A Randomized, Open-Label, Crossover Study Evaluating the Effect of Food on the Relative Bioavailability of Linagliptin in Healthy Subjects

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ABSTRACT

Objective: The objective of this study was to determine the relative bioavailability of the dipeptidyl-peptidase-4 (DPP-4) inhibitor linagliptin when administered with and without food, in accordance with regulatory requirements to support dosing recommendations for patients.

Methods: This was a randomized, open-label, crossover study involving 32 healthy white male and female subjects. All subjects received a single dose of 5 mg linagliptin after an overnight fast of at least 10 hours, or immediately after ingestion of a high-fat, high-calorie breakfast. These treatments were separated by a period of 5 weeks. Plasma samples for pharmacokinetic analysis were collected before dosing and at prespecified time points after dosing. The concentration of linagliptin in these samples was analyzed by high-performance liquid chromatography coupled to tandem mass spectrometry. Relative bioavailability was assessed by the total area under the curve between 0 and 72 hours (AUC_{0-72}) and maximum measured plasma concentration (C_{max}) of linagliptin. Tolerability was also assessed.

Results: In 32 subjects (mean age, 34.8 years; weight, 74.3 kg; male, 53%; white race, 100%), intake of a high-fat meal resulted in comparable bioavailability with regard to AUC_{0-72} (geometric mean ratio [GMR] between the fed and fasted group means was 103.5%; 90% CI, 98.1%–109.2%). Individuals' responses to food ranged from a maximum increase in exposure of 38% to a decrease of 32% relative to the fasted state. The concurrent intake of food increased the time to reach maximum plasma concentration (T_{max}) by approximately 2 hours and reduced C_{max} by about 15% (GMR 84.7%; 90% CI, 75.9%–94.6%). Since adequate drug exposure for inhibition of DPP-4 was still given for the entire 24-hour dosing interval, this result was considered to be of no clinical relevance. Linagliptin was well tolerated during the study.

Conclusions: Intake of a high-fat meal reduced the rate of linagliptin absorption but had no influence on the extent of absorption; this finding suggests that food has no relevant influence on the efficacy of linagliptin. (*Clin Ther.* 2011;33:1096–1103)

Key words: bioavailability, DPP-4 inhibitor, food, linagliptin, pharmacokinetics, type 2 diabetes.

INTRODUCTION

Linagliptin is a structurally novel dipeptidyl-peptidase-4 (DPP-4) inhibitor currently in late-stage development for the treatment of type 2 diabetes,[1,2] with high selectivity for DPP-4 relative to other dipeptidyl-peptidases.[1] In an extensive multinational program of Phase III studies, linagliptin was well tolerated and improved glycemic control in patients with type 2 diabetes as monotherapy or in combination with other antihyperglycemic agents.[3–9]

Linagliptin has nonlinear pharmacokinetics owing to the high-affinity binding of linagliptin to DPP-4 in plasma and tissues.[10,11] The high affinity of linagliptin for DPP-4 is also responsible for the long terminal half-life ($t_{½}$) of the drug, at >130 hours.[10,12] However, these binding sites are present at low concentrations (5–6 nM in human plasma) and are therefore readily saturated at human therapeutic dose levels.[13–16] Once the

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DPP-4 binding sites have been saturated, the unbound linagliptin is eliminated quickly ($t_{½}$ of 11.1 h).[16] This leads to an accumulation $t_{½}$ of linagliptin of approximately 11 hours, minimal accumulation of the drug, and a less-than-proportional increase in drug exposure with increasing doses.[12,17] Linagliptin has a predominantly nonrenal route of excretion; >90% of an oral dose is excreted unchanged, primarily in feces.[18] Metabolism of the drug has been reported to be minimal, with pharmacologically inactive metabolites.[18]

The present study investigated whether exposure to linagliptin would be affected by food intake, in accordance with regulatory requirements to support dosing recommendations for patients with type 2 diabetes. Depending on the characteristics of the drug, food may either increase or decrease the drug's exposure, or it may have no effect at all.[19] Because linagliptin is characterized by high aqueous solubility at physiologic pH values (pH 7.4, >5 g/L),[20] it was not expected that food would increase or accelerate absorption, but effects associated with a food-related delay in gastric emptying could not be ruled out. Therefore, the objective of this study was to investigate the effects of food on the pharmacokinetics of single doses of linagliptin in healthy male and female subjects.

SUBJECTS AND METHODS

Study Participants

Subjects were recruited from the pool of volunteers at the Human Pharmacology Center, Department of Clinical Research, Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach, Germany, depending on their availability. Participants were compensated according to inconvenience, discomfort, and loss of time, as approved by the Independent Ethics Committee. The study aimed to recruit 32 healthy male and female subjects aged 18 to 50 years with a body mass index of 18.5 to 29.9 kg/m^2. Subjects were in good general health according to routine medical history, physical examination, vital signs (blood pressure and pulse rate), and laboratory data (clinical chemistry, hematology, urinalysis, drug screening, and serology for hepatitis B and C, and HIV). Female subjects were required to use appropriate birth control measures until 2 months after completion of the study.

All subjects gave written informed consent. The study was conducted in compliance with the guidelines on good clinical practice and with ethical standards for human experimentation established by the Declaration of Helsinki (1996 version) and in accordance with applicable regulatory requirements. Approval was obtained from the local Independent Ethics Committee (Ethik-Kommission bei der Landesärztekammer Baden-Württemberg, Stuttgart, Germany) and the Federal Institute for Drugs and Medical Devices (Bundesinstitut für Arzneimittel und Medizinprodukte, Bonn, Germany). On-site monitoring was performed by the Clinical Research Organization, CenTrial GmbH, Tübingen, Germany.

Study Design

This was a single-center, open-label, 2-way crossover study conducted in healthy male and female subjects. Following a 21-day screening period and baseline evaluation, subjects were randomized in a 1:1 ratio to 1 of 2 study period sequences: fed–fasted or fasted–fed. In the "fed" study period, subjects received a single oral dose of 5 mg linagliptin following a high-fat, high-calorie breakfast (test treatment). In the "fasted" study period, dosing occurred after an overnight fast of at least 10 hours (reference treatment). The randomization list was generated using a validated pseudo-random number generator and a supplied seed number so that the allocation of medication numbers would be both reproducible and not predictable. In each case, linagliptin was administered between 8 AM and 9 AM with 240 mL of water. Subjects randomized to receive linagliptin in the fed state took the drug immediately after consuming a standard US Food and Drug Administration high-fat breakfast of approximately 945 Kcal.[21] This consisted of 2 eggs (120 g), 2 strips of bacon (30 g), 2 slices of toast (60 g), butter (30 g), hash brown potatoes (120 g), and whole milk (240 mL). Subjects in both treatment arms were required to fast for a further 4 hours after administration of the drug.

A period of 5 weeks separated the treatments when no medication was taken. The subjects then crossed over to the alternate treatment regimen. The crossover design removes intersubject variability of the treatment comparisons, since each subject served as his or her own control. End-of-study medical evaluations were performed within 7 to 14 days after the final treatment with linagliptin. Subjects admitted to the study center were not permitted to smoke or consume any food or drink other than that provided by the staff, and had to avoid excessive physical activity.

Pharmacokinetic Sampling

Blood samples for pharmacokinetic analysis of linagliptin were taken before dosing on Day 1 and at set time points (0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 48, 72, and 96 h) after dosing. Day 1 samples were obtained using an indwelling catheter (Optiva 2, 32-mm, 18-gauge; Medex Medical GmbH, Klein-Winternheim, Germany) without flushing, so no blood had to be discarded. After sampling, the cannula was locked using a mandrin obturator for Optiva 2 indwelling cannulae (Medex Medical GmbH, Klein-Winternheim, Germany). At ambulatory visits, blood was collected using a Safety-Multifly 21-gauge cannula (Sarstedt AG & Co, Nümbrecht, Germany). Blood samples (4.9 mL) were taken from a forearm vein into an EDTA anticoagulant blood-drawing tube and immediately chilled in an ice bath. Samples were then centrifuged at 2500 × g for 10 minutes at 4°C. Plasma was collected in 2 aliquots of at least 1 mL and frozen at or below −18°C. Centrifugation and freezing steps were performed within 60 and 90 minutes of sampling, respectively. Each subject provided a total of approximately 200 mL of blood for study purposes.

Analytical Methods

Plasma concentrations of linagliptin were analyzed using high-performance liquid chromatography coupled to tandem mass spectrometry (HPLC–MS/MS) by Covance Laboratories Ltd (Harrogate, United Kingdom), as described previously.[22] The calibration curves for undiluted plasma samples exhibited linear responses over the range of concentrations from 0.1 to 20 nmol/L using a plasma volume of 150 μL. In-study assay validation at nominal linagliptin concentrations of 0.250, 1.00, and 15.0 nmol/L showed an assay inaccuracy and imprecision from −6.1% to −8.7% and 6.0% to 7.4%, respectively.

Pharmacokinetic Methods

Pharmacokinetic parameters for linagliptin were determined for each individual by noncompartmental analysis[23] using the WinNonlin software program (Pharsight Corporation, Cary, North Carolina). Only concentrations within the validated concentration range of 0.1 to 20 nmol/L were used to calculate pharmacokinetic parameters. Actual sampling times were used. The apparent terminal rate constant (λ_z) was estimated by regression of the terminal log-linear portion (determined by inspection) of the plasma concentration-time profile using the last 3 available data points; $t_{½}$ was calculated as the quotient of ln(2) and λ_z. C_{max} and T_{max} values were obtained by inspection of the plasma concentration data. AUC over a time interval was calculated using the trapezoid rule and the extra- or interpolated concentration at that time point. Owing to the long $t_{½}$ of linagliptin of >100 hours,[12] the percentage of the total AUC generated by extrapolation exceeds 20%, despite long sampling periods. Therefore, a truncated AUC over a time interval of 0 to 72 hours was considered more appropriate to assess the relative bioavailability of linagliptin, which was determined primarily on the basis of the parameters AUC_{0-72} and C_{max}. This is in line with the US Food and Drug Administration and European Medicines Agency guidelines for bioavailability studies of orally administered drugs with a long terminal $t_{½}$.[24,25]

Safety

Evaluations of routine clinical chemistry (sodium, potassium, calcium, aspartate transaminase, alanine transaminase, alkaline phosphatase, γ-glutamyltransferase, glucose, total cholesterol, C-reactive protein, creatinine, bilirubin [total and direct], triglycerides, total protein, and thyroid-stimulating hormone), hematology (hematocrit; hemoglobin; red and white blood cell counts; platelets; absolute and differential counts of neutrophils, eosinophils, basophils, lymphocytes, and monocytes; prothrombin time; prothrombin time/international normalized ratio; and activated partial thromboplastin time), urine pH analysis, vital signs (blood pressure and pulse rate), 12-lead ECG, and physical examinations were performed prestudy and poststudy. Tolerability was assessed based on adverse events (AEs). The investigator (M.I.) documented the time of onset, end time, intensity (mild, moderate, or severe), relationship to study drug, and outcome of each AE and any treatment or action required. All AEs persisting after trial completion were followed up until the subject had recovered or the AE had been sufficiently characterized. A serious AE was defined as any AE that (1) resulted in death, or persistent or significant disability or incapacity, (2) consisted of a congenital anomaly or birth defect, (3) required hospitalization or prolongation of hospitalization, (4) was immediately life-threatening, or (5) was deemed serious for any other reason representing a comparably significant hazard.

Statistical Analysis

Data analyses were conducted as described by Patterson and Jones.[26] The effect of food on the bioavail-

ability of linagliptin was determined by comparing the AUC from 0 to 72 hours (AUC_{0-72}) and C_{max} in the fed and fasted state. Point estimators (geometric means [gMeans]) of the median intrasubject ratios of AUC_{0-72} and C_{max} and their 2-sided 90% CIs were calculated. These parameters were logarithmically transformed to ensure additivity of the model effects and were then analyzed by an ANOVA model with effects due to sequence, subjects, period, and regimen (ie, fasted or fed). CIs were based on the residual error from ANOVA. Descriptive statistics for all other parameters were calculated. Safety data were evaluated descriptively, and AEs were described in their entirety. All subjects who received at least 1 dose of study drug were included in the safety evaluation.

The sample size was selected to achieve a desired precision of the estimate of the test/reference ratio of the pharmacokinetic end points. Based on an intraindividual geometric coefficient of variation (gCV) for C_{max} of 25% to 30% as seen in previous trials,[22,27] the sample size of 28 evaluable subjects ensured that the 2-sided 90% CI of the test/reference ratio on log scale should be smaller than 0.1, with a probability of at least 90%. To account for potential dropouts, it was planned for 32 subjects to be randomized into the study.

RESULTS

The study population comprised 17 male and 15 female subjects, aged between 21 and 49 years. All subjects were white (reflecting the local, general population), with a mean body mass index of 24.21 kg/m^2. Five subjects were smokers, and 26 subjects drank alcohol but at a level that was deemed a priori to be insufficient to affect the study (≤10 cigarettes, ≤3 cigars, or ≤3 pipes/day; average alcohol consumption ≤30 g/day in males and ≤20 g/day in females). There were no concomitant diagnoses and no concomitant therapies that were considered relevant to the analyses.

Data from all subjects were included in the pharmacokinetic set, since all predose concentrations of linagliptin were lower than 5% of C_{max}. One subject in the fed–fasted sequence discontinued the study after the fed treatment owing to an AE (severe acute tonsillitis, not considered by the investigator to be related to study medication). He did not complete the fasted treatment period of the study; therefore, the pharmacokinetic analysis is on the remaining 31 subjects.

The arithmetic mean plasma concentration-time profiles of linagliptin after fed and fasted conditions are shown in Figure 1. Under fasted conditions, linagliptin was rapidly absorbed and reached C_{max} about 1 hour after dosing. The intake of food prolonged the T_{max} by approximately 2 hours. The prolonged absorption seen after the high-fat meal resulted in lower C_{max} but higher plasma concentrations compared with the fasted state beyond 8 hours after dosing. The decline in plasma concentrations during the terminal phase was similar under fasted and fed conditions.

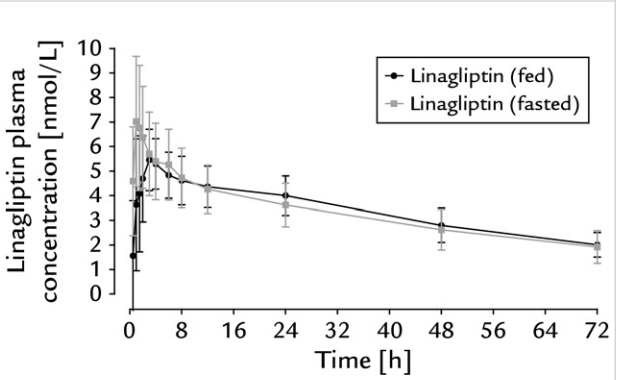

Figure 1. Arithmetic mean plasma concentration-time profiles of linagliptin after single oral administration of linagliptin under fed and fasted conditions.

The pharmacokinetic parameters of linagliptin for both treatment conditions are shown in the Table. Inferential analysis of bioavailability based on the ANOVA model for AUC_{0-72} revealed no difference under fed and fasted conditions; the gMean ratio (GMR) was 103.5, and the 90% CIs of 98.1% to 109.2% met the standard bioequivalence criteria of 80% to 125%. The C_{max} of linagliptin was reduced from 7.04 to 5.97 nmol/L (GMR 84.7%; 90% CI, 75.9%–94.6%). The percentage of total AUC that was obtained by extrapolation exceeded 25% in both treatment arms, supporting the selection of a truncated AUC_{0-72} for the primary analysis. In accordance with the lowered C_{max} but comparable AUC observed in fed subjects, the concentrations of linagliptin >12 hours after administration were found to be slightly higher than those in fasted subjects. Overall, administration of linagliptin with a high-fat meal did not influence the extent of exposure to linagliptin. Figures 2A and 2B show the intraindividual comparisons of AUC_{0-72} and C_{max}; intraindividual variability, gCV, was 12.4% for AUC_{0-72} and 26.1% for C_{max}, respectively.

Table. Geometric mean (gMean) and geometric coefficient of variation (gCV) of pharmacokinetic parameters of linagliptin 5 mg administered in fed and fasted conditions. For AUC_{0-72} and C_{max}, gMean ratios (GMR; fed–fasted) and 90% CIs are given.

	gMean (gCV [%])		
Pharmacokinetic Parameter	Linagliptin 5 mg (fasted) (n = 31)	Linagliptin 5 mg (fed) (n = 32)	GMR (90% CI)
AUC_{0-72} (nmol · h/L)	229 (25.9)	236 (20.0)	103.5 (98.1,109.2)
C_{max} (nmol/L)	7.04 (34.0)	5.97 (19.5)	84.7 (75.9, 94.6)
C_{24} (nmol/L)	3.52 (24.7)	4.28 (18.9)	
T_{max}* (h)	1.02 (0.52–8.00)	2.99 (0.50–8.00)	
$t_{1/2}$ (h)	59.4 (16.1)	55.4 (16.0)	

*Median and range are shown for T_{max}; AUC_{0-72}, area under the concentration-time curve from 0 to 72 hours; C_{max}, maximum measured plasma concentration; C_{24}, plasma concentration at 24 hours post-dose; T_{max}, time from dose to reach maximum plasma concentration; $t_{1/2}$, terminal half-life of linagliptin in plasma.

Seventeen subjects experienced at least 1 AE during the study, and 11 of these subjects experienced an AE during a treatment period (the 7-day period starting with treatment). The same number of subjects experienced an AE whether linagliptin was administered with food (7/32 subjects) or without food (7/31 subjects). The most common AEs were headache (9 subjects), nasopharyngitis (4 subjects), and vomiting (2 subjects, experienced at 10 hours and 12 hours after dosing, respectively). Three subjects had an AE classed as severe, 1 subject experienced nausea 12 hours after dosing, and 1 subject had a joint sprain. The third subject had severe acute tonsillitis 29 days after the first treatment with linagliptin, and this led to the subject discontinuing the study. No serious AEs were reported, and the investigator did not consider any AEs to be related to the study medication. Overall, single oral doses of linagliptin were well tolerated, whether administered with or without food.

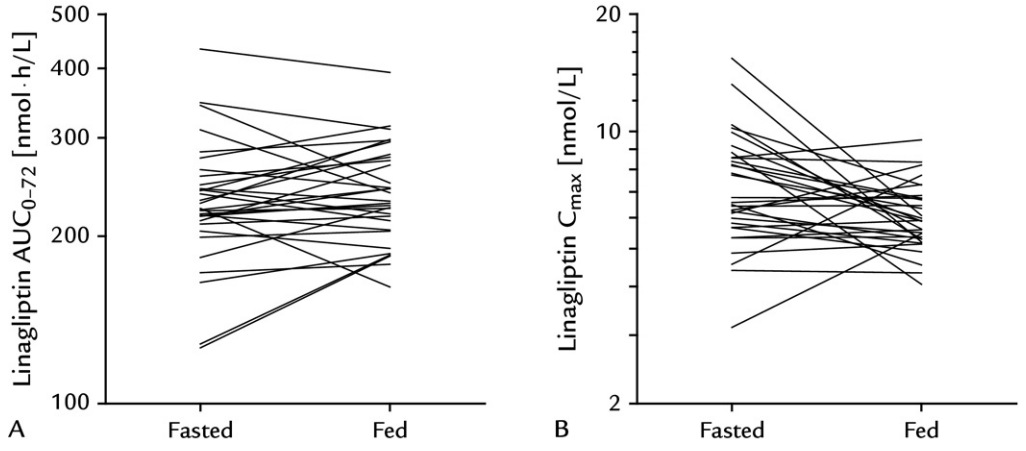

Figure 2. (A) Intraindividual comparisons of single-dose AUC_{0-72} values of linagliptin, given in fed or fasted conditions. (B) Intraindividual comparisons of single-dose C_{max} values of linagliptin, given in fed or fasted conditions.

DISCUSSION

The objective of this study was to investigate the effect of food on the bioavailability of single doses of linagliptin in healthy male and female subjects. The similarity of plasma concentration-time profiles for fasted and fed subjects indicated no clinically relevant changes in the pharmacokinetics of linagliptin.

Administration of linagliptin 5 mg with a high-fat meal had no influence on the extent of exposure as determined by the AUC over various time intervals compared with administration of the drug in the fasted state. However, intake of food caused an increase in the median T_{max} (from 1.02 h fasted to 2.99 h fed) associated with a reduction in C_{max} (from 7.04 nmol/L to 5.97 nmol/L). This suggests that food delayed the absorption, and thus the rate of absorption was decreased, which is in line with the observation that, although maximum concentrations were lower in the fed state, linagliptin plasma concentrations beyond 12 hours after dosing were slightly higher with food. The 15% reduction in C_{max} observed in this study is not expected to influence the efficacy of linagliptin. Since stimulation of glucagon-like peptide-1 secretion occurs several times per day (after each intake of food), adequate inhibition of DPP-4 is necessary for the entire 24-hour dosing interval. The linagliptin concentration that results in >80% DPP-4 inhibition (EC_{80}; the clinically effective concentration) was reported to be 5.6 nM.[11] Under multiple-dose conditions, linagliptin trough concentrations measured 24 hours after last dosing following 24 weeks of 5 mg linagliptin monotherapy treatment were about 8 nM.[4] Therefore, a 15% reduction in C_{max} is not considered to be of clinical relevance. In turn, no effect of food on tolerability associated with a reduction in C_{max} would be expected.

Consequently, based on the results from this small group of healthy white volunteers, it is anticipated that linagliptin can be taken with or without food. Linagliptin was well tolerated in this study, and there were no AEs related to medication. These results are in line with the safety and tolerability profile of linagliptin observed in the global Phase III program, in which the overall safety profile of linagliptin was comparable to that of placebo.[3–9]

CONCLUSIONS

Linagliptin was well tolerated in this study. Intake of a high-fat meal reduced the rate of absorption but had no influence on the extent of absorption; therefore, it is expected that food will have no clinically relevant influence on the efficacy or tolerability of linagliptin. This suggests that linagliptin tablets can be administered without regard to meals.

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REFERENCES

1. Thomas L, Eckhardt M, Langkopf E, et al. (R)-8-(3-amino-piperidin-1-yl)-7-but-2-ynyl-3-methyl-1-(4-methyl-quinazolin-2-ylmethyl)-3,7-dihydro-purine-2,6-dione (BI 1356), a novel xanthine-based dipeptidyl peptidase 4 inhibitor, has a superior potency and longer duration of action compared with other dipeptidyl peptidase-4 inhibitors. *J Pharmacol Exp Ther*. 2008;325:175–182.
2. Deacon CF, Holst JJ. Linagliptin, a xanthine-based dipeptidyl peptidase-4 inhibitor with an unusual profile for the treatment of type 2 diabetes. *Expert Opin Investig Drugs*. 2010;19:133–140.
3. Kawamori R, Inagaki N, Araki E, et al. Linagliptin provides superior glycemic control compared to voglibose as monotherapy in Japanese patients with type 2 diabetes. Poster 632, presented at the American Diabetes Association 70th Scientific Sessions, June 25–29, 2010; Orlando, Fla. Abstract available at http://professional.diabetes.org/

Abstracts_Display.aspx?TYP=1&CID=79579. Accessed October 21, 2010.
4. Del Prato S, Barnett AH, Huisman H, et al. Effect of linagliptin monotherapy on glycaemic control and markers of β-cell function in patients with inadequately controlled type 2 diabetes: a randomized controlled trial. *Diab Obes Metab*. 2011;13:258–267.
5. Gomis R, Espadero R-M, Jones R, et al. Efficacy and safety of initial combination therapy with linagliptin and pioglitazone in patients with inadequately controlled type 2 diabetes: a randomized, double-blind, placebo-controlled study. *Diabetes Obes Metab*. 2011;13:653–661.
6. Owens DR, Swallow R, Dugi KA, Woerle H-J. Efficacy and safety of linagliptin in persons with type 2 diabetes inadequately controlled by a combination of metformin and sulfonylurea: a 24-week randomized study. *Diabet Med*. 2011; in press.
7. Taskinen MR, Rosenstock J, Tamminen I, et al. Safety and efficacy of linagliptin as add-on therapy to metformin in patients with type 2 diabetes: a randomized, double-blind, placebo-controlled study. *Diabetes Obes Metab*. 2011;13:65–74.
8. Lewin AJ, Arvay L, Liu D, et al. Safety and efficacy of linagliptin as add-on therapy to a sulphonylurea in inadequately controlled type 2 diabetes. Poster 821, presented at the European Association for the Study of Diabetes 46th Annual Meeting, September 20–24, 2010; Stockholm, Sweden.
9. Barnett AH, Harper R, Toorawa R, et al. Linagliptin monotherapy improves glycaemic control in type 2 diabetes patients for whom metformin therapy is inappropriate. Poster 823, presented at the European Association for the Study of Diabetes 46th Annual Meeting, September 20–24, 2010, Stockholm, Sweden.
10. Hüttner S, Graefe-Mody EU, Withopf B, et al. Safety, tolerability, pharmacokinetics, and pharmacodynamics of single oral doses of BI 1356, an inhibitor of dipeptidyl peptidase 4, in healthy male volunteers. *J Clin Pharmacol*. 2008;48:1171–1178.
11. Retlich S, Duval V, Ring A, et al. Pharmacokinetics and pharmacodynamics of single rising intravenous doses (0.5 mg to 10 mg) and determination of absolute bioavailability of the dipeptidyl peptidase-4 inhibitor linagliptin (BI 1356) in healthy male volunteers. *Clin Pharmacokinet*. 2010;49:829–840.
12. Heise T, Graefe-Mody EU, Hüttner S, et al. Pharmacokinetics, pharmacodynamics and tolerability of multiple oral doses of linagliptin, a dipeptidyl peptidase-4 inhibitor in male type 2 diabetes patients. *Diabetes Obes Metab*. 2009;11:786–794.
13. Fuchs H, Binder R, Greischel A. Tissue distribution of the novel DPP-4 inhibitor BI 1356 is dominated by saturable binding to its target in rats. *Biopharm Drug Dispos*. 2009;30:229–240.
14. Fuchs H, Tillement JP, Urien S, et al. Concentration-dependent plasma protein binding of the novel dipeptidyl peptidase 4 inhibitor BI 1356 due to saturable binding to its target in plasma of mice, rats and humans. *J Pharm Pharmacol*. 2009;61:55–62.
15. Retlich S, Withopf B, Greischel A, et al. Binding to dipeptidyl peptidase-4 determines the disposition of linagliptin (BI 1356)—investigations in DPP-4 deficient and wildtype rats. *Biopharm Drug Dispos*. 2009;30:422–436.
16. Retlich S, Duval V, Graefe-Mody U, et al. Impact of target-mediated drug disposition on linagliptin pharmacokinetics and DPP-4 inhibition in type 2 diabetic patients. *J Clin Pharmacol*. 2010;50:873–885.
17. Forst T, Uhlig-Laske B, Ring A, et al. The oral DPP-4 inhibitor linagliptin significantly lowers HbA_{1c} after 4 weeks of treatment in patients with type 2 diabetes mellitus. *Diab Obes Metab*. 2011;13:542–550.
18. Blech S, Ludwig-Schwellinger E, Gräfe-Mody EU, et al. The metabolism and disposition of the oral dipeptidyl peptidase-4 inhibitor, linagliptin, in humans. *Drug Metab Dispos*. 2010;38:667–678.
19. Singh BN. Effects of food on clinical pharmacokinetics. *Clin Pharmacokinet*. 1999;37:213–255.
20. Eckhardt M, Langkopf E, Mark M, et al. 8-(3-(R)-aminopiperidin-1-yl)-7-but-2-ynyl-3-methyl-1-(4-methyl-quinazolin-2-ylmethyl)-3,7-dihydropurine-2,6-dione (BI 1356), a highly potent, selective, long-acting, and orally bioavailable DPP-4 inhibitor for the treatment of type 2 diabetes. *J Med Chem*. 2007;50:6450–6453.
21. Food Effect Working Group of the Biopharmaceutics Coordinating Committee, Office of Pharmaceutical Science. Guidance for industry: food-effect bioavailability and fed bioequivalence studies, 2002. http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm064964.htm. Accessed October 21, 2010.
22. Graefe-Mody EU, Padula S, Ring A, et al. Evaluation of the potential for steady-state pharmacokinetic and pharmacodynamic interactions between the DPP-4 inhibitor linagliptin and metformin in healthy subjects. *Curr Med Res Opin*. 2009;25:1963–1972.
23. Gabrielsson J, Weiner D. *Pharmacokinetic and Pharmacodynamic Data Analysis: Concepts and Applications*. 4th ed. Stockholm, Sweden: Swedish Pharmaceutical Press; 2007.
24. US Food and Drug Administration. Guidance for industry: bioavailability and bioequivalence studies for orally administered drug products—general considerations, 2003. http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm070124.pdf. Accessed October 21, 2010.

25. European Medicines Agency. Committee for medicinal products for human use: guideline on the investigation of bioequivalence, 2008. http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003011.pdf. Accessed October 21, 2010.
26. Patterson S, Jones B. *Bioequivalence and Statistics in Clinical Pharmacology*. London, United Kingdom: Chapman & Hall/CRC Press; 2005.
27. Graefe-Mody U, Hüttner S, Stähle H, et al. Effect of linagliptin (BI 1356) on the steady-state pharmacokinetics of simvastatin. *Int J Clin Pharmacol Ther*. 2010;48:367–374.

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