to calculate the following sitagliptin pharmacokinetic parameters: the area under the plasma concentration–time curve ($AUC_{0-\infty}$), the maximum concentration observed in plasma (C_{max}) and its time of occurrence (t_{max}), and the apparent terminal half-life ($t_{½}$) for each subject after each dose. The half-life was estimated as the quotient of ln(2) and the apparent terminal rate constant (λ), estimated by regression of the terminal log-linear portion of the plasma sitagliptin concentration–time profile. $AUC_{0-\infty}$ was calculated by use of the linear trapezoidal method (ascending concentrations) and log trapezoidal method (descending concentrations) up to the last measured concentration, and the extrapolated area was given by the quotient of the last measured concentration and λ. The C_{max} and its t_{max} were obtained by inspection of the concentration-time data.

Urinary sitagliptin concentrations and urine volumes were used to calculate renal clearance (Cl_R) and the fraction of dose excreted unchanged in urine extrapolated to infinity ($f_{e,\ 0-\infty}$). The amount of sitagliptin

excreted unchanged in urine was calculated from the product of urine concentration of sitagliptin and volume. In addition, $f_{e,\ 0\text{-}\tau}$ was determined by the quotient of the sum of sitagliptin collected over all dosing intervals and the dose administered, and $f_{e,\ 0\text{-}\infty}$ was determined as the product of $f_{e,\ 0\text{-}\tau}$ and $AUC_{0\text{-}\tau}/AUC_{0\text{-}\infty}$, where τ represents the stop time of the final urine collection interval.

Pharmacodynamic assessments

Blood for enzyme activity and hormone and glucose analyses was obtained at selected time points. For the DPP-IV activity assay, blood samples were collected for all doses before dosing and at 0.5, 1, 2, 4 (premeal), 6, 8, 12, 16, and 24 (premeal) hours after dosing and only at 36 and 48 hours for sitagliptin doses greater than 25 mg. For hormone and glucose assays, blood samples were obtained for placebo and sitagliptin in doses of 12.5 to 600 mg before dosing; before the meals at 4, 10, and 24 hours after dosing; and at 0.25, 0.5, 1, and 2 hours after the meals at 4, 10, and 24 (24-hour meal for doses above 100 mg) hours after dosing. Blood was collected into tubes containing ethylenediaminetetraacetic acid (EDTA) alone (plasma DPP-IV assay and glucagon assay) or EDTA, DPP-IV inhibitor (Linco Research, St Charles, Mo), and aprotinin (plasma active and total GLP-1 assay). Blood for serum glucose, insulin, and C-peptide assays was collected in serum tubes containing clot activator (Sarstedt, Newton, NC). After centrifugation, plasma and serum samples were stored at −70°C or below until assayed. For glucagon samples, 50 μL of 0.4% aprotinin was added for each milliliter of plasma, and this plasma was stored in glass transfer vials at −20°C until analysis.

DPP-IV enzyme assay. DPP-IV enzyme activity was measured by incubating 40 μL EDTA-treated human plasma (2.5-fold dilution in assay) with the substrate glycyl-prolyl-paranitroaniline (400 μmol/L in assay) at 30°C and measuring the release of paranitroaniline by an increase in absorbance at 390 nm over time. The change in absorbance between each 30-second interval was averaged over a 10-minute period to calculate the slope for each individual sample. Enzyme activity was defined as the slope (in milli–optical density units per minute) from 4 to 14 minutes. The lower limit of reliable quantitation (ie, 20% interassay coefficient of variation [CV]) was a slope of 0.6 mOD per minute based on an analysis of an extra-low quality control that reads near this lower limit. For each subject, percent inhibition of plasma DPP-IV activity was plotted against plasma sitagliptin concentration and a simple E_{max} (maximum drug effect) model was used to determine the EC_{50} (concentration that achieves 50% of the maximum drug effect) by use of the Gauss-Newton method.[26]

Active and total GLP-1 assays. Active GLP-1 (GLP-1-[7-36] amide and GLP-1-[7-37]) was assayed by use of an enzyme-linked immunosorbent assay kit according to manufacturer specifications (Linco Research). There was lot-to-lot variability between different kits. The lower limit of reliable quantitation was estimated to be 0.70 pmol/L for study 001 and 2.0 pmol/L for study 002. Total GLP-1 was assayed by use of a radioimmunoassay (RIA) kit (Linco Research) with a modified assay procedure. For RIA, plasma (300 μL) was mixed with 0.03% N-octyl BD-glucopyranoside and 0.5% bovine serum albumin (final concentrations) and extracted with 1.0 mL of 95% ethanol. The resulting precipitant was re-extracted with 500 μL of 95% ethanol. The supernatants were then pooled and dried under a stream of nitrogen for 3 hours at room temperature. Dried extract was rehydrated in 300-μL sample hydrating solution and assayed in the RIA in a 96-well plate format. Separation of free and bound radioactive ligand was performed by filtration onto a 96-well glass fiber filter plate and counted. The lower limit of reliable quantitation was estimated to be 4.45 pmol/L, which was based on day-to-day precision for the low total GLP-1 quality control sample provided with the assay.

Glucose, insulin, glucagon, and C-peptide assays. Serum glucose concentrations were quantitated by a hexokinase enzymatic assay on a Hitachi 747 automated analyzer (Roche Diagnostics, Indianapolis, Ind). This assay was linear in the range from 0 to 750 mg/dL, with an interassay CV of 1.3%. Serum insulin concentrations were quantitated by electrochemiluminescence on the ELECSYS automated analyzer (Roche Diagnostics). This assay had a working range of 0.2 to 100 μIU/mL and an interassay CV of 5.5%. Plasma glucagon concentrations were measured by double-antibody RIA (Diagnostic Products, Los Angeles, Calif). The lower limit of detection was 13 pg/mL, and the interassay CV was 9%. Serum C peptide levels were measured by double-antibody RIA (Diagnostic Products). The lower limit of detection was 0.22 ng/mL, and the interassay CV was 7.1%.

Safety and tolerability

Physical examinations, vital signs, 12-lead electrocardiograms (including assessment for QTc- and PR-interval prolongation), and safety laboratory measurements comprising routine hematology, serum chemistry (including liver transaminases), and urinalysis were performed before the study, at various time points after dosing, and after the study. Hypoglycemia was also

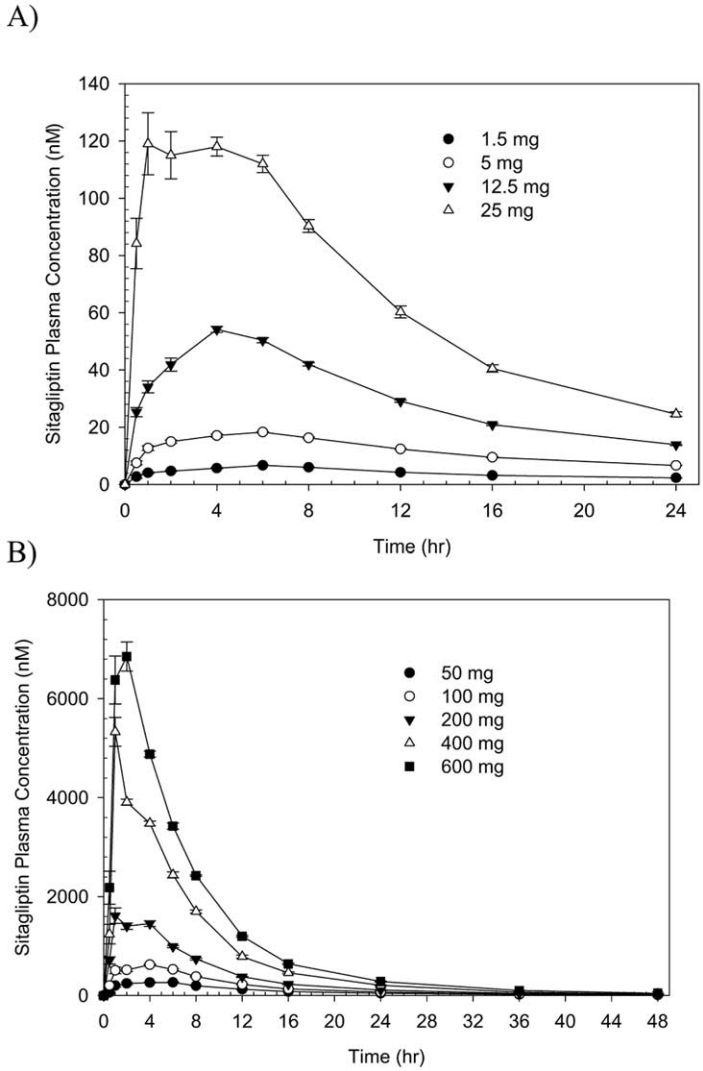

Fig 1. Plasma concentration–time profiles of sitagliptin (in nanomoles per liter) after ingestion of single oral doses of sitagliptin, 1.5 to 25 mg (**A**) and 50 to 600 mg (**B**). Data represent mean values (±SE) in 6 healthy male subjects who fasted.

monitored by frequent glucometer measurements over the first 24-hour period after dosing, as well as by clinical assessment. Adverse experiences were monitored throughout the study. Investigators evaluated all clinical adverse experiences in terms of intensity (mild, moderate, or severe), duration, severity, outcome, and relationship to study drug.

Statistical analysis

Sitagliptin pharmacokinetic parameters ($AUC_{0-\infty}$, C_{max}, t_{max}, and $t_{½}$) were analyzed by use of a mixed-effect ANOVA model appropriate for an alternating-panel, rising-dose design. The effects of a standardized breakfast on log-transformed $AUC_{0-\infty}$ and C_{max} values for a single sitagliptin dose were also assessed by use of an ANOVA model. Linear contrasts were used to estimate the 95% confidence intervals (CIs) for the geometric mean ratio of fed versus fasted. ANOVA was also used to compare the effect of sitagliptin versus placebo on the weighted average inhibition (WAI) of DPP-IV activity. WAI was the percent inhibition calculated for the weighted average DPP-IV activity through 12 and 24 hours relative to the baseline activity (area under DPP-IV inhibition-time curve divided by

Table I. Pharmacokinetic parameters for sitagliptin in healthy young men after administration of single oral doses of sitagliptin (1.5-600 mg)

Parameter	*1.5 mg*	*5 mg*	*12.5 mg*	*25 mg*
$AUC_{0-\infty}$ (μmol/L · h)*	0.158 ± 0.029	0.434 ± 0.029	0.948 ± 0.112	1.96 ± 0.35
C_{max} (nmol/L)*	7.28 ± 0.86	19.5 ± 1.66	52.7 ± 6.78	137 ± 43
t_{max} (h)†	6	6	4	1.5
Apparent $t_{½}$ (h)‡	14.1 ± 3.4	13.7 ± 2.1	11.4 ± 0.6	9.51 ± 0.4
$f_{e,\ 0-\infty}$§	—	—	0.706 ± 0.071	0.752 ± 0.067
Cl_R (mL/min)	—	—	376 ± 16.3	396 ± 82.2

$AUC_{0-\infty}$, Area under plasma concentration–time curve; C_{max}, maximum concentration observed in plasma; t_{max}, time to maximum concentration observed in plasma; $t_{½}$, half-life; $f_{e,\ 0-\infty}$, fraction of sitagliptin dose excreted unchanged in urine; Cl_R, renal clearance.
*Geometric least squares mean back-transformed from log scale ± SD.
†Median.
‡Harmonic mean ± jackknife SD.
§Arithmetic least squares mean ± SD.

12 or 24 hours). For each dose within a panel, a 2-sided 95% CI was calculated by the method of Wong et al.[27] The effect of sitagliptin on plasma concentrations of active GLP-1 and total GLP-1 and glucagon and serum concentrations of glucose, insulin, and C peptide was determined for the 2 hours after standardized meals at 4, 10, and 24 (for doses above 100 mg) hours after dosing with a similar procedure (area under concentration-time curve divided by 2 hours). For all ANOVA analyses, assumptions of normality and homogeneity of variances were tested by use of the Shapiro-Wilk test and Levene test, respectively.

RESULTS

Pharmacokinetics

The plasma sitagliptin concentration profile and the principal sitagliptin pharmacokinetic parameters after single oral doses of sitagliptin (1.5-600 mg) are summarized in Fig 1 and Table I. Plasma sitagliptin $AUC_{0-\infty}$ increased approximately dose-proportionally over the dose range studied (1.5-600 mg). The apparent $t_{½}$ for sitagliptin was determined to be approximately 8 to 14 hours. Median t_{max} values across doses ranged from 1 to 6 hours. A trend toward a shorter t_{max} was apparent with increasing sitagliptin dose.

The fraction of the sitagliptin dose excreted unchanged in urine was generally independent of dose and averaged approximately 80% (Table I). The renal clearance of sitagliptin was also generally independent of dose and averaged approximately 388 mL/min (pooled across doses) (Table I).

A standard high-fat breakfast did not have a clinically meaningful effect on the extent or rate of absorption of a single oral 25-mg dose of sitagliptin. Mean sitagliptin plasma concentrations after a 25-mg dose of sitagliptin in the fed or fasted state are shown in Fig 2. The ratio of the least squares means (fed/fasted) was 1.01 (95% CI, 0.94-1.10) for $AUC_{0-\infty}$ and 1.21 (95% CI, 1.00-1.45) for C_{max}. The administration of food before dosing produced no significant difference in median t_{max} (2 hours versus 1.5 hours), harmonic mean $t_{½}$ (9.9 hours versus 9.5 hours), Cl_R (405 mL/min versus 396 mL/min), and $f_{e,\ 0-\infty}$ (0.78 versus 0.75) compared with values obtained in the fasted state.

Pharmacodynamics

The time profiles for mean plasma DPP-IV inhibition after administration of single oral doses of sitagliptin are shown in Fig 3. A dose-related increase in percent inhibition of plasma DPP-IV enzyme activity occurred over the sitagliptin dose range from 1.5 to 600 mg. DPP-IV WAI was significantly different ($P < .001$) between all doses of sitagliptin and placebo at 12 and 24 hours after dosing (Table II). The lower bounds of the 95% CI were greater than 0 for all doses, indicating that the plasma DPP-IV activity was significantly inhibited at all doses. The lower bounds of the 95% CI of DPP-IV WAI (difference from placebo) over a 12-hour period were greater than 80% for sitagliptin doses of 50 mg or higher, and the lower bounds of the 95% CI of DPP-IV WAI (difference from placebo) over a 24-hour

50 mg	*100 mg*	*200 mg*	*400 mg*	*600 mg*
4.13 ± 0.24	7.76 ± 1.10	15.4 ± 1.62 (panel A) 16.2 ± 1.33 (panel C)	36.8 ± 2.57	55.0 ± 3.65 (panel C) 51.6 ± 4.57 (panel D)
320 ± 97	747 ± 232	1720 ± 485 (panel A) 2380 ± 538 (panel C)	5000 ± 1710	8440 ± 2340 (panel C) 7000 ± 1840 (panel D)
6	4	1 (panel A) 1.5 (panel C)	1	1.5 (panel C) 2 (panel D)
11.7 ± 1.2	10.1 ± 0.9	9.45 ± 1.18 (panel A) 8.84 ± 0.59 (panel C)	8.14 ± 0.62	8.00 ± 0.55 (panel C) 8.64 ± 0.75 (panel D)
0.801 ± 0.056	0.796 ± 0.065	0.815 ± 0.073 (panel A) 0.803 ± 0.089 (panel C)	0.887 ± 0.048	0.856 ± 0.082 (panel C) 0.662 ± 0.066 (panel D)
395 ± 39.6	416 ± 75.3	440 ± 56.3 (panel A) 407 ± 58.1 (panel C)	397 ± 25.8	378 ± 28.9 (panel C) 315 ± 41.8 (panel D)

period were approximately greater than 80% for 100 mg or higher (the lower bound was 77.4% for 100 mg).

The pharmacokinetic-pharmacodynamic relationship between plasma concentrations of sitagliptin (pooled across all subjects) and inhibition of plasma DPP-IV activity is shown in Fig 4. Inspection of individual plots of DPP-IV activity versus time suggested that there was no hysteresis (data not shown); that is, for each subject, inhibition of plasma DPP-IV activity was described by plasma sitagliptin concentration alone and was independent of time. Approximately 50% of DPP-IV inhibition occurred at a plasma sitagliptin concentration of approximately 26 nmol/L, and 80% of inhibition occurred at a concentration of approximately 100 nmol/L.

Active GLP-1 concentrations over the 2-hour period after standardized meals at 4, 10, and 24 hours were augmented by sitagliptin compared with placebo (Fig 5). The weighted average active GLP-1 concentrations were significantly higher than placebo for sitagliptin doses of 12.5 mg or greater ($P < .05$). The 95% CI for the geometric mean ratio (active dose/placebo) for sitagliptin doses of 12.5 mg or higher contained the value 2.00, indicating that these doses produced a 2-fold increase in weighted average active GLP-1 levels compared with placebo (Table III).

No significant differences in total GLP-1 concentrations occurred with doses of sitagliptin compared with placebo. The weighted average ratio of active to total GLP-1 levels was significantly greater than placebo at doses of sitagliptin of 12.5 mg or greater ($P < .05$). Sitagliptin doses at 12.5 mg or higher were generally associated with an increase of approximately 2-fold from placebo in weighted average ratio of active to total GLP-1 levels after standardized meals at 4, 10, and 24 hours.

Sitagliptin (12.5-600 mg) produced no clinically meaningful effect on glucose, insulin, glucagon, or C-peptide levels over a 2-hour period after standardized meals at 4, 10, and 24 hours (Table IV).

Safety and tolerability

Sitagliptin was generally well tolerated. No subject had serious clinical or laboratory adverse experiences or discontinued the study because of a clinical adverse experience. A total of 82 nonserious clinical adverse experiences were reported; 3 (common cold, eye irritation, and headache) occurred before the study, and 79 occurred during the study. Of the adverse experiences occurring during the study, a total of 19 (14 in the sitagliptin group and 5 in the placebo group) were considered to be possibly related to study drug.

Adverse experiences were mild to moderate, were transient, and resolved without treatment. There were no reports of clinical or laboratory adverse experiences of hypoglycemia, as assessed by frequent glucometer measurements or signs and symptoms of hypoglycemia. In one subject a laboratory adverse experience developed that preceded administration of active drug (leukocyturia before and 24 and 48 hours after the 200-mg and 600-mg sitagliptin doses); this was considered by the investigator to be definitely not drug-related. Another subject had symptomatic hypotension, including a brief episode of syncope after a 600-mg

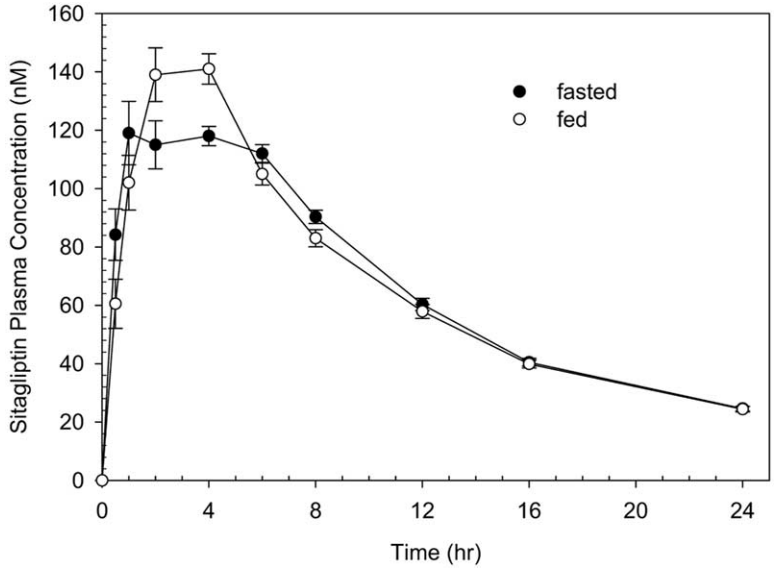

Fig 2. Plasma concentration–time profiles of sitagliptin (in nanomoles per liter) in fasted state and after standard high-fat breakfast after ingestion of single oral dose of 25 mg sitagliptin. Data represent mean values (±SE) in 6 healthy male subjects.

single oral dose. Because the adverse event occurred in the setting of excessive temperature in the unit, it was speculated that it may have been a result of environmental circumstances. Subsequently, this episode was determined unlikely to be related to the study drug because the subject showed no orthostatic signs or symptoms when rechallenged with the same dose of sitagliptin when temperatures returned to normal.

DISCUSSION

The combined results of 2 double-blind, placebo-controlled, single-dose studies are the first clinical data for sitagliptin in humans. By showing sustained inhibition of plasma DPP-IV activity and augmentation of postmeal active GLP-1 levels, these results provide proof of pharmacology for sitagliptin in humans and also indicate that sitagliptin has the pharmacokinetic and pharmacodynamic characteristics that support a once-daily dosing regimen and was generally well tolerated in healthy male subjects.

Absorption of sitagliptin was extensive, with approximately 80% of the oral dose excreted unchanged in the urine. The plasma $AUC_{0\text{-}\infty}$ and C_{max} increased with dose over the range of sitagliptin doses evaluated. $AUC_{0\text{-}\infty}$ for sitagliptin increased in an approximately dose-dependent manner, whereas C_{max} increased greater than dose-proportionally. The apparent terminal $t_{½}$ of sitagliptin ranged from 8 to 14 hours and appeared to decrease with increasing dose. The renal clearance of sitagliptin was estimated to be 388 mL/min and was largely uninfluenced by the dose administered. Sitagliptin is likely excreted through an active renal secretion process because the renal clearance of sitagliptin exceeded the typical glomerular filtration rate of normal healthy subjects (125 mL/min). Given that the plasma $AUC_{0\text{-}\infty}$ for sitagliptin increased dose-dependently and the renal clearance did not, the small differences observed in $t_{½}$ across doses are likely the result of nonlinearities in the distribution of sitagliptin rather than differences in renal clearance.

The rate of absorption but not the extent of absorption appeared to be impacted slightly by consumption of a standard high-fat breakfast. The $AUC_{0\text{-}\infty}$ for sitagliptin was largely uninfluenced by food, but a slight increase of approximately 20% in the plasma C_{max} occurred in the fed state compared with the fasted state, a difference that is not likely to be clinically meaningful with long-term dosing.

Sitagliptin demonstrated marked inhibition of plasma DPP-IV activity in a dose-dependent manner. Sitagliptin doses of 50 mg or higher produced at least 80% inhibition of DPP-IV activity over a 12-hour period, and doses of 100 mg or higher produced 80% inhibition over a 24-hour period. These findings, together with the observation that

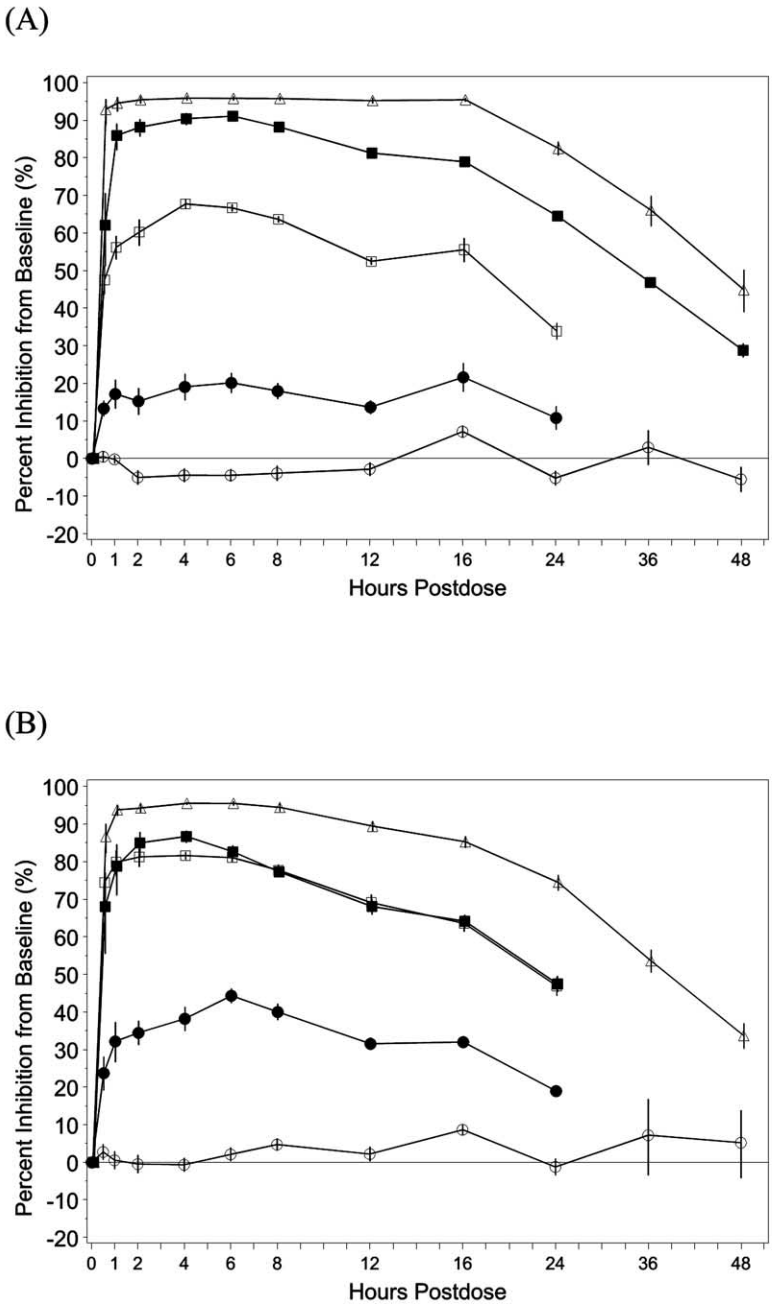

Fig 3. Time course of inhibition of plasma dipeptidyl peptidase IV (DPP-IV) activity after administration of placebo (*open circles*) or single oral doses of sitagliptin—1.5 (*solid circles*), 12.5 (*open squares*), 50 (*solid squares*), and 200 (*open triangles*) mg (**A**) and 5 (*solid circles*), 25 (fed [*solid squares*] and fasted [*open squares*]), and 100 (*open triangles*) mg (**B**)—to healthy young male subjects. Data represent mean percent inhibition from baseline values (±SE).

the apparent terminal $t_{½}$ of sitagliptin ranges from 8 to 14 hours, are consistent with a once-daily dosing regimen. Data in rodent models found that near-maximal glucose lowering is correlated with inhibition of plasma DPP-IV activity of 80% or greater.[21] Analysis of the pharmacokinetic-pharmacodynamic relationship between plasma sitagliptin concentration and inhibition of plasma DPP-IV activity indicates inhibition of plasma DPP-IV

Table II. Summary statistics for weighted average inhibition (percent) of plasma DPP-IV activity through 12- and 24-hour periods after administration of single oral doses of 1.5 to 600 mg sitagliptin or placebo in healthy young male subjects

	12 h After dosing				*24 h After dosing*			
Treatment	*No.*	*Percent inhibition mean*†*	*Mean difference from placebo†*	*95% CI‡*	*No.*	*Percent inhibition mean*†*	*Mean difference from placebo†*	*95% CI‡*
Panel A								
Placebo	8	−3.6			8	−1.2		
1.5 mg	6	17.7	21.4	10.0-32.8	6	17.0	18.2	9.5-26.8
12.5 mg	6	59.6	63.3	54.5-72.2	6	53.6	54.8	47.9-61.8
50 mg	6	84.2	87.8	80.7-95.3	6	79.3	80.4	74.8-86.2
200 mg	6	93.1	96.8	90.2-100.0	6	92.0	93.1	88.1-98.4
Panel B								
Placebo	6	3.3			6	4.0		
5 mg	6	36.3	33.0	22.5-43.7	6	32.4	28.5	20.2-36.8
25 mg	6	75.9	72.6	64.8-80.8	6	67.3	63.3	56.8-70.0
25 mg (fed)	6	75.9	72.5	64.7-80.7	6	67.3	63.4	56.9-70.0
100 mg	6	91.3	88.0	81.2-95.2	6	86.9	82.9	77.4-88.6
Panel C								
Placebo	6	−2.9			6	−1.2		
200 mg	6	93.0	96.0	88.8-100.0	6	90.1	91.3	85.7-97.2
600 mg	6	94.5	97.3	90.2-100.0	6	94.2	95.4	89.9-100.0
Panel D								
Placebo	6	−4.4			6	−1.3		
400 mg	6	93.8	98.2	91.0-100.0	6	93.4	94.7	89.2-100.0
600 mg	6	93.9	98.2	91.0-100.0	6	94.4	95.7	90.2-100.0

DPP-IV, Dipeptidyl peptidase IV; CI, confidence interval.
*Percent inhibition mean is calculated as (1 − GMR) × 100, where GMR is the geometric mean ratio of the weighted average DPP-IV enzyme activity through the 12-hour period after dosing relative to plasma DPP-IV enzyme activity before dosing.
†Back-transformed from the log percent scale.
‡CIs were calculated by method 5.[27]

activity of 80% or greater when sitagliptin plasma concentrations are at least 100 nmol/L. Because no corrections for dilution during the assay procedure were used, the extent of inhibition of DPP-IV activity in vivo would be higher (eg, 80% inhibition in this assay would be estimated to represent >90% inhibition in vivo if the Michaelis-Menten equation is applied). If the degree of DPP-IV inhibition that produced near-maximal glycemic efficacy in rodent models is reasonably predictive, then once-daily doses of 100 mg or higher would be expected to be associated with near-maximal glucose lowering in diabetic subjects.

Consistent with marked inhibition of DPP-IV activity, sitagliptin resulted in increases in the postprandial rise in active GLP-1 concentrations, an incretin hormone that is degraded by plasma DPP-IV.[4,8,9] Glucose-dependent insulinotropic polypeptide (GIP) also has insulinotropic activity and is inactivated by DPP-IV.[8] Although increases in active GIP levels would also be expected, the effect of DPP-IV inhibition on GIP levels was not assessed in our study. In a subsequent study in patients with type 2 diabetes, sitagliptin was also shown to enhance active GIP levels (Herman GA, unpublished data, 2005). Sitagliptin produced postmeal increases in GLP-1 that were approximately 2-fold higher than corresponding values for placebo. This increase is also consistent with near-maximal short-term glucose-lowering efficacy in preclinical studies.[21] Inhibition of sitagliptin appeared to increase incretin levels by stabilizing the active form of GLP-1 rather than increasing secretion. This assertion is supported by the finding that sitagliptin increased postmeal active GLP-1 levels, as well as the ratio of active to total GLP-1 levels, but had no impact on total GLP-1 levels. The sustained inhibition of DPP-IV and augmentation of GLP-1 levels provide pharmacologic proof of concept for sitagliptin in humans.

There was no clinically meaningful effect of sitagliptin on fasting and postmeal levels of glucose, insulin, glucagon, and C peptide in healthy men with normal

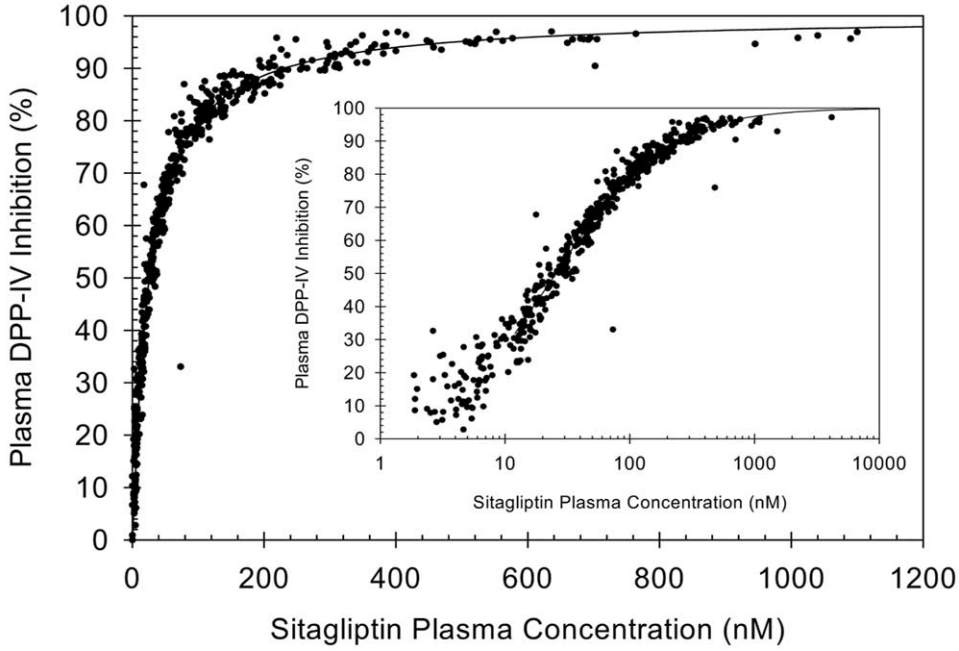

Fig 4. Individual values for plasma sitagliptin concentrations and percent inhibition of plasma DPP-IV activity after administration of single oral doses of sitagliptin over range of 1.5 to 600 mg to healthy male subjects. *Inset* represents plot on a semilogarithmic scale.

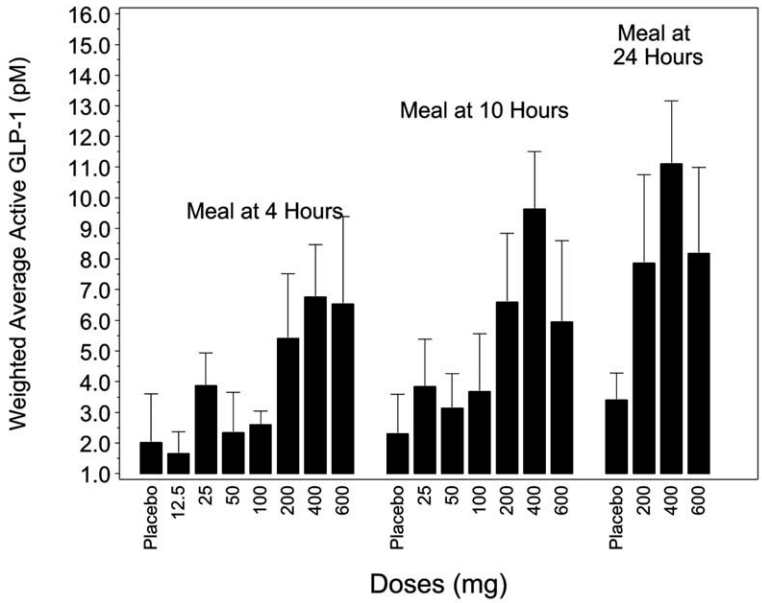

Fig 5. Weighted average active glucagon-like peptide 1 (GLP-1) plasma concentration (in picomoles per liter) over 2-hour interval after standardized meals at 4, 10, and 24 hours after administration of single oral doses of 12.5 to 600 mg sitagliptin to healthy young male subjects. Data are expressed as mean ± SE.

Table III. Mean weighted average active GLP-1 plasma concentrations (in picomoles per liter) over 2-hour interval after standard meals at 4, 10, and 24 hours after dosing with single oral doses of sitagliptin or placebo in healthy young male subjects

	Meal at 4 h after dosing				*Meal at 10 h after dosing*				*Meal at 24 h after dosing*			
Treatment	*No.*	*Mean**	*GMR†*	*95% CI†*	*No.*	*Mean**	*GMR†*	*95% CI†*	*No.*	*Mean**	*GMR†*	*95% CI†*
Panel A												
Placebo	4	0.83			2	1.16			—	—	—	—
12.5 mg	6	1.61	1.94	1.28-2.93	—	—	—	—	—	—	—	—
50 mg	6	2.34	2.82	1.86-4.27	6	3.13	2.69	1.50-4.83	—	—	—	—
Panel B												
Placebo	4	1.59			4	1.74			—	—	—	—
25 mg	6	3.87	2.44	1.61-3.69	6	3.75	2.15	1.44-3.21	—	—	—	—
100 mg	6	2.53	1.59	1.05-2.41	6	3.65	2.09	1.40-3.12	—	—	—	—
Panel C												
Placebo	6	2.90			6	2.86			6	3.16		
200 mg	6	5.34	1.84	1.28-2.66	6	6.51	2.27	1.60-3.23	6	7.81	2.47	1.84-3.31
600 mg	6	5.79	2.00	1.38-2.89	6	5.90	2.06	1.45-2.93	6	6.78	2.14	1.60-2.88
Panel D												
Placebo	6	3.09			3	3.67			6	3.76		
400 mg	6	6.85	2.22	1.53-3.20	6	9.62	2.62	1.58-4.35	6	11.33	3.01	2.24-4.04
600 mg	6	7.20	2.33	1.61-3.36	—	—	—	—	6	9.36	2.49	1.85-3.34

GLP-1, Glucagon-like peptide 1; GMR, geometric mean ratio based on least squares means; dash, active GLP-1 was not measured for this dose at this specific time.
*Least squares means were used because of the unbalanced design and back-transformed from the log scale.
†GMR and 95% CI were determined for the comparisons between active doses and placebo within panels.

Table IV. Glucose, insulin, C-peptide, and glucagon area under the plasma concentration–time curve values over 2-hour interval after a standardized meal at 4 hours after dosing with single oral doses of sitagliptin or placebo in healthy male subjects

Parameter	*Placebo (n = 20)**	*12.5 mg (n = 6)*	*25 mg (n = 6)*	*50 mg (n = 6)*	*100 mg (n = 6)*	*200 mg (n = 12)†*	*400 mg (n = 6)*	*600 mg (n = 12)*
Glucose (mg · h/dL)	206.7 (21.5)	173.3 (20.3)	170.3 (32.6)	—	188.4 (31.4)	186.1 (18.0)	196.5 (13.1)	189.7 (17.6)
Insulin (μIU/mL)	31.8 (12.5)	24.7 (9.2)	26.0 (6.3)	31.4 (12.2)	36.5 (14.8)	32.7 (10.7)	32.9 (18.1)	30.1 (14.8)
C-peptide (ng/mL)	6.4 (1.6)	—	—	—	—	6.5 (1.1)	7.2 (2.9)	7.4 (2.0)
Glucagon (pg · h/mL)	95.1 (50.1)	—	—	—	—	79.7 (44.6)	124.1 (25.8)	103.1 (40.6)

Data are given as mean with SD in parentheses.
Dash, No measurements or missing data at the dose for this specific parameter.
*n = 22 for insulin and n = 12 for C peptide and glucagon.
†n = 6 for C peptide and glucagon.

glucose concentrations. An explanation for the lack of response to enhanced GLP-1 levels with sitagliptin may be related to the actual increase in circulating active GLP-1 levels. Pharmacologic doses but not physiologic doses of GLP-1-[7-36] amide via subcutaneous injection have been shown to increase insulin and C-peptide levels and lower glucose and glucagon levels in the fasted state in healthy men.[28] Similar efficacy was noted with intravenous infusion of single doses of GLP-1-[7-36] amide at pharmacologic levels but not at physiologic levels on fasting and post–glucose challenge glycemic responses in healthy male subjects.[29] In our study active GLP-1 levels were enhanced with sitagliptin, but these levels were within the physiologic range. Thus treatment with sitagliptin would not be expected to influence premeal and postmeal glycemic efficacy in healthy, nondiabetic men. In contrast, single doses of sitagliptin (25 mg and 200 mg) were insulinotropic and

glucagonostatic and also reduced postprandial glucose concentrations in response to an oral glucose tolerance test in patients with type 2 diabetes.[30]

Administration of single doses of sitagliptin over a wide dose range (1.5-600 mg) did not result in serious adverse experiences or discontinuations. Clinical adverse experiences associated with sitagliptin were qualitatively similar to those seen with placebo and were generally mild, transient, and self-limited. In particular, there were no episodes of hypoglycemia, on the basis of either laboratory or glucometer assessment or adverse experiences, a finding consistent with expectations, because GLP-1–mediated insulin release is glucose-dependent.[14] Moreover, the absence of an effect of sitagliptin on glycemic parameters in healthy subjects in this study, together with the observed lack of effect on signs and symptoms of hypoglycemia, is consistent with the notion that DPP-IV inhibition, even if maximal, carries a low risk of hypoglycemia. Sitagliptin was not associated with any clinically significant treatment-related adverse effects as assessed by measurement of blood cell counts, transaminase and serum creatinine levels, vital signs, or electrocardiographic parameters such as QTc- or PR-interval prolongation.

In summary, sitagliptin has an apparent terminal half-life and an inhibitory effect on plasma DPP-IV activity of a duration and magnitude that should permit a once-daily dosing regimen and was generally well tolerated across the dose range studied in healthy male volunteers.

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