Pharmacokinetic, Pharmacodynamic, and Tolerability Profiles of the Dipeptidyl Peptidase-4 Inhibitor Linagliptin: A 4-Week Multicenter, Randomized, Double-Blind, Placebo-Controlled Phase IIa Study in Japanese Type 2 Diabetes Patients*

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ABSTRACT

Background: The dipeptidyl-peptidase-4 (DPP-4) inhibitor linagliptin is under clinical development for treatment of type 2 diabetes mellitus (T2DM). In previous studies in white populations it showed potential as a once-daily oral antidiabetic drug.

Objectives: In compliance with regulatory requirements for new drugs intended for use in the Japanese population, this study investigated the pharmacokinetics, pharmacodynamics, and tolerability of multiple oral doses of linagliptin in Japanese patients with T2DM.

Methods: In this randomized, double-blind, placebo-controlled multiple dose study, 72 Japanese patients with T2DM were assigned to receive oral doses of linagliptin 0.5, 2.5, or 10 mg or placebo (1:1:1:1 ratio) once daily for 28 days. For analysis of pharmacokinetic properties, linagliptin concentrations were determined from plasma and urinary samples obtained throughout the treatment phase, with more intensive samplings on days 1 and 28. DPP-4 inhibition, glycosylated hemoglobin A1c (HbA_{1c}) levels, and plasma glucose and glucagon-like peptide-1 (GLP-1) levels were compared by mixed effect model. Tolerability was assessed throughout the study by physical examination, including blood pressure and pulse rate measurements, 12-lead ECG, and laboratory analysis.

Results: Baseline demographic characteristics were well balanced across the 4 treatment groups (mean [SD] age, 59.7 [6.4] years in the placebo group, 60.8 [9.2] years in the 0.5 mg group, 60.2 [6.4] years in the 2.5 mg group, and 59.1 [8.6] years in the 10 mg group; mean [SD] weight, 67.2 [10.0] kg in the placebo group, 64.5 [9.0] kg in the 0.5 mg group, 69.6 [9.4] kg in the 2.5 mg group, and 63.5 [12.2] kg in the 10 mg group; mean [SD] duration of T2DM diagnosis, 5.1 [4.2] years in the placebo group, 5.2 [4.7] years in the 0.5 mg group, 5.9 [4.8] years in the 2.5 mg group, and 2.6 [2.3] years in the 10 mg group). The majority of the patients treated were male (76.4%). Use of previous antidiabetic medication was more common in the 2.5 mg linagliptin group (44%) than in the 0.5 or 10 mg linagliptin (15.8% and 22.2%, respectively) or placebo groups (35.3%). Total systemic exposure in terms of linagliptin AUC and C_{max} (which occurred at 1.25–1.5 hours) increased in a less than dose-proportional manner. The terminal half-life was long (223–260 hours) but did not reflect the accumulation half-life (10.0–38.5 hours), resulting in a moderate accumulation ratio of <2.9 that decreased with increasing dose.

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Urinary excretion increased with linagliptin doses but was <7% at steady state for all dose groups. Inhibition of plasma DPP-4 at 24 hours after the last dose on day 28 was approximately 45.8%, 77.8%, and 89.7% after linagliptin 0.5, 2.5, and 10 mg, respectively. At steady state, linagliptin was associated with dose-dependent increases in plasma GLP-1 levels, and the postprandial GLP-1 response was enhanced. Statistically significant dose-dependent reductions were observed in fasting plasma glucose levels at day 29 for all linagliptin groups (–11.5, –13.6, and –25.0 mg/dL for the 0.5, 2.5, and 10 mg groups, respectively; $P < 0.05$ for all linagliptin groups). Linagliptin also produced statistically significant dose-dependent reductions from baseline for glucose area under the effect curve over 3 hours after meal tolerance tests (–29.0 to –68.1 mg × h/dL; $P < 0.05$ for all 3 linagliptin groups). For the 0.5 and 10 mg linagliptin-treated groups, there were statistically significant reductions in HbA_{1c} from baseline compared with placebo, despite the relatively low baseline HbA_{1c} (7.2%) and small sample size ($P < 0.01$ for both groups). The greatest reduction in HbA_{1c} (–0.44%) was seen in the highest linagliptin dose group (10 mg). On dosing for up to 28 days, linagliptin was well tolerated with no reported serious adverse events or symptoms suggestive of hypoglycemia. Overall, fewer adverse events were reported by patients after linagliptin than after placebo (11 of 55 [20%] vs 6 of 17 [35%]).

Conclusions: Linagliptin demonstrated a nonlinear pharmacokinetic profile in these Japanese patients with T2DM consistent with the findings of previous studies in healthy Japanese and white patients. Linagliptin treatment resulted in statistically significant and clinically relevant reductions in HbA_{1c} as soon as 4 weeks after starting therapy in these Japanese patients with T2DM, suggesting that clinical studies of longer duration in Japanese T2DM patients are warranted. (*Clin Ther*. 2011;33:973–989)

Key words: BI 1356, dipeptidyl-peptidase-4, incretin, linagliptin, pharmacodynamics, pharmacokinetics, type 2 diabetes.

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a chronic progressive disease caused by a combination of insulin resistance and β-cell dysfunction, resulting in hyperglycemia.[1] Dipeptidyl-peptidase-4 (DPP-4) inhibitors are a class of oral antihyperglycemic agents that have been introduced as a new treatment option for monotherapy and combination therapy use in T2DM.[2,3] DPP-4 inhibitors lower blood glucose by preventing the degradation of glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide.[4] These peptides, released by gut endocrine cells in response to food intake, play an important role in glucose homeostasis by stimulating glucose-dependent insulin secretion from pancreatic islet β-cells. Prolonging the effects of endogenous GLP-1 by inhibition of DPP-4 was clinically validated as a glucose-dependent therapeutic approach to improve fasting and postprandial plasma glucose levels, leading to decreases in glycosylated hemoglobin (HbA_{1c}).[2]

Linagliptin is an orally active DPP-4 inhibitor[5] that was approved in the US for the treatment of T2DM.[6,7] In preclinical studies, linagliptin exhibited high-potency inhibition of DPP-4 (K_i ~1 nM)[8] and improved glycemic homeostasis in a variety of rodent models of T2DM.[8,9] Linagliptin showed high selectivity for DPP-4 versus DPP-8 (40,000-fold) and DPP-9 (>10,000-fold).[8] In a Phase I pharmacokinetic (PK) and pharmacodynamic (PD) study in 64 healthy white male volunteers, single oral doses of linagliptin up to 120 times the proposed clinically effective dose level (ie, 5 mg/d) were well tolerated with an adverse event (AE) profile similar to that of placebo.[10] Compared with other DPP-4 inhibitors, linagliptin showed a unique PK and PD profile with a mainly nonrenal route of elimination.[11,12] In a multiple dose study in 48 white male patients with T2DM, once-daily dosing of linagliptin for 12 days (1–10 mg) resulted in maximal inhibition of plasma DPP-4 of >90% with the 5 and 10 mg doses at steady state, with ~85% inhibition remaining at 24 hours post-dose (5 mg).[13] In studies performed in T2DM patients in Europe and North America, oral dosing with linagliptin was well tolerated and resulted in significant improvements of glucose parameters ($P < 0.05$).[6,7,14] Guidance from the Pharmaceutical and Medical Devices Agency in Japan requires that the dose–response relationship for any new drug is confirmed in the Japanese population and that treatment is evaluated in "adequate numbers of Japanese cases."[15] In a Phase I study of 56 healthy male Japanese volunteers, the tolerability, PK, and PD profiles of linagliptin were consistent with previous observations in white patients[10,13,16]; however, these profiles have not

been previously investigated in Japanese patients with T2DM. Therefore, the aim of the present study was to evaluate the safety profile, tolerability, PKs, and PDs of linagliptin in Japanese patients with type 2 diabetes.

PATIENTS AND METHODS

Study Design

This randomized, double-blind, placebo-controlled multiple-dose study was conducted at 5 centers in Japan in 2007 to evaluate the PKs, PDs, safety profile, and tolerability of linagliptin administered orally once daily for 28 days in Japanese patients with T2DM. No payments were received by the investigators or patients for enrollment or completion of treatment. At the initial screening visit, each patient provided written informed consent before enrollment. Testing for use of illicit drugs (amphetamines, barbiturates, benzodiazepines, cocaine, cannabinoids, methadone [as 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine], opiates) was done for all patients at screening. After screening, a washout phase of 14 days before first drug administration was mandatory for each patient, in which no antidiabetic drugs were to be taken. Patients whose fasted blood glucose levels did not exceed 240 mg/dL (13.3 mmol/L) on 2 consecutive days during the washout period were eligible to be randomized. Patients were assigned in a 1:1:1:1 ratio to 1 of 4 treatment groups: placebo, linagliptin 0.5, 2.5, or 10 mg/d for days 1 to 28 (treatment phase).

The randomization procedure was based on a table of computer-generated pseudorandom numbers; seed numbers were used to set the starting point for a series of random numbers to make the assignment list reproducible and unpredictable. To maintain blinding of the patients and investigators, study medications were identical in appearance and provided in identical packaging.

Patients were admitted into each study center on day –2 for baseline assessments and were discharged after dosing on day 2. They were readmitted into the study center on the evening of day 27 and were discharged on day 30 after completion of all study-related assessments. To ensure a dose interval of 24 hours, the investigational products were administered at the same time (8:00 AM) daily.

Patients were kept under close observation by medically qualified staff for the first days of medication (from day –2 to the morning of day 2) and the last 3 days (from the afternoon of day 27 to day 30) during the in-house period. Water was allowed at all times, except for 1 hour before and after drug administration. The patients were instructed to swallow the investigational products with water after an overnight fast. Food and beverages were not allowed for 1 hour after drug administration in the admission periods.

During the in-house stay, compliance was ensured by administration of all study medication under supervision of the investigator or another member of staff at the study sites. Tablet intake at home was recorded in a study diary by each patient. Compliance was controlled by checking the diary. The numbers of administered tablets were checked at the visits on day 14 (ambulatory visit) and on day 27 (readmission to the study center). The measured plasma concentrations provided additional information concerning compliance.

The study protocol was designed by the sponsor, Nippon Boehringer Ingelheim Co, Ltd, Hyogo, Japan, and was approved by the ethics committee or institutional review board at each study center. The study was conducted in compliance with the ethical standards for human experimentation established by the Declaration of Helsinki at the time the study was initiated[17] and in accordance with the International Conference on Harmonisation: Harmonised Tripartite Guideline for Good Clinical Practice and the Japanese Good Clinical Practice regulations.[18]

Patients

Eligible male and female Japanese patients, aged between 21 and 70 years, had to have type 2 diabetes that was treated with diet and/or exercise only or with 1 or 2 oral hypoglycemic agents (other than thiazolidinediones). Additional inclusion criteria included body mass index (BMI) $\geq$17.6 and $\leq$35.0 kg/m^2, and HbA_{1c} $\leq$8.5% for patients treated with $\leq$1 oral hypoglycemic agent or $\leq$8.0% for patients treated with 2 oral hypoglycemic agents. Patients were excluded if they had a relevant history of hepatic, renal, neurologic, cardiovascular, gastrointestinal, metabolic, or hormonal disorders; hyperlipidemia; or hypertension. Patients were also excluded if they had donated blood ($\geq$100 mL within 4 weeks before planned drug administration), participated in another clinical trial within 2 months before study start, tested positive for illicit drug use, or had a medical history that included a drug or alcohol abuse problem.

Pharmacokinetic End Points and Assessments

PK end points were assessed based on measured plasma and urinary linagliptin concentrations performed throughout the treatment phase, with more intensive sampling on days 1 (ie, at single dose conditions) and 28 (steady-state conditions). Blood sampling for PKs was also performed on the morning of days 2, 14, 29, 30, 33, 35, 38, 41, and 43. PD end points were based on assay of plasma DPP-4 inhibition; in addition, GLP-1 levels, plasma glucose, plasma fructosamine level, and HbA_{1c} levels were determined for exploratory purposes. DPP-4 inhibition was assessed at the same time point as linagliptin concentrations in plasma. HbA_{1c} was measured before the first drug administration (day –1) and 24 hours after final dosing (day 29). Fasting plasma glucose (FPG) was measured on days 1, 2, 14, 28, and 29 (ie, 24 hours after the last linagliptin dose). On days –1, 1, and 29, meal tolerance tests (MTTs) were performed in the morning using an MTT standard meal, Calorie Mate (Otsuka Pharmaceutical Co Ltd, Tokyo, Japan). Seven-point glucose profiles (premeal, postmeal, and bedtime) were measured on the same days as the MTT for each patient. GLP-1 concentrations were also determined on these days (ie, days −1, 1, and 29) for blood samples taken 30 minutes before intake of a standardized drink for the MTT (on day 1, the samples were drawn 30 minutes after drug administration; on day 29, the samples were drawn 24 hours after the last drug dose). A further blood sample for GLP-1 assay was then taken 30 minutes after the drink intake on these days. Throughout the in-house period, patients received standardized meals suitable for patients with T2DM, and a snack before bedtime as described.[19]

Noncompartmental analysis of the plasma and urinary linagliptin concentration–time data was performed according to standard methods[20] using WinNonlin Professional (version 5.0.1; Pharsight Corporation, Mountain View, California). Actual sampling times were used for PK analysis. From the individual plasma PK parameters and urinary excretion data, the following descriptive statistics were calculated per treatment group for each PK parameter: N, arithmetic mean, geometric mean (gMean), SD, minimum, median, maximum, and arithmetic CVs, and geometric coefficient of variation (gCV). The apparent terminal rate constant (λ) at steady state was estimated by regression of the terminal log-linear portion of the plasma concentration–time curve (determined by direct inspection); the $t_{½}$ was calculated as the quotient of ln(2) and λ. The AUC to the last measured concentration was calculated using the linear trapezoidal method for ascending concentrations and the log trapezoidal method for descending concentrations. The C_{max} and t_{max} were determined by direct inspection of the plasma concentration data.[20]

The apparent CL/F was determined as the quotient of the drug dose and AUC within a specific time interval. Renal clearance (CL_R) was determined as the quotient of the amount excreted unchanged in urine and AUC over the respective interval. The apparent volume of distribution during the terminal phase after oral administration (V_d/F) was calculated by dividing the CL/F by λ.[20]

The fraction of dose excreted unchanged in urine (fe) over the 24-hour interval on day 1 ($fe_{0-24,1}$) and on day 28 ($fe_{0-24,ss}$) was calculated from the sum of the amounts of linagliptin collected in the urine in each collection interval (the product of the linagliptin urine concentration in each interval and the weight of the urine collected in each interval, with weight set equal to volume [ie, 1 kg = 1 L], without correction for specific gravity of urine). Plasma accumulation of linagliptin after multiple dosing was assessed by calculating the accumulation ratios (R_A) (day 28/day 1) for AUC and C_{max} for each patient. Accumulation $t_{½}$ was calculated from the R_A for AUC by the following formula: $k = \ln[RA/(RA - 1)]/\tau$, where $k = \ln(2)/\text{accumulation } t_{½}$.

Laboratory analyses were carried out at central laboratories: Covance Laboratories Ltd, Harrogate, United Kingdom (measurement of linagliptin in plasma and urine); the Institut für Klinische Forschung unt Entwicklung (Institute for Clinical Research and Development) GmbH, Mainz, Germany (plasma DPP-4 activity, plasma glucose, and plasma GLP-1); and SRL Inc., Hachioji Laboratory, Tokyo, Japan (HbA_{1c} measurement [after screening and enrollment], laboratory tests for tolerability, including hematology, blood biochemistry, and urinanalysis; quality control and performance for the assays for this laboratory were validated by The Japan Accreditation Board for Conformity Assessment). Urine and plasma samples were collected and stored by the logistics clinical research organization, SRL Medisearch Inc., which shipped samples on dry ice to the respective laboratories.

Blood Sampling and Analytical Procedures

For quantification of linagliptin plasma concentrations and measurement of DPP-4 activity, 4 mL of blood was taken from a forearm vein in an EDTA-2K anticoagulant blood-drawing tube. Immediately after collection, blood samples were centrifuged (KUBOTA 5900, RS-720M; Kubota Corporation, Tokyo, Japan) at 4°C for ~10 minutes at 1750*g*. Three aliquots of EDTA plasma samples were obtained (0.5 mL for DPP-4 activity measurement, 0.6 mL for PK analysis, and a backup sample of ≥0.6 mL). Until shipment to the analytical laboratory, plasma samples were stored at –20°C or below at the clinical site and at the analytical laboratory until analysis. The backup aliquot was stored at –20°C or below until the clinical trial report was finalized. Time points for blood sampling were 30 minutes before drug administration and at 30 minutes, 1, 1.5, 2, 4, 6, 8, and 12 hours after drug administration on days 1 (at single dose conditions) and 28 (at steady conditions) and at 30 minutes before drug administration on days 2 and 14. As repeated small aliquots were taken, indwelling cannulas were used for blood sampling on days 1 and 28. A total amount of ~120 mL blood was collected per patient during the whole course of the study for PK purposes.

A blank urine sample was collected for quantification of linagliptin urine concentrations before administration of study drug, and two 0.5 mL aliquots were retained to check for analytical interference. For patients, all urine voided during the sampling intervals 0 to 4, 4 to 8, 8 to 12, and 12 to 24 hours after administration on days 1 and 28 was collected. Patients emptied their bladders at the end of each sampling interval. The sampling containers (3 L) contained a premetered amount of citric acid solution (15 mL of 2 M citric acid solution) and were stored in a refrigerator from sampling times until 24 hours after drug administration. The urine weight (without correction for specific gravity of urine) for each collection interval was documented. Until shipment to the analytical laboratory, the urine samples were stored at –20°C or below at the clinical site and stored at the analytical laboratory at –20°C or below until analysis. The second aliquot was stored at –20°C until the clinical trial report was finalized.

Plasma and urinary concentrations of linagliptin were determined using a validated HPLC-MS/MS method, using [$^{13}C_3$]-linagliptin as an internal standard (Department of Bioanalytical Services, Covance Laboratories Ltd).[10] The inaccuracy and imprecision of the assay in quality control samples spiked, with 3 linagliptin concentrations between –5.2% and 0.4% and 3.4% and 7.1% for plasma, and between 1.4% and 8.4% and 3.1% and 5.8% for urine, respectively. No interference of endogenous compounds was observed in the blank plasma of humans. The linear range for quantitation of linagliptin in plasma was 0.100 to 100 nmol/L and 1.00 to 1000 nmol/L in urine.

Plasma DPP-4 activity before and after drug administration was measured using a validated method that employed a semi-quantitative enzyme activity assay with fluorescence detection (substrate: H-Ala-Pro-7-aminoamido-4-trifluoromethylcoumarin) at the Institute for Clinical Research and Development.[10] Fluorescence was detected at 535 nm (emission) using 405 nm excitation wavelength after 10 minutes of incubation at amplification/gain 60 using a GENios FL fluorescence reader (Tecan; Durham, North Carolina). Assay performance was evaluated during the study by coanalysis of 6 in-house standards in each run/plate. The imprecision of the assay was between 1.88% and 7.03%.

Blood samples for GLP-1 measurement were collected before intake of a standardized drink for the MTT and at 0.5 hour after the drink intake. About 3.0 mL of blood was taken into a collection tube, BD P700 v1.0, EDTA-2K-containing iced tube (Becton, Dickinson and Company, New Jersey). This tube also contained a proprietary DPP-4 inhibitor that enabled preservation of GLP-1. After blood was collected, the tube was inverted several times to mix the content and then cooled in an ice bath. The test tube was immediately centrifuged (4°C, 10 minutes, 1750*g*) (KUBOTA 5900, RS-720M; Kubota Corporation). Plasma was dispensed into 2 tubes (approximately 0.75 mL/tube) and immediately frozen (at −50°C or below) until shipment to the analytical laboratory. For quantification of biologically active forms of GLP-1 (ie, GLP-1 [7-36 amide] and GLP-1 [7-37]) in plasma, a validated fluorescence-based direct ELISA (Linco Research, St. Charles, Illinois) was used at the Institute for Clinical Research and Development as previously described.[10] Calibration was performed for each GLP-1 plate by coanalysis of 6 calibrators in duplicates. The calibrators supplied with the ELISA kit ranged from 2 pmol/L (lower limit of quantification) to 100 pmol/L (upper limit of quantification). Two commercial quality con-

trols were used for determination of interassay accuracy and interassay precision.

HbA_{1c} at screening was performed within the safety laboratory measurements analyzed by the respective contract laboratory of each study site. This result was not included into the PDs biomarker analysis. During the study, blood samples for the determination of HbA_{1c} were taken in the morning on days −1, 29, and at the end of study examination. Approximately 1.2 mL of blood was collected in EDTA plasma tubes and shipped within 5 days to a central laboratory for analysis (SRL Inc.). Samples were stored at 4°C to 8°C if shipment took ≥2 days.

For plasma glucose measurements, approximately 2.0 mL of blood were collected in NaF plasma tubes and centrifuged immediately (4°C, 10 minutes, 1750*g*) (KUBOTA 5900, RS-720M; Kubota Corporation). Plasma was collected in 2 aliquots (each containing at least 0.2 mL plasma) and immediately frozen (at −18°C or below) until shipment on dry ice to the analytical laboratory (Institute for Clinical Research and Development).

Plasma glucose was quantitatively determined using an electrochemical, enzymatic-amperometric measuring principle (SuperGL; Hitado Diagnostic Systems GmbH, Möhnesee, Germany).[10] Commercial quality controls were used to determine in-study accuracy and precision. In addition, 2 in-house precision controls were used for determination of interassay precision. The inaccuracy and imprecision of the assay was between 0.4% and 2.5% and 1.7% and 6.3%, respectively.

Tolerability Methods

All patients who received 1 dose of the study drug were included in the tolerability evaluations. Tolerability observations consisting of physical examination, vital signs (systolic and diastolic blood pressures, and pulse rate), and 12-lead ECG; laboratory tests consisted of hematology, blood biochemistry (including blood glucose), and urinalysis; and AEs were performed before treatment, during the study, and at evaluation on study completion. The amount of blood collected per patient for the laboratory tests on tolerability was ~101 mL. Blood samples for laboratory tests were collected after the patient fasted for 10 hours. Laboratory tests were conducted by SRL Hachioji Laboratory.

During the study, including the 14-day washout period before the first study drug administration and the postobservation period, patients used finger sticks and a self-blood glucose measurement glucometer (Accu-Check Aviva, Roche Diagnostics, Indianapolis, Indiana) to check their fasting blood glucose levels every morning before breakfast. These values were documented in a trial diary kept by each patient and checked by the investigator on their next visit to the study site.

ECG records were comprehensively evaluated by the principal investigator in each of the study sites together with the clinical findings and laboratory test results. If any ECG abnormality was observed, the patient was carefully monitored, withdrawn from the study, and treated as necessary. As part of the analysis of all 12-lead ECG records, examination was made for QTc. These intervals were determined from 4 waveforms in the second lead. If a flattened T wave was not observed or was not able to be measured in the second lead, the first lead was used. If it was not measured in the first lead, the V_5 lead was used.

All AEs were recorded throughout the study and were coded for system organ class and preferred term according to the *Medical Dictionary for Regulatory Activities* (MedDRA) Version 10.0, and all records were checked and confirmed by the trial principal investigator. The investigators evaluated all clinical AEs in terms of intensity (mild, moderate, or severe), duration, severity, outcome, and relationship to study drug. All AEs occurring throughout the study were recorded on electronic case report forms provided by the study sponsor and were reported to the study sponsor.

Statistical Analyses

The planned number of 60 enrolled patients was not based on a power calculation. The sample size of having at least 15 patients in each group was considered sufficient for the exploratory evaluation of the safety profile and PKs of multiple doses in a study and reflected previous experience in similar studies.[21]

The dose-proportionality of linagliptin's PKs was explored using a regression model that described the relationship between the dose and PK end points ($C_{max,1}$ and $AUC_{\tau,1}$, after the first dose, and steady-state parameters $C_{max,ss}$ and area under the steady-state plasma concentration–time curve to the dosing interval [$AUC_{\tau,ss}$] after the last dose).[22] A 2-sided 95% CI was computed for the slope. To determine attain-

ment of steady state, trough concentrations of the analyte in plasma immediately before drug administration were used.[23] The assumption of a linear relationship between the log-transformed PK parameter and the log-transformed dose was examined. Pairwise comparisons of the differences between all the time points were then performed by *t*-tests.[22] The relationship between linagliptin plasma concentrations and a PD parameter (DPP-4 inhibition) was assessed in an exploratory manner based on GLP-1 concentrations.

Baseline DPP-4 activity (in plasma samples taken before the administration of the study medication) was compared with the enzyme activity at defined time points after drug administration; the baseline value was set to 100% and all other values were calculated as the respective percentage of DPP-4 activities. The percent inhibition of DPP-4 was calculated by subtracting the percentage of plasma DPP-4 activity from 100%. The linagliptin half-maximal inhibitory concentration (IC_{50}) for plasma DPP-4 activity was calculated using a sigmoid E_{max} model.

For the MTT comparison, data were analyzed using a mixed linear model with "patient" as a random effect and "treatment" as a fixed effect.[23] All statistical analyses were done using SAS (version 8.02; SAS Institute Inc, Cary, North Carolina). All patients who completed all treatments as planned in the protocol were included in the PK analysis.

Tolerability data were evaluated descriptively, and adverse experiences were described in their entirety. A serious AE was defined as any AE that resulted in death, was immediately life-threatening, resulted in persistent or significant disability or incapacity, required or prolonged patient hospitalization, possibly led to disability, was deemed serious for any other reason representing a significant hazard that was comparable to the aforementioned criteria, or was a congenital anomaly/birth defect.

The safety profile and tolerability of the dosing groups of linagliptin (0.5, 2.5, and 10 mg) were determined on the basis of the investigated parameters in comparison with placebo. No statistical analysis was performed on the tolerability data. Instead, AEs were described in their entirety and evaluated by descriptive statistical methods. All patients who received 1 dose of study drug were included in the tolerability evaluation. The 3 active drug groups were compared with the placebo group in a descriptive manner. Time course descriptive statistics of the laboratory values and those for the differences from the baseline were calculated.

RESULTS

Patient Disposition and Demographic Characteristics

Of the 98 patients with T2DM screened, 73 were randomized, and, of these, 72 received at least 1 dose of linagliptin or placebo (1 patient withdrew consent after randomization but before receiving medication). Sixty-nine patients completed the study. One patient from the linagliptin 2.5 mg group withdrew consent on day 26 (on request of the patient to discontinue the study for personal reasons other than those specified in the trial protocol). One patient in the linagliptin 0.5 mg group withdrew on day 2 owing to lost study medication. One patient in the placebo group withdrew on day 38 because of elevated FPG (>240 mg/dL) on 2 consecutive days. Thus, 72 patients were included in both the PK and safety profile analyses, respectively. For those patients who withdrew from the study before completion, only data from samples taken before withdrawal were included in the PK and PD analyses.

Baseline demographics (mean [SD] age, 60.0 [7.7] years; BMI, 24.3 [3.5] kg/m^2) were well balanced across the 4 treatment groups. Most of the participants (72.2% to 78.9%) in each group were male (**Table I**). Mean baseline values for glycemic parameters were also similar for the 4 treatment groups (HbA_{1c}, 6.9%–7.2% for the linagliptin groups, 7.0% for the placebo group; FPG, 147.9–158.4 mg/dL for the linagliptin groups, 155.5 mg/dL for the placebo group) (**Table I**). Use of previous antidiabetic medication was slightly more common in the 2.5 mg linagliptin group (8 of 18 patients [44.4%]) than in the 0.5 or 10 mg linagliptin (3 of 19 [15.8%]; 4 of 18 [22.2%], respectively) or placebo groups (6 of 17 [35.3%]) (**Table I**).

Pharmacokinetic Analysis

The mean plasma concentration–time profiles of linagliptin after a single oral dose on day 1 and at steady state on day 28 are shown in **Figure 1**. Linagliptin was rapidly absorbed in all patients with a median time to first occurrence of t_{max} at ~1.5 hours across doses (range, 0.5–12 hours after drug intake on day 1; 0.5–8 hours on day 28) (**Table II**). A less than proportional increase of linagliptin $C_{max,1}$ was observed between 0.5 and 2.5 mg (2.81 vs 8.84 nmol/L; factor of 3.1). In contrast, a dose-proportional increase of

Table I. Patient demographic characteristics and antidiabetic medication taken before the start of study—treated set.

Demographic Characteristics	Placebo	0.5 mg	2.5 mg	10 mg
No. of patients	17	19	18	18
Sex, no. (%)				
Male	13 (76.5)	15 (78.9)	14 (77.8)	13 (72.2)
Female	4 (23.5)	4 (21.1)	4 (22.2)	5 (27.8)
Age, y				
Mean (SD)	59.7 (6.4)	60.8 (9.2)	60.2 (6.4)	59.1 (8.6)
Range	47–67	29–69	42–68	40–69
Weight, kg				
Mean (SD)	67.2 (10.0)	64.5 (9.0)	69.6 (9.4)	63.5 (12.2)
Range	50.2–83.0	48.3–75.4	45.5–82.1	47.4–89.4
BMI, kg/m^2				
Mean (SD)	24.9 (3.0)	22.8 (2.1)	26.0 (3.2)	23.8 (4.5)
Range	20.8–31.4	18.4–26.5	19.7–33.3	18.4–34.4
<25 kg/m^2	10 (58.8)	15 (78.9)	6 (33.3)	13 (72.2)
≥ 25 kg/m^2	7 (41.2)	4 (21.1)	12 (66.7)	5 (27.8)
GLP-1, pmol/L				
Mean (SD)	1.7 (1.1)	3.7 (7.2)	2.7 (3.4)	2.2 (2.2)
Range	1.0–4.5	1.0–31.6	1.0–13.2	1.0–8.1
HbA_{1c} %				
Mean (SD)	7.0 (0.5)	6.9 (0.9)	7.1 (0.5)	7.2 (0.9)
Range	5.9–7.8	5.4–9.5	6.3–7.6	5.7–8.6
No of antidiabetic medications				
0	11 (64.7)	16 (84.2)	10 (55.6)	14 (77.8)
1	4 (23.5)	3 (15.8)	7 (38.9)	4 (22.2)
2	2 (11.8)	0 (0.0)	1 (5.6)	0 (0.0)
Antidiabetic therapy				
Metformin	0 (0.0)	1 (5.6)	0 (0.0)	1 (5.6)
Sulfonylurea	5 (29.4)	2 (10.5)	6 (33.3)	2 (11.1)
α-Glucosidase inhibitor	3 (17.6)	0 (0.0)	3 (16.7)	1 (5.6)

BMI = body mass index; GLP-1 = glucagon-like peptide-1; HbA_{1c} = glycosylated hemoglobin.

$C_{max,1}$ was observed between 2.5 and 10 mg (8.84 to 35.1 nmol/L; factor of 4.0). The total exposure on day 1 ($AUC_{\tau,1}$) of linagliptin increased dose dependently, but less than dose proportionally from 0.5 to 2.5 mg (29.9 to 129 nmol × h/L; a factor of 4.3), and from 2.5 to 10 mg (129 to 323 nmol × h/L; a factor of 2.5) (Table II). In the statistical analysis for dose proportionality using a power model for the PK parameters $AUC_{\tau,1}$, $AUC_{\tau,ss}$, $C_{max,1}$, and $C_{max,ss}$ of linagliptin, none of the PK parameters showed dose proportionality (95% CI for the slope did not include 1). At steady state, the t_{max} and $t_{½}$ did not increase with dose.

Linagliptin $CL/F_{,ss}$ and volumes of distribution ($Vd_z/F_{,ss}$) increased with dose. Moderate accumulation of linagliptin was observed after once daily dosing. The $R_{A,AUC}$ decreased with increasing doses, from 2.9-fold

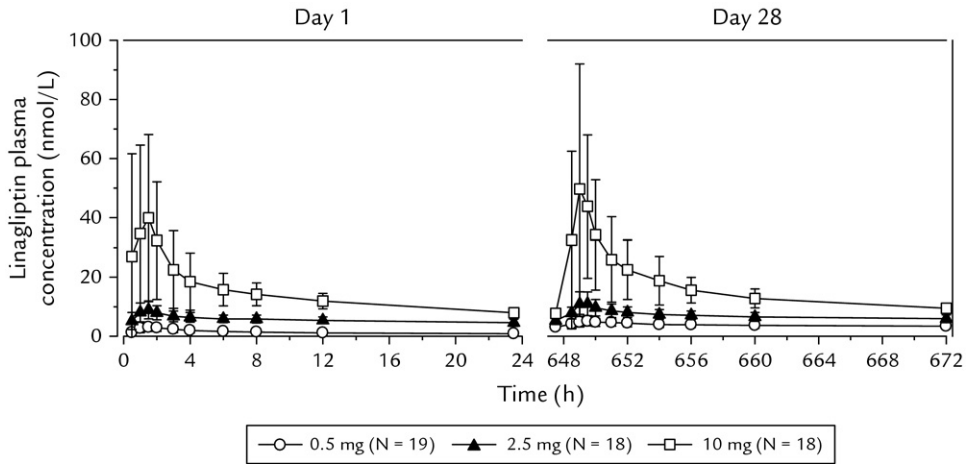

Figure 1. Mean (SD) plasma concentration–time profiles of linagliptin after oral dosing (0.5, 2.5, and 10 mg/d for 28 days) in male and female Japanese patients with type 2 diabetes mellitus (left panel: day 1; right panel: day 28). Lower limit of quantitation = 0.05 nmol/L.

for the 0.5 mg dose group to 1.2-fold for the 10 mg dose group. The terminal half-life of linagliptin ranged from 223 to 260 hours, which did not reflect the accumulation half-life of the drug. The accumulation half-life values for the 0.5, 2.5, and 10 mg linagliptin doses, as determined from the accumulation ratios after multiple dosing, were 38.5, 10.7, and 10.0 hours, respectively (**Table II**).

Renal excretion of the parent compound seemed to be only a minor route of elimination. Steady-state CL_R of linagliptin was negligible with 4.50 mL/min in the 0.5 mg dose group and increased with dose to 65.0 mL/min in the 10 mg dose group. Even at the highest dose group of 10 mg, the steady-state urinary excretion over 24 hours ($fe_{0-24,SS}$) was <7% of the administered dose.

Pharmacodynamic Analysis

In this multiple-dose study, linagliptin administration resulted in a dose-dependent DPP-4 inhibition in plasma, an effect not observed among patients on placebo (**Figure 2** and **Table III**). Maximum DPP-4 inhibition after 28 days treatment with linagliptin was increased compared with that on day 1. Maximum inhibition (percent relative to baseline) for linagliptin 0.5, 2.5, and 10 mg dose groups was 66.7%, 89.6%, and 92.9%, respectively, on day 28. Inhibition of plasma DPP-4 at 24 hours after the last dose on day 28 was approximately 46%, 78%, and 90% after linagliptin 0.5, 2.5, and 10 mg, respectively (**Table III**). Steady-state trough plasma DPP-4 activity was inhibited by >80% in none of the 17 patients in the 0.5 mg dose group, in 9 of the 17 patients in the 2.5 mg dose group, and all 18 patients in the 10 mg dose group. Interindividual variability of plasma DPP-4 inhibition was low to moderate over time and dose (gCV was <50% for all sampling times for the patients in the placebo and linagliptin 0.5 mg groups; gCV was also <50% for all sampling times in the linagliptin 2.5 and 10 mg groups, with the exception of the initial 2 sampling times in the 2.5 mg group, and the first sample time in the 10 mg group).

Linagliptin plasma concentration was well correlated with DPP-4 inhibition, and an 80% inhibition of DPP-4 activity in plasma was achieved with linagliptin concentrations of approximately 6 nmol/L (**Figure 3**). The IC_{50} of linagliptin on plasma DPP-4 activity was 3.15 (0.05) nmol/L, consistent with previous observations in healthy male subjects from the Japanese population.[16] In 1 patient, 80% inhibition of DPP-4 activity was observed at 2 of the plasma sampling times (days 14 and 33), despite plasma concentrations of linagliptin being <1 nmol/L. This patient was in the group receiving 2.5 mg linagliptin, and by day 35 (7 days after the final dose of drug), the patient's plasma linagliptin levels were below the limit of detection (0.1 nmol/L), whereas for all other patients receiving linagliptin, the drug was still detectable in plasma until day

Table II. Pharmacokinetic parameters of linagliptin after administration of single doses and at steady state (day 28) after administration of multiple oral doses in male and female Japanese patients with type 2 diabetes mellitus.

Parameter	0.5 mg (n = 19) gMean (gCV)	2.5 mg (n = 18) gMean (gCV)	10 mg (n = 18) gMean (gCV)
$AUC_{\tau,1}$, nmol × h/L	29.9 (45.7)	129 (23.7)	323 (32.6)
$AUC_{\tau,ss}$, nmol × h/L	89.4 (27.2)†	164 (23.4)†	373 (33.5)
$C_{max,1}$, nmol/L	2.81 (55.4)	8.84 (35.1)	35.1 (80.1)
$C_{max,ss}$, nmol/L	5.02 (33.9)†	11.0 (40.9)†	44.0 (80.4)
$t_{max,1}$, h*	1.50 (1.00–2.00)	1.50 (0.500–8.00)	1.50 (0.500–12.0)
$t_{max,ss}$, h*	1.50 (1.00–8.00)†	1.50 (0.500–4.00)†	1.25 (0.500–2.00)
$T_{1/2,ss}$, h	240 (33.1)†	223 (23.0)‡	260 (32.3)
$CL/F_{,ss}$, mL/min	197 (27.2)†	537 (23.4)†	945 (33.5)
$CL_{R,ss}$, mL/min	4.50 (76.6)‡	22.8 (54.7)‡	65.0 (30.0)†
$V_d/F_{,ss}$, L	4090 (45.0)†	10400 (31.2)‡	21200 (55.5)
$fe_{0-24,1}$, %	NC	0.227 (145)	4.08 (94.7)
$fe_{0-24,ss}$, %	2.26 (93.1)‡	4.25 (72.4) ‡	6.79 (51.6)†
$R_{A,AUC}$	2.88 (28.3)†	1.27 (21.4)†	1.16 (27.8)
$R_{A,Cmax}$	1.71 (35.8)†	1.23 (40.4)†	1.25 (78.0)
Accumulation $t_{1/2}$, h	38.5 (36.7)†	10.7 (50.9)§	10.0 (54.0)‖

$AUC_{\tau,ss}$ = area under the steady-state plasma concentration–time curve to the dosing interval; $CL/F_{,ss}$ = apparent oral clearance at steady-state; $C_{max,ss}$ = steady-state maximum plasma concentration; $fe_{0-24,1}$ = fraction of dose excreted unchanged in urine over the 24 h interval on day 1; $fe_{0-24,ss}$ = fraction of dose excreted unchanged in urine over the 24 h interval on day 28; gCV = geometric coefficient of variation; gMean = geometric mean; NC = not calculated as most values below lower limit of quantification; $R_{A:Cmax}$ = accumulation ratio C_{max}; $R_{A,AUC}$ = accumulation ratio AUC; $t_{1/2,ss}$ = terminal elimination half-life at steady-state; $t_{max,ss}$ = time to reach C_{max} at steady state; $V_d/F_{,ss}$ = apparent volume of distribution at steady-state.

Values are geometric mean (geometric %CV), unless otherwise specified.

*Values are given as median and range (min–max).

†n = 17.

‡n = 16.

§n = 15.

‖n = 14.

43, the final sampling time. The low plasma concentration affected this PK/PD relationship. This patient showed a similar profile of DPP-4 inhibition as the other patients until 24 hours post-treatment. However, the profile of DPP-4 inhibition was quite different after 24 hours post-treatment. The DPP-4 inhibition profile in this patient seemed to be irreversible inhibition, and the inhibition continued for 2 weeks after completion of drug treatment.

During the trial, linagliptin dose dependently decreased FPG concentrations, leading to a statistically significant change from baseline of −12.1 mg/dL ($P < 0.05$) and −22.8 mg/dL ($P < 0.01$) in the 2.5 and 10 mg dose groups after 14 days of treatment (**Table IV**). On day 29, statistically significant and clinically relevant changes from baseline of −11.5 ($P < 0.05$), −13.6 ($P < 0.05$), and −25.0 ($P < 0.01$) mg/dL were observed for the 0.5, 2.5, and 10 mg dose groups, respectively, whereas FPG in the placebo group remained almost unchanged relative to the day 1 baseline level (−3.2 mg/dL on day 29) (**Figure 4**).

Linagliptin reduced the $AUEC_{0-3h}$ during the MTT, both on day 1 ($P < 0.05$ for 10 mg dose; P = NS for lower doses) and more markedly on day 29 ($P < 0.05$ for all linagliptin doses tested) (**Table IV**). A dose-dependent reduction in glucose AUC based on

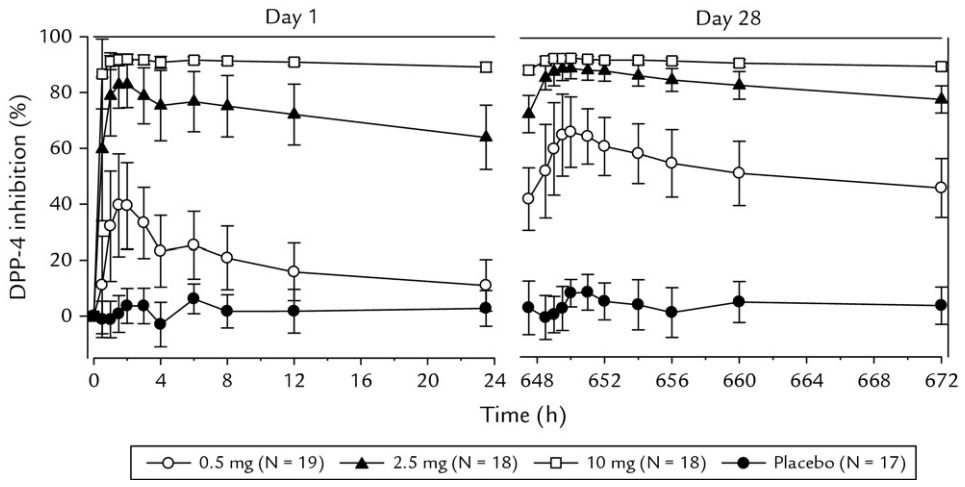

Figure 2. Mean (SD) percent inhibition of plasma dipeptidyl-peptidase-4 (DPP-4) activity after single oral doses of linagliptin (0.5, 2.5, 10 mg) and placebo once daily in male and female Japanese patients with type 2 diabetes mellitus (left panel: day 1), and after multiple dosing (right panel: day 28).

7-point measurements was also observed on days 1 and 29, reaching statistical significance for the 2.5 and 10 mg dose groups on day 29 ($P < 0.01$) (data not shown).

Despite the relatively short 4-week duration of treatment in this study, linagliptin demonstrated a significant effect to decrease HbA_{1c}. By day 29, treatment with linagliptin resulted in statistically and clinically significant reductions in HbA_{1c} relative to placebo ($P < 0.05$ for the 0.5 and 10 mg dose groups; P = NS for the 2.5 mg group) (**Table IV** and **Figure 5**). However, no clear effect of linagliptin was noted on fructosamine, and no trend was found in mean values and changes for the linagliptin dose groups in this trial.

Linagliptin dose dependently increased fasting plasma GLP-1 concentrations after a single dose on day 1 and at steady state (day 29). A statistically significant postmeal increase of about 2- and 3-fold versus placebo was already observed on day 1 for the 2.5 ($P < 0.05$) and 10 mg ($P < 0.01$) doses, and on day 29 for the 10 mg dose ($P < 0.001$), respectively. On day 29, postmeal GLP-1 concentrations were about 2-, 3-, and 4-fold higher compared with placebo for the 0.5, 2.5, and 10 mg linagliptin doses, respectively (**Table IV** and **Figure 6**).

Table III. Mean (SD) maximum inhibition of plasma dipeptidyl-peptidase-4 (DPP-4) activity (maximum pharmacodynamic effect and maximum pharmacodynamic effect at steady state) and the plasma DPP-4 inhibition 24 hours after dosing on days 1 and 28.

Dose	E_{max}, %	E_{24}, %	$E_{max,ss}$, %	$E_{\tau,ss}$, %
Placebo	8.9 (3.6)	2.8 (6.4)	9.7 (5.1)	2.4 (8.1)
0.5 mg	42.1 (17.5)	11.0 (9.2)	66.7 (12.0)	45.8 (10.6)
2.5 mg	84.7 (7.9)	63.9 (11.5)	89.6 (3.8)	77.8 (4.9)
10 mg	92.5 (1.2)	89.1 (1.8)	92.9 (1.0)	89.7 (1.4)

E_{24} = effect at 24 hours; E_{max} = maximum effect; $E_{max,ss}$ = maximum effect at steady state; $E_{\tau,ss}$ = effect at 24 hours at steady state.

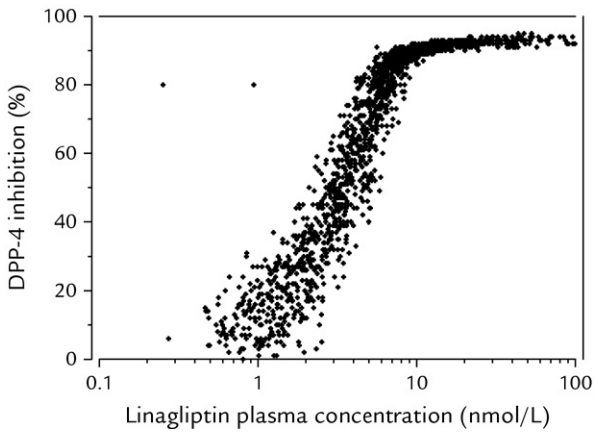

Figure 3. Scatterplot of the correlation between percent inhibition of plasma dipeptidyl-peptidase-4 (DPP-4) and plasma concentrations of linagliptin after multiple oral doses (0.5, 2.5, and 10 mg/d for 28 days) in male and female Japanese patients with type 2 diabetes mellitus.

Tolerability

Linagliptin administered once daily for 28 days over the dose range from 2.5 to 10 mg was generally well tolerated, and no significant tolerability concerns emerged during this study. No serious AEs, deaths, or significant AEs were reported, and there were no discontinuations due to AEs (**Table V**). Of the 72 patients who received linagliptin (n = 55) or placebo (n = 17), 17 (23.6%) reported at least 1 AE. No differences were noted in the overall incidence of AEs between the patients in the linagliptin dose groups (11 [20.0%] of the 55 patients) and those in the placebo group (6 [35.3%] of the 17 patients). Two patients in the linagliptin dose groups experienced an AE of moderate intensity (3.6%): 1 case of nasopharyngitis in a patient who received linagliptin 10 mg and 1 case of constipation in a patient who received linagliptin 0.5 mg. All other AEs were of mild intensity, and no AEs were noted during the washout phase.

Of the 55 patients who received linagliptin, 3 (5.5%) patients experienced an AE that the investigator considered related to the investigational product: 1 (5.3%) of the 19 patients in the 0.5 mg group (constipation) and 2 (11.1%) of the 18 patients in the 2.5 mg group (infrequent bowel movement for 1 patient and early satiety for 1 patient). The most frequent reported AE was nasopharyngitis (10 of 72 patients; 13.9% overall). The incidence of nasopharyngitis was higher in the placebo group (11.8%) than in the linagliptin dose groups (5.3%–11.2%) (**Table V**).

Clinical laboratory parameters were stable throughout the treatment period, and no patient experienced symptoms of hypoglycemia. No blood glucose levels <70 mg/dL were recorded from daily self-blood glucose measurements. No clinically relevant changes were observed in any ECG parameters. Mean body weight did not significantly change in any of the treatment groups during the study. After 28 days of treatment, mean weight was 65.9 (10.1) kg for those in the placebo group, a reduction of 1.3 kg from the baseline weight at screening. In the linagliptin groups, mean weights at day 28 were 64.5 (8.5) kg, 69.3 (9.6) kg, and 62.6 (12.5) kg for those in the 0.5, 2.5, and 10 mg groups, respectively (ie, all changes observed were reductions of <1 kg from baseline for all of the linagliptin groups).

DISCUSSION

This randomized, double-blind, placebo-controlled, multiple-dose design study was the first study to assess the PK, PD, and tolerability profiles of multiple doses of linagliptin in Japanese patients with type 2 diabetes. The PK profile of multiple doses of linagliptin in this study (nonlinear PKs, long terminal half-life that was not accumulation half-life, and low [<7%] urinary excretion) was similar to that previously observed in healthy Japanese[16] and white patients.[10,24]

After oral administration, linagliptin was rapidly absorbed and inhibited plasma DPP-4 activity in a dose-dependent manner. It is believed that DPP-4 inhibition of ≥80% results in a clinically meaningful glucose-lowering effect in diabetic patients.[25] Therefore, the sustained inhibition of plasma DPP-4 activity by >80% at 24 hour postdosing with the highest linagliptin dose evaluated (10 mg) supported a once daily dosing regimen in Japanese type 2 diabetes patients. Consistent with previous studies in white and Japanese patients,[10,16] with daily doses of 2.5 mg linagliptin, DPP-4 inhibition remained slightly below this efficacy threshold (ie, inhibition of ≥80% for 24 hours at steady state) in this Japanese patient population. Studies are ongoing to determine if a 5 mg/d dose of linagliptin would be effective for improving and maintaining glycemic control in patients with type 2 diabetes in the Japanese population, as observed in studies in white T2DM patients.[6,7]

Table IV. Analysis of change from baseline of fasting plasma glucose (FPG), plasma blood glucose levels during a meal tolerance test (MTT), glycosylated hemoglobin (HbA_{1c}) and plasma glucagon-like peptide-1 (GLP-1) after administration of linagliptin in Japanese patients with type 2 diabetes

Characteristic	Placebo Mean (SD)	0.5 mg Mean (SD)	2.5 mg Mean (SD)	10 mg Mean (SD)
Mean change from baseline of FPG, mg/dL				
Day 1 (baseline)	155.5 (21.3)	147.9 (25.4)	154.7 (25.1)	158.4 (28.6)
Day 14	0.3 (27.0)	–3.8 (10.8)	–12.1 (15.3)	–22.8 (15.9)
P Placebo vs active	—	0.2881	0.0462	0.0005
Day 29	–3.2 (22.5)	–11.5 (8.3)	–13.6 (15.2)	–25.0 (12.3)
P Placebo vs active	—	0.0191	0.0479	0.0001
Mean change from baseline of glucose $AUEC_{0-3}$ after MTT, mg × h/dL*				
Day –1 (baseline)	380.9 (51.2)	320.4 (53.3)	383.9 (82.6)	372.8 (80.2)
Day 1	5.6 (36.3)	–4.7 (17.5)	–18.6 (16.5)	–24.6 (36.5)
P Placebo vs active	—	0.1547	0.0576	0.0119
Day 29	–8.0 (25.0)	–29.0 (26.4)	–45.6 (53.1)	–68.1 (47.7)
P value Placebo vs active	—	0.0441	0.0282	0.0004
Mean change from baseline of HbA_{1c}, %				
Day –1 (baseline)	7.04 (0.50)	6.94 (0.90)	7.07 (0.45)	7.22 (0.92)
Day 29	0.04 (0.55)	–0.31 (0.19)	–0.20 (0.39)	–0.44 (0.28)
P Placebo vs active	—	0.0086	0.0759	0.0005
Mean change from baseline of GLP-1, nmol/L				
Day –1 Post meal	2.2 (2.1)	2.8 (3.9)	2.6 (1.1)	2.2 (1.5)
P Placebo vs active	—	0.7483	0.8433	0.8843
Day 1 Post meal	2.6 (2.1)	3.1 (3.2)	5.0 (2.9)	5.2 (2.8)
P Placebo vs active	—	0.5455	0.0144	0.0072
Day 29 Post meal	2.5 (1.6)	3.4 (6.3)	4.1 (4.5)	7.2 (6.9)
P Placebo vs active	—	0.1668	0.0610	0.0005

For FPG and plasma glucose $AUEC_{0-3}$, the differences are between levels measured on day –1 or 1 (pre-treatment baseline) and those measured on days 14 and 29 or 1 and 29 (post-treatment), respectively. For GLP-1, the differences are between pre- and post-meal GLP-1 levels on the days shown. *P* values are for ANCOVA between placebo and active groups, which included baseline value as a covariate.

*The AUC (0–3 hours after drug administration) between zero and the glucose concentration at each time point was analyzed and $AUEC_{0-3}$ was corrected for baseline ($AUEC_{0-3,\ norm}$) (ie, the actual glucose concentrations at each time point were corrected for the individual pre-dose measurements on each day).

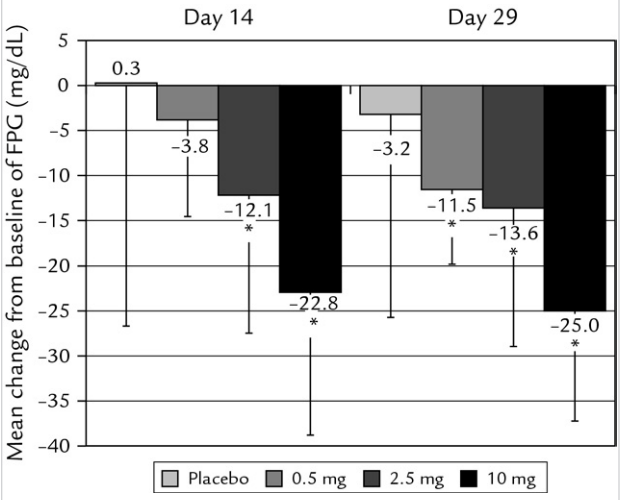

Figure 4. Mean (SD) change from baseline (SD) of fasting plasma glucose (FPG) (milligrams per deciliter) on days 14 and 29 in male and female Japanese patients with type 2 diabetes mellitus. $*P < 0.05$.

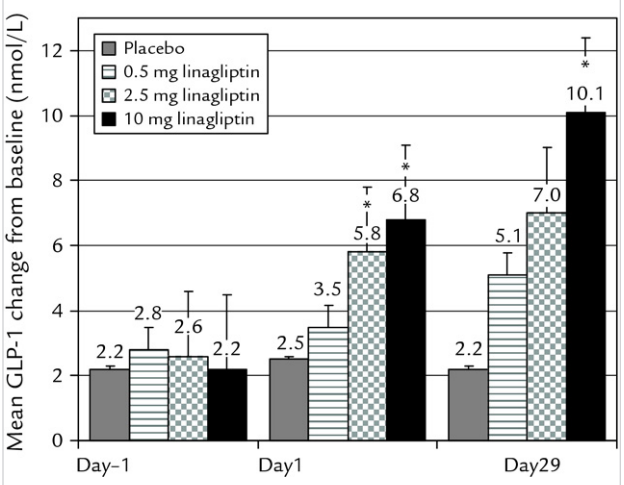

Figure 6. Mean change from baseline (day −1) of glucagon-like peptide-1 (GLP-1) measured 30 minutes after a meal tolerance test (MTT; picomoles per liter) on days 1 and 29 (mean [SD]) $*P < 0.05$ comparison with placebo.

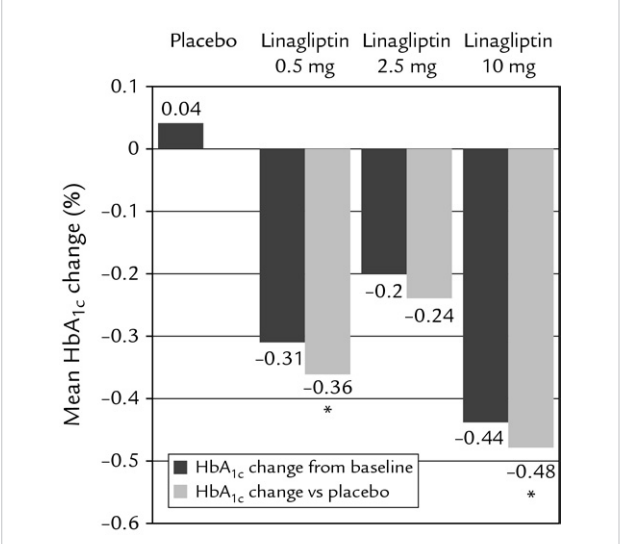

Figure 5. Comparison of mean change (percent) in glycosylated hemoglobin (HbA_{1c}) at day 29 of linagliptin therapy with baseline and placebo in male and female Japanese patients with type 2 diabetes mellitus. $*P < 0.05$.

The analyses performed in this study centered on the relationship between linagliptin PDs and plasma concentrations rather than between body weight adjusted dose and PDs. This was because an exploratory population PK analysis of the impact of body weight on PK and PD parameters demonstrated that body weight did not have any clinically meaningful impact (data not shown). In addition, other studies showed that the PK properties of linagliptin were such that dose adjustment based on body weight was not required to achieve a therapeutic plasma concentration.[10,12–14] Analysis of the relationship between DPP-4 inhibition and plasma drug concentration levels in the present study showed that 1 patient had unusually high DPP-4 inhibition and low linagliptin plasma concentrations (<1 nmol/L) at 2 sampling times (days 14 and 33). In this patient, when the final PK sample was taken (day 43), the inhibition of DPP-4 activity remained >80%, despite the level of drug having fallen below the limit of detection in the patient's plasma. It was not clear why this individual showed such a prolonged response.

Improvement in blood glucose levels during an MTT were observed after the first intake of linagliptin at the 10 mg dose. At 24 hours after the last drug intake, this effect was even more apparent, with improvements for all linagliptin doses studied. Linagliptin reduced FPG in a dose-

Table V. Adverse events (AEs) reported in ≥1 patient by treatment group

System Organ Class/ Preferred Term	Placebo (N = 17) n (%)	0.5 mg (N = 19) n (%)	2.5 mg (N = 18) n (%)	10 mg (N = 18) n (%)	Total for Linagliptin (N = 55) n (%)
Any AE	6 (35.3)	2 (10.5)	5 (27.8)	4 (22.2)	11 (20.0)
Severe	0	0	0	0	0
Drug-related AE	0	1 (5.3)	2 (11.1)	0	3 (5.5)
AEs leading to discontinuation	0	0	0	0	0
Any serious AE	0	0	0	0	0
Gastroenteritis	0	0	1 (5.6)	0	1 (1.8)
Nasopharyngitis	2 (11.8)	1 (5.3)	1 (5.6)	2 (11.2)	4 (7.3)
Pharyngitis	2 (11.8)	0	0	0	0
Seasonal allergy	1 (5.9)	0	0	0	0
Eye discharge	1 (5.9)	0	0	0	0
Ocular hyperemia	1 (5.9)	0	0	0	0
Hot flush	0	0	0	1 (5.6)	1 (1.8)
Constipation	0	1 (5.3)	0	0	1 (1.8)
Diarrhea	1 (5.9)	0	0	0	0
Infrequent bowel movements	0	0	1 (5.6)	0	1 (1.8)
Lower gastrointestinal hemorrhage	1 (5.9)	0	0	0	0
Dermatitis contact	0	0	0	1 (5.6)	1 (1.8)
Eczema	0	0	1 (5.6)	0	1 (1.8)
Chills	0	0	0	1 (5.6)	1 (1.8)
Early satiety	0	0	1 (5.6)	0	1 (1.8)

dependent manner and the magnitude of these reductions increased with time. A particularly notable finding given the brief duration of treatment and low mean baseline HbA_{1c} (6.9%–7.2%) values for the treated groups, was that by the end of the study, significant reductions in placebo-adjusted HbA_{1c} levels were observed in the linagliptin groups. The reduction in mean HbA_{1c} level observed for the linagliptin 2.5 mg group was less than that for the other linagliptin groups (ie, 0.5 and 10 mg doses); this might at least partially be explained by the fact that this group had a higher proportion of patients who had previously received antidiabetic medication than the other linagliptin-treated groups. In patients who received antidiabetic treatment before the study, it would be expected that the HbA_{1c} value at baseline, which followed a washout of just 2 weeks, would be likely to increase over the course of the study as the effects of the previous treatment on HbA_{1c} wore off. This was seen for the placebo group, where the proportion of patients who received previous antidiabetic medication was also high and the mean HbA_{1c} rose over time.

The data for improvements in glycemic control (FPG, 3 hours postprandial glucose levels after MTT, and HbA_{1c}) reported were compatible with the mechanism of action of linagliptin, which inhibits DPP-4 activity and the degradation of GLP-1 and glucose-dependent insulinotropic polypeptide. The data from measurement of the mean change from baseline in GLP-1 level after an MTT on days 1 and 29 suggested that linagliptin significantly increased plasma GLP-1 levels. On day 29, when plasma GLP-1 levels were assayed before the MTT (but 24 hours after the last linagliptin dose), the baseline GLP-1 levels were found to be >3-fold higher in the linagliptin-treated patients compared with the placebo group. This suggested a

long duration of action for linagliptin, and was consistent with a once daily dosing regimen.

Linagliptin was well tolerated with a tolerability profile similar to that of placebo at multiple doses up to 10 mg/d for 28 days in the Japanese patients with type 2 diabetes. In the dose range investigated (0.5–10 mg), symptoms of hypoglycemia were not reported with linagliptin. Larger studies of longer duration will determine if the risk of hypoglycemia for linagliptin when used as monotherapy in Japanese patients with type 2 diabetes is similar to that for placebo, as was reported for white patients.[6]

In this study, there was no indication for a clinical effect of linagliptin on any ECG parameter investigated. A previous thorough QT study for linagliptin performed in accordance with the International Conference on Harmonisation E14 guideline found that single dose administration of therapeutic (5 mg) and supratherapeutic (100 mg) doses of linagliptin did not prolong the QT interval in healthy volunteers.[26]

In Japan, type 2 diabetes has been recognized as the leading cause for dialysis since 1998.[27] The primarily nonrenal elimination pathway of linagliptin, and, potentially, no need for dose adjustment, may offer advantages in diabetic patients with renal impairment.[12,28]

The inclusion and exclusion criteria adhered to in this trial might limit extrapolation of the results to certain patient groups not studied here; however, further investigations to evaluate the long-term tolerability and efficacy potential for linagliptin in the Japanese population are either ongoing or planned. These include a Phase IIb/III trial that is currently under way (Clinicaltrials.gov identifier: NCT00654381).[24,29]

CONCLUSIONS

In this randomized, double-blind, placebo-controlled multiple-dose design study in male and female Japanese type 2 diabetes patients, linagliptin demonstrated statistically significant and clinically meaningful improvements in glucose control. The nonlinear PKs, long terminal half-life, low accumulation, and low urinary excretion observed were consistent with findings in Japanese healthy volunteers and white patients. Linagliptin was well tolerated at all doses studied (0.5, 2.5, and 10 mg).

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