Linagliptin, a Dipeptidyl Peptidase-4 Inhibitor in Development for the Treatment of Type 2 Diabetes Mellitus: A Phase I, Randomized, Double-Blind, Placebo-Controlled Trial of Single and Multiple Escalating Doses in Healthy Adult Male Japanese Subjects

Akiko Sarashina, MSc[1]; Shogo Sesoko, MD[2]; Mitsuyoshi Nakashima, MD[3]; Naoyuki Hayashi, MSc[4]; Atsushi Taniguchi, MSc[5]; Yoshiharu Horie, PhD[5]; Eva U. Graefe-Mody, PhD[6]; Hans-Juergen Woerle, MD[6]; and Klaus A. Dugi, MD[7]

[1]Pharmacokinetics and Non-clinical Safety Department, Nippon Boehringer Ingelheim Co., Ltd., Hyogo, Japan; [2]Iryohoujin-shadan Shinpukai Maruyama Hospital, Shizuoka, Japan; [3]Hamamatsu Institute of CTP, Shizuoka, Japan; [4]Clinical Research Department, Nippon Boehringer Ingelheim Co., Ltd., Tokyo, Japan; [5]Biostatistics Group, Nippon Boehringer Ingelheim Co., Ltd., Tokyo, Japan; [6]Therapeutic Area Metabolism, Boehringer Ingelheim GmbH, Ingelheim, Germany; and [7]Medical Affairs, Boehringer Ingelheim GmbH, Ingelheim, Germany

ABSTRACT

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

Objective: This study investigated the tolerability, pharmacokinetics, and pharmacodynamics of linagliptin in healthy adult male Japanese volunteers, in compliance with Japanese regulatory requirements for new drugs intended for use in humans.

Methods: This was a Phase I, randomized, double-blind, placebo-controlled study in healthy volunteers. Linagliptin or placebo was administered as single escalating doses of 1, 2.5, 5, and 10 mg, or as multiple escalating doses of 2.5, 5, and 10 mg once daily for 12 days. Three quarters of subjects in each dose group were randomized to active drug and one quarter to placebo. Blood and urine samples for determination of pharmacokinetic parameters were obtained before administration of the first dose of study drug and at regular time points after administration, with more frequent blood sampling on days 1 and 12 in subjects receiving multiple doses. Inhibition of DPP-4 activity and plasma concentrations of glucagon-like peptide-1 (GLP-1) and glucose were also determined. Tolerability was assessed throughout the study based on physical examinations, 12-lead ECGs, and standard laboratory tests.

Results: Eight subjects were enrolled in each dose group, 6 receiving active drug and 2 receiving placebo. Baseline demographic characteristics were comparable in the single-dose groups (mean [SD] age, 24.5 [3.6] years; mean weight, 61.2 [6.2] kg; mean height, 171.5 [5.3] cm) and multiple-dose groups (mean age, 25.4 [3.7] years; mean weight, 61.6 [5.2] kg; mean height, 170.9 [4.9] cm). Linagliptin displayed nonlinear pharmacokinetics. Total systemic exposure (AUC and C_{max}) increased in a manner that was less than dose proportional. T_{max} ranged from 1.50 to 6.00 hours, and elimination $t_{1/2}$ ranged from 96.9 to 175.0 hours. Total CL increased with increasing dose (from 140 mL/min in the 1-mg group to 314 mL/min in the 10-mg group), as did apparent V_d (from 1260 to 3060 L with doses up to 10 mg). Steady state was attained within 2 to 3 days. The accumulation $t_{1/2}$ ranged from ~10 to 15 hours. The accumulation ratio with multiple dosing was <1.5 and decreased with increasing dose (~1.2 in the 10-mg dose). Urinary excre-

Data from this study have been presented in part at the 68th Scientific Sessions of the American Diabetes Association, June 9–13, 2008, San Francisco, California (poster 0534P), and at the 52nd Annual Meeting of the Japan Diabetes Society, May 21–24, 2009, Osaka, Japan (poster III-P-96).

Accepted for publication April 20, 2010.
doi:10.1016/j.clinthera.2010.06.004
0149-2918/$ - see front matter

tion increased with increasing dose and over time in all dose groups, although it did not exceed 7% in any dose group on day 12. Linagliptin inhibited plasma DPP-4 activity in a dose-dependent manner. Mean DPP-4 inhibition was ≥80% over 24 hours after a single dose of 10 mg and after multiple doses of 5 and 10 mg for 12 days. Postprandial plasma GLP-1 concentrations increased from preprandial concentrations by 2- to 4-fold after administration of single doses and by 2- to 2.5-fold on day 12 after administration of multiple doses. Baseline (premeal) plasma GLP-1 concentrations were higher on day 12 than on day 1 in all linagliptin groups. A total of 3 adverse events were reported in 1 subject each: an increase in histamine concentration in a subject receiving a single dose of linagliptin 5 mg, vasovagal syncope in a subject receiving a single dose of linagliptin 10 mg, and pharyngitis in a subject receiving multiple doses of linagliptin 10 mg. None of these events was considered drug related. No episodes of hypoglycemia occurred during the study.

Conclusions: In this short-term study in healthy adult male Japanese volunteers, multiple oral doses of linagliptin inhibited plasma DPP-4 activity and elevated active GLP-1 concentrations in a dose-dependent manner, with no episodes of hypoglycemia. Multiple dosing of linagliptin for 12 days was well tolerated and exhibited a pharmacokinetic/pharmacodynamic profile consistent with a once-daily regimen. Clinical studies in Japanese patients with T2DM appear to be warranted. (*Clin Ther.* 2010;32:1188–1204) © 2010 Excerpta Medica Inc.

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

INTRODUCTION

Diabetes mellitus (DM) affects ~171 million people worldwide, and its prevalence is expected to double by the year 2030.[1] Type 2 DM (T2DM) accounts for between 90% and 95% of all cases of DM.[2] According to the 2007 National Health and Nutrition Survey from the Japanese Ministry of Health, Labour and Welfare, 8.9 million members of the Japanese population were considered likely to have diabetes, and 13.2 million were considered candidates for diabetes based on their risk profiles.[3] Changes in the Japanese lifestyle and diet are believed to have contributed to the increase in diabetes; in general, the Japanese have become more sedentary and their dietary fat intake has increased since the end of World War II.[4,5] Over this period, these changes have been associated with a steady increase in body mass index (BMI) among Japanese men, a known risk factor for the development of insulin resistance, impaired glucose tolerance, and diabetes. Genetic characteristics common to many Japanese may also contribute to the high prevalence of diabetes.[4,5] For example, unlike Westerners, up to half of Japanese individuals lack the catalytically active mitochondrial aldehyde dehydrogenase-2 isozyme.[6,7]

Long-term complications of DM include retinopathy, with potential loss of vision; nephropathy, leading to renal failure; peripheral neuropathy, with a risk for foot ulcers, amputations, and Charcot joints; and autonomic neuropathy, leading to gastrointestinal, genitourinary, and cardiovascular symptoms and sexual dysfunction.[2] Despite the availability of various antidiabetic treatments, the majority of patients fail to attain or maintain tight glycemic control over time, increasing their risk for serious microvascular and macrovascular complications.[8–10] Given a progressive loss of β-cell function and insulin secretion over time, single therapies for T2DM may be replaced by more complex combination therapies aimed at limiting further deterioration in glycemic control and preventing development of microvascular and/or microvascular complications.[2,11,12] Limitations in the long-term efficacy, tolerability, and dosing convenience of existing antihyperglycemic therapies lead to a continuing need for well-tolerated and effective treatments for T2DM.[13,14]

Dipeptidyl peptidase-4 (DPP-4) inhibitors have recently been introduced into the antidiabetic armamentarium.[15] Sitagliptin was approved by the US Food and Drug Administration in 2006, and vildagliptin, saxagliptin, and alogliptin were subsequently approved in various markets. Sitagliptin was approved in Japan in 2009, followed by vildagliptin and alogliptin in 2010. DPP-4 inhibitors act by increasing active (intact) levels of incretin peptides, including glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide. GLP-1 and other incretins augment insulin secretion in a glucose-dependent manner, whereas DPP-4 rapidly inactivates these peptides, attenuating their beneficial effects on glucose homeostasis. DPP-4 inhibitors delay the breakdown of incretins, thereby prolonging and enhancing their action. In patients with T2DM, treatment with DPP-4 inhibitors has been associated with improvements in glycemic control and postprandial glucose excursions.[15]

Inhibition of DPP-4 offers several advantages in the treatment of T2DM. Because incretin stimulation of insulin release is glucose dependent,[16] the risk of hypoglycemic episodes with DPP-4 inhibitors is reported to be low. DPP-4 inhibitors also appear to have a neutral effect on body weight,[17,18] compared with the weight gain that has been associated with the use of insulin, sulfonylureas, and thiazolidinediones.[19] In mouse and rat models of T2DM, administration of DPP-4 inhibitors for 2 to 3 months has been reported to have beneficial effects on β-cell mass and function.[20–22] Similar benefits on β-cell function have been reported in patients with T2DM,[23,24] along with improvements in glycemic control and insulin sensitivity.[23] Finally, unlike GLP-1 analogues,[25] DPP-4 inhibitors can be administered orally and may not be associated with the gastrointestinal adverse effects that have been noted with pharmacologic concentrations of GLP-1 analogues.[26] Although use of α-glucosidase inhibitors appears to be declining in Europe and the United States, they continue to be widely used in Japanese patients with T2DM to achieve greater glycemic control, particularly control of postprandial hyperglycemia.[27] Improvements in fasting and postprandial glucose concentrations and glycosylated hemoglobin have been reported in a review of clinical trials of monotherapy with various DPP-4 inhibitors,[15] without the gastrointestinal adverse effects (eg, flatulence, diarrhea) seen with α-glucosidase inhibitors.

Linagliptin (BI 1356) is an orally active DPP-4 inhibitor[28] that is currently in Phase III development for the treatment of T2DM. In preclinical studies, linagliptin exhibited high-potency inhibition of DPP-4 (half-maximal inhibitory concentration [IC_{50}], ~1 nM) and was reported to increase the $t_{1/2}$ of circulating active GLP-1 (AUC increased by 77% on oral glucose tolerance testing [OGTT] in Zucker fatty rats) and to improve glucose homeostasis (glucose excursion reduced by ~50% on OGTT in C57BL/6J mice).[29] Linagliptin has been found to enter the circulation rapidly (T_{max}, 1.5–2 hours), have a long duration of action (>80% DPP-4 inhibition maintained at 24 hours after dosing), and be excreted primarily by nonrenal elimination pathways (<10% renal elimination, the remainder eliminated fecally).[30–34] In a study of single escalating doses in 64 healthy white male volunteers, linagliptin was well tolerated at up to 120 times the proposed clinically effective dose of 5 mg once daily, with an adverse-event (AE) profile similar to that of placebo, suggesting a potentially wide safety margin.[30] In a multiple-dose study in 48 white male patients with T2DM, treatment with linagliptin 5 mg once daily for 12 days was well tolerated and was associated with a significant reduction in blood glucose concentrations ($P < 0.05$) on OGTT after the first dose of linagliptin; the reduction was more pronounced on day 13, 24 hours after the last dose of linagliptin.[31]

Because DPP-4 inhibitors such as linagliptin augment insulin secretion through their effects on concentrations of GLP-1 and glucose-dependent insulinotropic peptide, they may be beneficial in Japanese patients with T2DM. In a study examining the genetic susceptibility to T2DM in 4 US populations, patients of Japanese descent were reported to have a lower BMI (mean [SD], 25.84 [4.01] kg/m^2) and lower fasting insulin levels (8.74 [6.91] μU/mL) compared with individuals of European descent (30.43 [6.72] kg/m^2 and 14.01 [13.26] μU/mL, respectively), Mexican Americans (31.61 [8.18] kg/m^2 and 15.71 [11.55] μU/mL), and African Americans (33.04 [8.69] kg/m^2 and 17.39 [21.42] μU/mL).[35] Furthermore, compared with obese patients with T2DM, lean patients with T2DM have been found to display certain pathophysiologic characteristics, notably lower insulin resistance and poorer insulin secretory capacity.[36–38] Decreased β-cell function may be one of the major reasons that Asians, including Japanese, are at increased risk for DM, despite lower rates of obesity, compared with Westerners.[39,40]

Guidelines for the evaluation of new drugs from the Japanese Pharmaceutical and Medical Devices Agency recommend that the dose–response relationship of any new drug be confirmed in the Japanese population and that treatment be evaluated in "adequate numbers of Japanese cases."[41] In accordance with these guidelines, the present study was designed to evaluate the tolerability, pharmacokinetics, and pharmacodynamics of single and multiple escalating doses of linagliptin in healthy adult male Japanese subjects.

SUBJECTS AND METHODS

Study Design

This Phase I, randomized, double-blind, placebo-controlled study was conducted at the Iryohoujin-shadan Shinpukai Maruyama Hospital, Shizuoka, Japan, in 2006. After an overnight fast of ≥10 hours, linagliptin or placebo was administered as single escalating oral doses of 1, 2.5, 5, and 10 mg, or as multiple escalating doses of 2.5, 5, and 10 mg once daily for 12 days. Three quarters of subjects in each dose group were randomized

to active drug and one quarter to placebo. The study began with the lowest dose group; once the safety profile of each dose had been established, the study proceeded to the next-highest dose group.

Subjects in the single-dose study were admitted to the study center on day –1 and discharged on day 10 (9 days after dosing on day 1). Subjects in the multiple-dose study were admitted to the study center on day –1 and discharged on day 21 (9 days after the last dose on day 12). Subjects were not to lie down for 2 hours after drug administration, except during medical examinations, ECGs, or measurement of blood pressure and heart rate. All subjects were closely monitored throughout their hospitalization and were followed for 192 hours after administration of the last dose of study drug.

The randomization procedure was based on a computerized table of pseudorandom numbers; seed numbers were used to set the starting point for generation of a series of random numbers to make the assignment list reproducible and unpredictable. To maintain blinding of subjects and investigators, study medications were identical in appearance and were provided in identical packaging. Although blinding of subjects' treatment assignment (active drug or placebo) was maintained within each dose group, the current dose level was known to both subjects and investigators.

The study protocol was designed by the sponsor and approved by the ethics committee of the study center. The study was conducted in compliance with the ethical standards for human experimentation established by the Declaration of Helsinki (1996), and in accordance with the Guideline for Good Clinical Practice (GCP) and the Japanese GCP regulations (Ministry of Health and Welfare Ordinance no. 28, March 27, 1997).

Subjects

Eligible subjects were nonsmokers, aged between 20 and 35 years, and with a BMI ≥17.6 and ≤29.9 kg/m^2. Subjects were to be in good general health, as determined by a medical history, physical examination, measurement of vital signs (blood pressure and heart rate), and laboratory safety tests (routine hematology; serum chemistry, including transaminases; and urinalysis). Subjects were excluded if they had a relevant history of renal, hepatic, respiratory, cardiovascular, gastrointestinal, immunologic, neurologic, psychiatric, metabolic, or hormonal disorder. Subjects were also excluded if they had donated blood within 4 weeks of giving informed consent, participated in another clinical trial within 8 weeks before the start of the present study, or anticipated needing any prescription or nonprescription drugs during the study. All subjects gave written informed consent.

Pharmacokinetic End Points and Assessments

Pharmacokinetic and pharmacodynamic end points were assessed based on measured plasma and urinary linagliptin concentrations, DPP-4 activity, and GLP-1 concentrations after administration of single and multiple doses of linagliptin.

Noncompartmental analysis of plasma and urinary linagliptin concentration–time data was performed according to standard methods[42] using WinNonlin Professional version 5.0.1 (Pharsight Corporation, Mountain View, California). The apparent terminal rate constant (λ) was estimated by regression of the terminal log-linear portion of the plasma concentration–time curve (determined by direct inspection); the $t_{1/2}$ 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 $AUC_{0-\infty}$ was estimated as the sum of AUC to the last measured concentration, with the extrapolated area calculated as the quotient of the last predicted concentration and λ. C_{max} and T_{max} were determined by direct inspection of the plasma concentration–time data. The fraction of dose excreted unchanged in urine (fe) was calculated as the sum of the amounts of linagliptin collected in urine during each collection interval (the product of the linagliptin urine concentration in each interval and the weight of urine collected in each interval, with weight set equal to volume). The apparent clearance after oral administration (CL/F) was determined as the quotient of the drug dose and AUC within a specific time interval. The apparent V_d during the terminal phase after oral administration (V_d/F) was calculated by dividing the CL/F by λ. Plasma accumulation of linagliptin after multiple dosing was assessed by calculating the accumulation ratios (R_A) (day 12/day 1) for AUC and C_{max} for each subject.

Blood Sampling and Analytic Procedures

For quantification of linagliptin plasma concentrations and measurement of DPP-4 activity, blood samples (3 mL each) were collected from an indwelling cannula inserted in a forearm vein into an EDTA-2K–containing tube. In those receiving single doses, blood samples were

obtained at 30 minutes before administration of the dose and at 30 minutes and 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 48, 72, 96, 144, and 192 hours after administration. In those receiving multiple doses, blood samples were obtained at 30 minutes before administration and at 30 minutes and 1, 1.5, 2, 3, 4, 6, 8, and 12 hours after administration on day 1 (single-dose conditions) and day 12 (steady-state conditions), and at 30 minutes before administration (for determination of trough concentrations) on days 2 through 11, 13 through 16, and 18 through 20. Over the course of the study, the total amounts of blood collected per subject receiving single and multiple doses were ~48 and ~108 mL, respectively.

Immediately after collection, blood samples were centrifuged (KUBOTA 5900 RS-720M, KUBOTA Corporation, Tokyo, Japan) at 4°C for ~10 minutes at 3500 rpm. The plasma recovered from each blood sample was separated into 2 aliquots, one of which was to contain at least 0.6 mL, and stored at the clinical site at –20°C or lower. One aliquot of each blood sample was then shipped on dry ice to the appropriate analytic laboratory (Department of Bioanalytical Services, Covance Laboratories Ltd., Harrogate, United Kingdom, for measurement of plasma and urinary linagliptin concentrations; Institute for Clinical Research and Development GmbH, Mainz, Germany, for DPP-4 activity), where it was stored under similar conditions until analyzed, and the other aliquot was retained at the laboratory until the clinical trial report was finalized.

A blank urine sample was collected for quantification of urinary linagliptin concentrations before administration of study drug, and two 0.5-mL aliquots were retained to check for analytic interference. In subjects receiving single doses, all urine voided was collected over the intervals from 0 to 4, 4 to 8, 8 to 12, 12 to 24, 24 to 48, 48 to 72, 72 to 96, 96 to 120, 120 to 144, 144 to 168, and 168 to 192 hours after administration. In subjects receiving multiple doses, all urine voided was collected over the intervals from 0 to 4, 4 to 8, 8 to 12, and 12 to 24 hours after administration on days 1 and 12, and at 24-hour intervals after administration on days 2 to 11 and 13 to 21. Subjects 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 2M citric acid solution). Samples were stored in a refrigerator from the time of sampling until 24 hours after drug administration. The urine weight was set equal to volume (ie, 1 kg = 1 L), without correction for specific gravity of urine, and the weight was recorded for each collection interval. After completion of urine collection in each 24-hour interval, 2 aliquots (≥0.5 mL each) were stored at the clinical site at –20°C or lower until shipment on dry ice to the analytic laboratory, where they were stored under the same conditions until analyzed. An aliquot of each urine sample was retained at the laboratory, where it 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, with [$^{13}C_3$]-linagliptin as internal standard, as has been described previously.[30] The inaccuracy and imprecision of the assay in quality-control samples spiked with 3 linagliptin concentrations were between –3.4% and 5.9% and 3.0% and 3.8%, respectively, in plasma and between –5.6% and 8.1% and 5.9% and 7.4% in urine.

DPP-4 activity after administration of single and multiple doses was measured using a validated method that employed a semiquantitative enzyme activity assay with fluorescence detection (substrate, H-Ala-Pro-7-aminoamido-4-trifluoromethylcoumarin). Fluorescence was detected at 535 nm (emission) using an excitation wavelength of 405 nm after 10 minutes of incubation at amplification/gain 60 on a GENios FL reader (Tecan US Inc., Durham, North Carolina).[30] The imprecision of the assay was between 6.29% and 9.56%.

Blood samples for measurement of GLP-1 concentrations were collected before and at 4 hours (immediately before the first meal), 4 hours and 45 minutes, and 5 hours and 15 minutes after administration of single doses and after the initial and final administration of multiple doses (days 1 and 12). Approximately 2.0 mL of blood was collected into an EDTA-2Na–containing iced tube, which also contained an appropriate amount of DPP-4 inhibitor. After blood was collected, the tube was inverted several times to mix the contents, cooled in an ice bath, and immediately centrifuged (KUBOTA 5900 RS-720M) at 4°C for 10 minutes at 3500 rpm. Plasma was separated into 2 aliquots (~0.8 mL per tube) and immediately frozen at –20°C or lower until shipped on dry ice for analysis to Mitsubishi Kagaku Bio-Chemical Laboratories Inc., Tokyo, Japan. One of the aliquots was retained at the laboratory until the clinical trial report was finalized.

Tolerability Assessments

Tolerability was assessed based on physical examinations, vital signs (blood pressure and heart rate), 12-lead

ECGs, laboratory tests (hematology; blood biochemistry, including blood glucose; urinalysis), and AEs. In addition, because this was the first study of linagliptin in Japanese subjects, plasma histamine concentrations were measured on the first and final days of drug administration as a marker of potential pseudoallergic AEs.

Blood samples for laboratory safety tests were collected after a ≥10-hour fast. The amount of blood collected for these tests, including the determination of plasma histamine concentrations, was 55.2 mL per subject in those receiving single doses and 84.8 mL per subject in those receiving multiple doses. For determination of plasma histamine concentrations, 1 mL of blood was collected from a forearm vein into a tube containing EDTA anticoagulant. Blood samples were centrifuged immediately after collection at 4°C for ~15 minutes at 3500 rpm (KUBOTA 5900 RS-720M). Plasma samples (0.3 mL) were stored at the study site at –20°C or lower until transportation on dry ice to the analytic laboratory. Laboratory safety tests were performed by Iryohoujinshadan Shinpukai Maruyama Hospital and Mitsubishi Kagaku Bio-Chemical Laboratories, Inc.

In the single-dose study, ECGs were obtained at the screening visit (day –21 to day –1); before dosing on day 1; at 1, 2, 4, and 12 hours after dosing; on days 2, 3, and 9; and at the follow-up visit (day 10 to day 16). In the multiple-dose study, ECGs were obtained at the screening visit (day –21 to day –1); before dosing on day 1; at 2, 4, and 12 hours after dosing; on days 2, 3, and 7; at the time of dosing on day 12; on days 13, 14, and 20; and at the follow-up visit (day 21 to day 28). In the case of an ECG abnormality, the subject was to be withdrawn from the study, carefully monitored, and/or treated as necessary. The corrected QT interval was determined from 4 waveforms in the second lead; if a flattened T wave was not observed or could not be measured in the second lead, the first lead was used. If a flattened T wave could not be measured in the first lead, the V5 lead was used. Along with the physical examination findings and laboratory test results, ECG recordings were evaluated by the principal investigator.

AEs were monitored throughout the single- and multiple-dose studies and at the relevant follow-up visit. Subjects were questioned to confirm clinically abnormal findings and symptoms. The principal investigator evaluated all clinical AEs in terms of their intensity (mild, moderate, or severe), duration, severity, outcome, and relationship to study drug. All AEs occurring throughout the study were recorded on the electronic case-report forms and were reported to the study sponsor.

Statistical Analyses

The planned number of 56 enrolled subjects was not based on a power calculation. The sample size of 8 subjects per group (6 receiving active drug and 2 receiving placebo) was based on previous studies of single and multiple escalating doses.[30,31] This planned sample size was considered sufficient for an exploratory evaluation of the pharmacokinetics and tolerability of linagliptin in a Phase I study. The trial was not powered to demonstrate significant differences in pharmacokinetic or pharmacodynamic variables between linagliptin and placebo, nor was this a study objective.

All subjects who received one dose of study drug were included in the safety evaluation. Subjects who received placebo in the single- and multiple-dose studies were pooled as separate placebo groups. Safety parameters for subjects receiving linagliptin 1, 2.5, 5, or 10 mg as single doses and linagliptin 2.5, 5, or 10 mg as multiple doses were compared descriptively with those of the corresponding placebo groups. The timing of abnormalities in laboratory values relative to the timing of drug administration was evaluated using descriptive statistics. No formal statistical analysis of the safety data was performed.

The dose-proportionality of linagliptin's pharmacokinetics was explored using a regression model.[43] A 2-sided 95% CI was computed for the slope. To determine attainment of steady state, trough concentrations of linagliptin were analyzed using a mixed linear model with time as a repeated effect.[44] Pairwise comparisons of the differences between all time points were then performed using *t* tests. The relationship between linagliptin plasma concentrations and DPP-4 inhibition was assessed in an exploratory manner based on GLP-1 concentrations.

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

RESULTS

Subject Disposition and Demographic Characteristics

Fifty-six healthy male Japanese volunteers were enrolled in the study, 32 randomized to receive single doses of linagliptin or placebo and 24 randomized to receive multiple doses of linagliptin or placebo. All subjects completed the single-dose portion of the study. Among subjects receiving multiple doses, 1 subject assigned to placebo withdrew his consent, although he completed drug administration per protocol and was included in the study analyses.

Subjects receiving single doses had a mean (SD) age of 24.5 (3.6) years (range, 20–34 years), mean weight of 61.2 (6.2) kg (range, 49.0–79.2 kg), mean height of 171.5 (5.3) cm, and mean BMI of 20.8 (1.9) kg/m^2 (range, 17.7–25.5 kg/m^2). Subjects receiving multiple doses had a mean age of 25.4 (3.7) years (range, 20–34 years), mean weight of 61.6 (5.2) kg (range, 51.7–71.0 kg), mean height of 170.9 (4.9) cm, and mean BMI of 21.1 (1.6) kg/m^2 (range, 18.1–23.7 kg/m^2).

Pharmacokinetic Analysis

Plasma and urinary linagliptin pharmacokinetic parameters after single and multiple doses are summarized in the **table**.

Across all doses, median T_{max} ranged from 1.50 to 6.00 hours. Exposure (C_{max} and AUC) increased in a manner that was less than dose proportional after single doses (**Figure 1**) and multiple doses (**Figure 2**). The interindividual variability in linagliptin pharmacokinetic parameters was low to moderate. The geometric %CV for AUC and C_{max} was <40% for all single doses of linagliptin and for the 2.5-, 5-, and 10-mg doses on day 12 after multiple dosing, with the exception of C_{max} on day 1 for the 10-mg multiple-dose group (geometric %CV, 64.2).

The CL/F after single dosing appeared to increase with increasing doses, ranging from 140 mL/min in the 1-mg group to 314 mL/min in the 10-mg group. The V_d/F ranged from 1260 L in the 1-mg group to 3060 L in the 10-mg group. Steady state was attained within 3 days for the 2.5- and 5-mg doses, and within 2 days for the 10-mg dose. The R_A for linagliptin after multiple doses was <1.5 and decreased with increasing doses to ~1.2 in the 10-mg dose group. The terminal $t_{1/2}$ of linagliptin did not reflect the accumulation $t_{1/2}$, which was ~10 to 15 hours. Urinary excretion increased with increasing dose and from day 1 to day 12. Renal excretion of linagliptin did not exceed 7% in any dose group on day 12.

Pharmacodynamic Analysis

Administration of linagliptin was associated with dose-dependent inhibition of plasma DPP-4 activity. Inhibition of DPP-4 activity reached steady-state conditions by day 4. The inhibitory effect of linagliptin on DPP-4 was sustained over the 12-day dosing period. Mean DPP-4 inhibition was ≥80% over the 24 hours after a single 10-mg dose of linagliptin (**Figure 3**) and after multiple 5- and 10-mg doses for 12 days (**Figure 4**). The interindividual variability in DPP-4 activity was low to moderate; the geometric %CV ranged from 10% to 50% for most dose groups in the single- and multiple-dose studies. The greatest interindividual variability in DPP-4 activity was seen at 24 hours after dosing in the 5-mg group in the single-dose study (geometric %CV, 55.5). The percentages of patients with ≥80% DPP-4 inhibition from baseline at 24 hours after the last dose were 33%, 100%, and 100% in the linagliptin 2.5-, 5-, and 10-mg multiple-dose groups, respectively. Maximum DPP-4 inhibition at steady state was ~92% to 93% after multiple doses of linagliptin 2.5, 5, and 10 mg. The correlations between linagliptin plasma concentrations and inhibition of plasma DPP-4 activity were comparable after single doses and multiple doses (**Figure 5**).

IC_{50} values appeared to be comparable after administration of single doses and on days 1 and 12 of multiple dosing (mean [SD], 3.03 [0.08], 2.65 [0.06], and 2.26 [0.04] nmol/L, respectively). Linagliptin concentrations of ~4 to 6 nmol/L resulted in ≥80% inhibition of plasma DPP-4 activity after administration of single and multiple doses.

Linagliptin was associated with increases in postprandial plasma GLP-1 concentrations (**Figure 6**). Plasma GLP-1 concentrations increased from baseline (premeal) concentrations by 2- to 4-fold after administration of single doses and by 2- to 2.5-fold on day 12 after administration of multiple doses. The greatest changes were observed in the groups that received single and multiple doses of linagliptin 5 mg. There was a 4.2-fold increase from baseline in postprandial GLP-1 concentrations after a single dose and a 2.4-fold increase on day 12 after multiple doses. Baseline (premeal) plasma GLP-1 concentrations were higher on day 12 than on day 1 in all linagliptin groups.

Tolerability

All 32 subjects in the single-dose study and all 24 in the multiple-dose study were included in the safety

Table. Pharmacokinetic parameters of linagliptin after administration of single doses and at steady state (day 12) after administration of multiple oral doses in healthy adult male Japanese subjects. Values are geometric mean (geometric %CV), unless otherwise specified.

	Single Doses				Multiple Doses		
Parameter*	1 mg (n = 6)	2.5 mg (n = 6)	5 mg (n = 6)	10 mg (n = 6)	2.5 mg (n = 6)	5 mg (n = 6)	10 mg (n = 6)
$AUC_{0-\infty}$, nmol/L	253 (26.4)	517 (17.7)	765 (34.2)	1120 (23.5)	NC	NC	NC
AUC_{0-24} or AUC_{τ}, nmol · h/L	62.0 (28.0)	108 (20.7)	159 (34.0)	294 (26.3)	133 (13.9)	193 (16.2)	285 (10.6)
C_{max}, nmol/L	4.27 (32.1)	5.92 (18.3)	9.00 (40.6)	23.1 (32.1)	7.79 (24.3)	12.0 (29.1)	21.8 (17.7)
T_{max}, median (range), h	1.77 (1.50–4.00)	2.00 (1.00–8.00)	6.00 (2.00–8.00)	1.50 (1.00–6.00)	3.75 (0.500–6.00)	2.25 (0.500–6.00)	4.00 (1.50–6.00)
$t_{1/2}$, h	104 (14.0)	96.9 (13.3)	105 (8.26)	113 (18.4)	142 (7.62)	143 (16.5)	175 (12.5)
fe_{0-tz} or fe_{0-24}, %	0.0510† (241)	0.273 (514)	1.15‡ (459)	7.06 (37.0)	4.20 (46.7)	4.88 (60.2)	6.88 (18.8)
CL/F, mL/min	140 (26.4)	171 (17.7)	231 (34.2)	314 (23.5)	664 (13.9)	913 (16.2)	1240 (10.6)
V_d/F, L	1260 (36.0)	1430 (14.4)	2090 (34.3)	3060 (22.1)	8180 (16.3)	11,300 (21.1)	18,700 (16.4)
R_A	NC	NC	NC	NC	1.31 (16.6)	1.28 (14.1)	1.14 (20.6)

NC = not calculated; fe_{0-tz} = fraction of dose excreted unchanged in urine from time zero to last quantifiable analyte concentration; R_A = ratio of AUC_{τ} to AUC_{0-24}.

*For parameters that include 2 terms (eg, AUC_{0-24} or AUC_{τ}), the first is the parameter after a single dose and the second is the parameter at steady state after multiple dosing.

†n = 4.

‡n = 5.

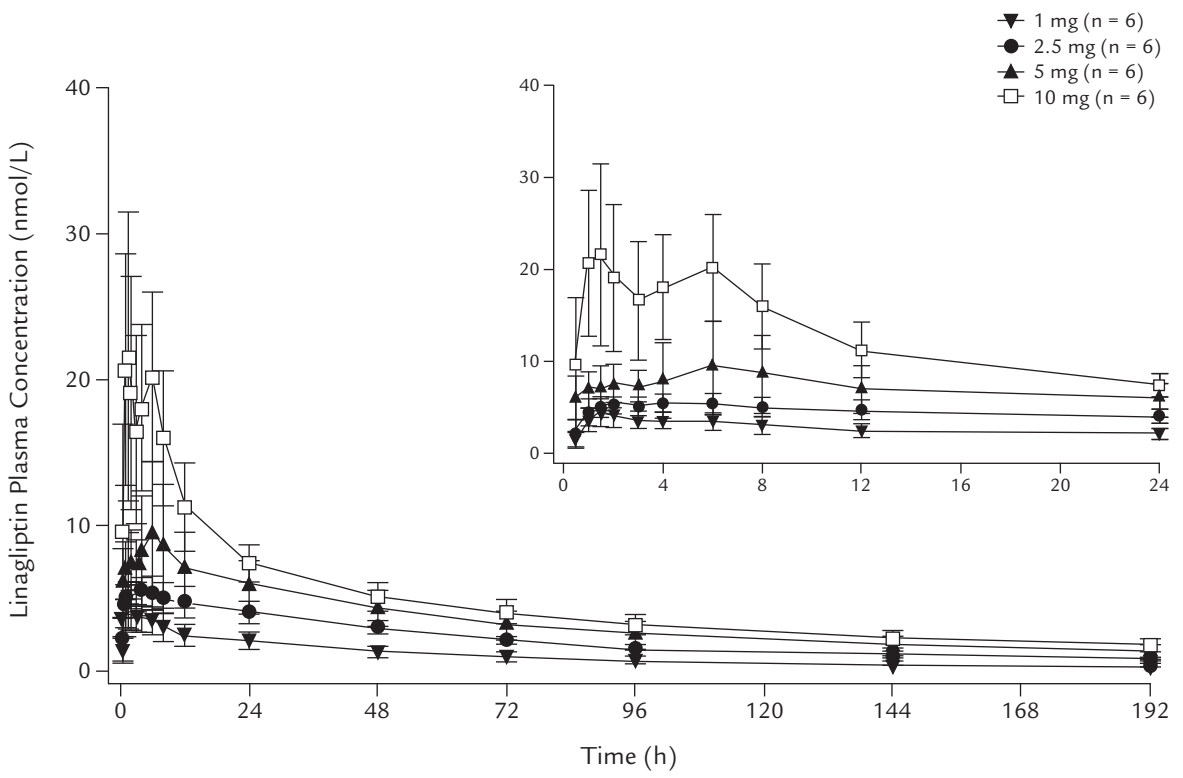

Figure 1. Mean (SD) plasma concentration–time profile of linagliptin up to 192 hours after administration of single oral doses in healthy adult male Japanese subjects (normal scale). The inset shows the period from zero to 24 hours after dosing (normal scale). Lower limit of quantitation = 0.05 nmol/L.

analysis. Two AEs were reported in the 24 subjects who received single doses of linagliptin. One subject in the 5-mg group had an increase in histamine concentrations that was not considered related to study drug. No temporal relationship was found between the event and administration of study drug, and no clinical signs or symptoms were observed that could be attributed to this event. A subject in the 10-mg group experienced vasovagal syncope, which was considered to have been caused by frequent blood sampling up to 1 hour after drug administration. Among the 18 subjects who received multiple doses of linagliptin, a single subject in the 10-mg group reported pharyngitis, which was considered unrelated to study drug.

No subjects experienced an episode of hypoglycemia during the study. No dose-dependent changes or specific trends in laboratory parameters were observed in the single- and multiple-dose groups. Changes from baseline in laboratory parameters were comparable between the linagliptin and placebo groups. There were no clinically relevant effects of linagliptin on any ECG parameter. With the exception of the elevated histamine concentration mentioned earlier, no AEs were associated with changes in laboratory values. All measures of hepatic and renal function were within normal ranges. There were no clinically relevant changes in blood pressure or heart rate in either group. Thus, single and multiple doses of linagliptin were generally well tolerated, with no significant safety concerns emerging during the study.

DISCUSSION

In the present study, linagliptin displayed nonlinear pharmacokinetics, with a less than dose-proportional increase at doses that included the expected therapeutic dose (ie, 5 mg). This finding can be explained by linagliptin's nonlinear plasma protein binding at the relevant range of concentrations due to its high affinity but readily saturable binding to DPP-4 at these concentrations.[45] Linagliptin's nonlinear pharmacokinetics, long

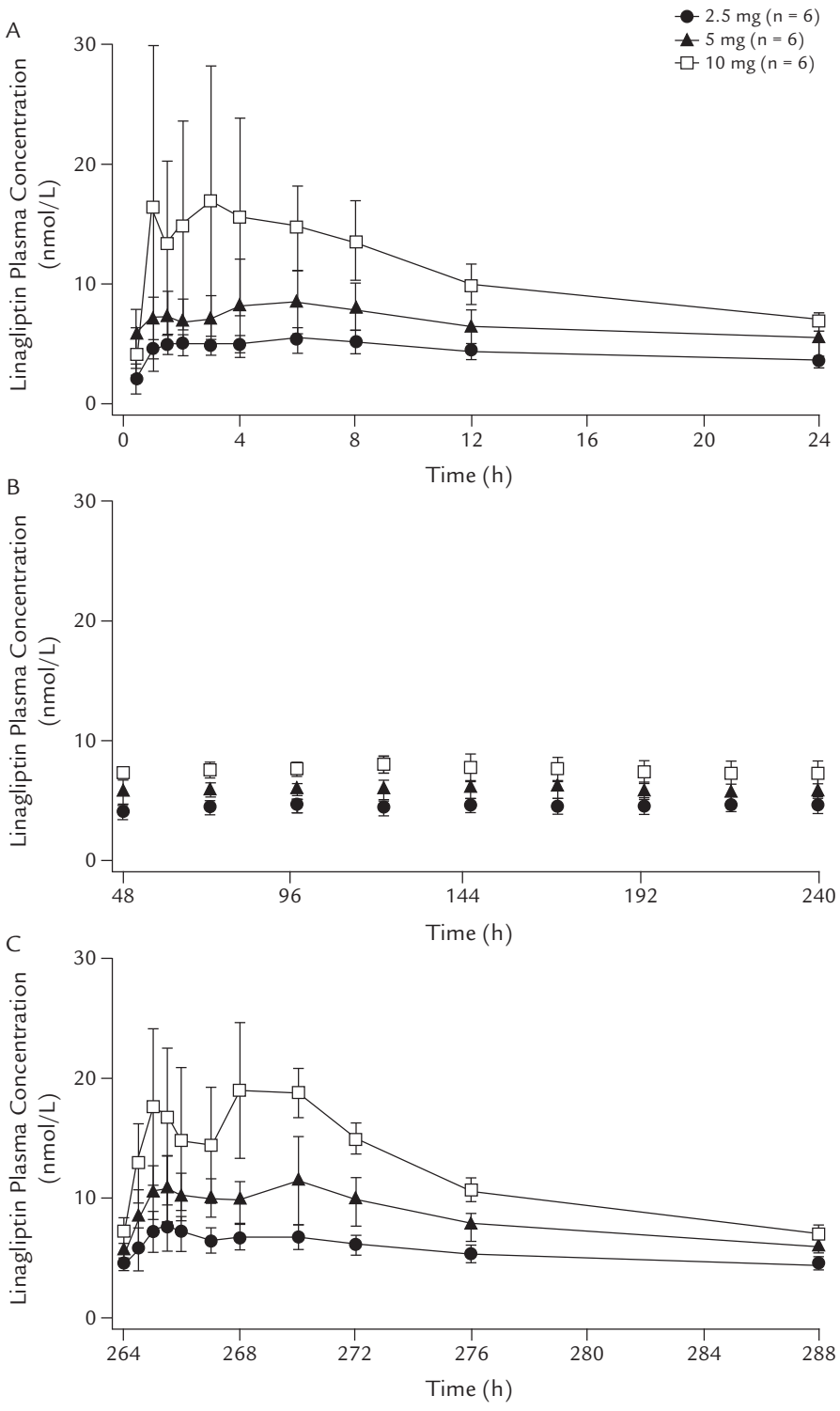

Figure 2. Mean (SD) plasma concentration–time profile of linagliptin during administration of multiple oral doses in healthy adult male Japanese subjects on (A) day 1 (before and after dosing), (B) days 2 to 11 (trough), and (C) day 12 (trough and after dosing) (normal scale). Lower limit of quantitation = 0.05 nmol/L.

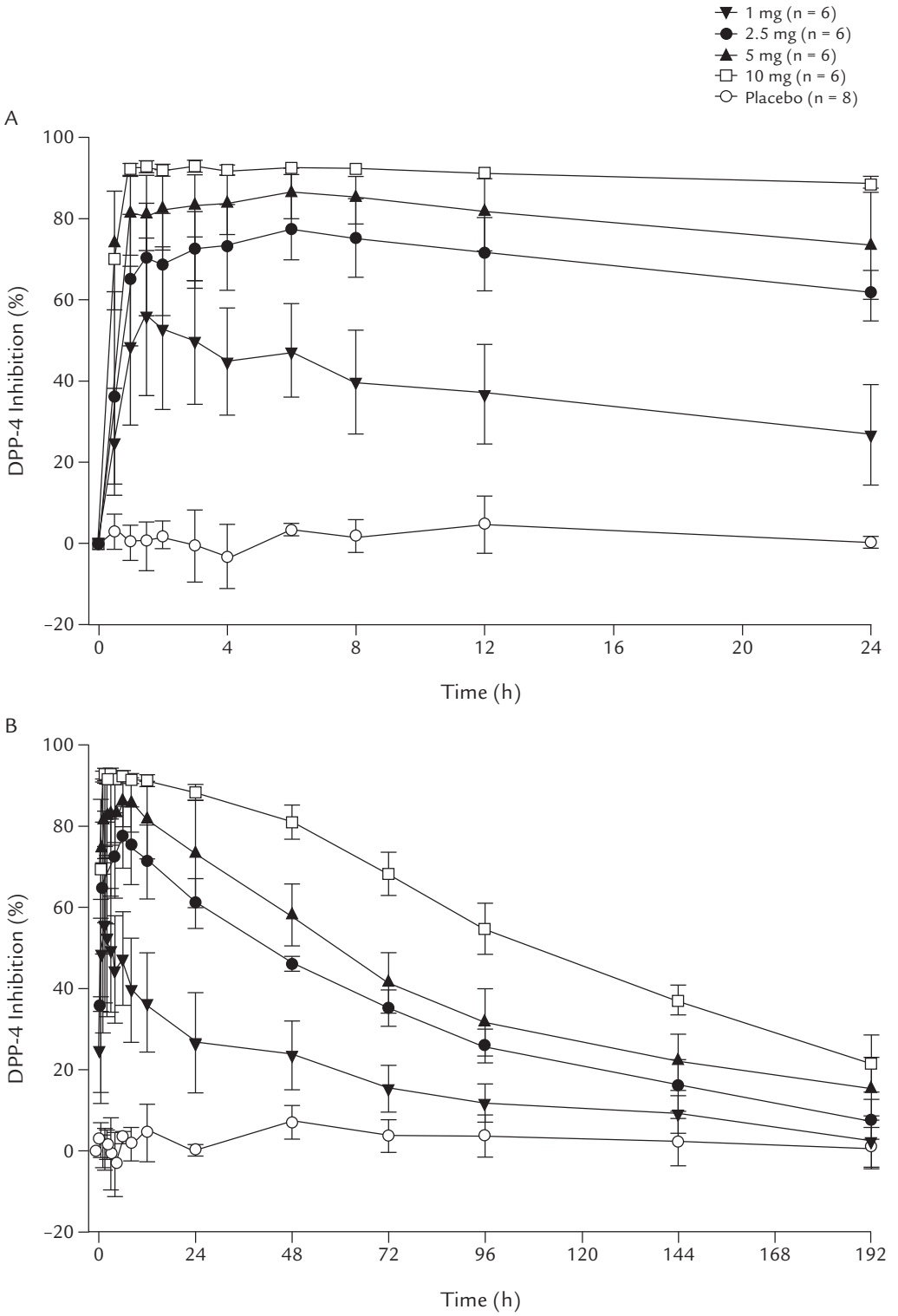

Figure 3. Mean (SD) percent inhibition from baseline of plasma dipeptidyl peptidase-4 (DPP-4) activity over (A) 24 hours and (B) 192 hours after administration of single oral doses of linagliptin or placebo in healthy adult male Japanese subjects.

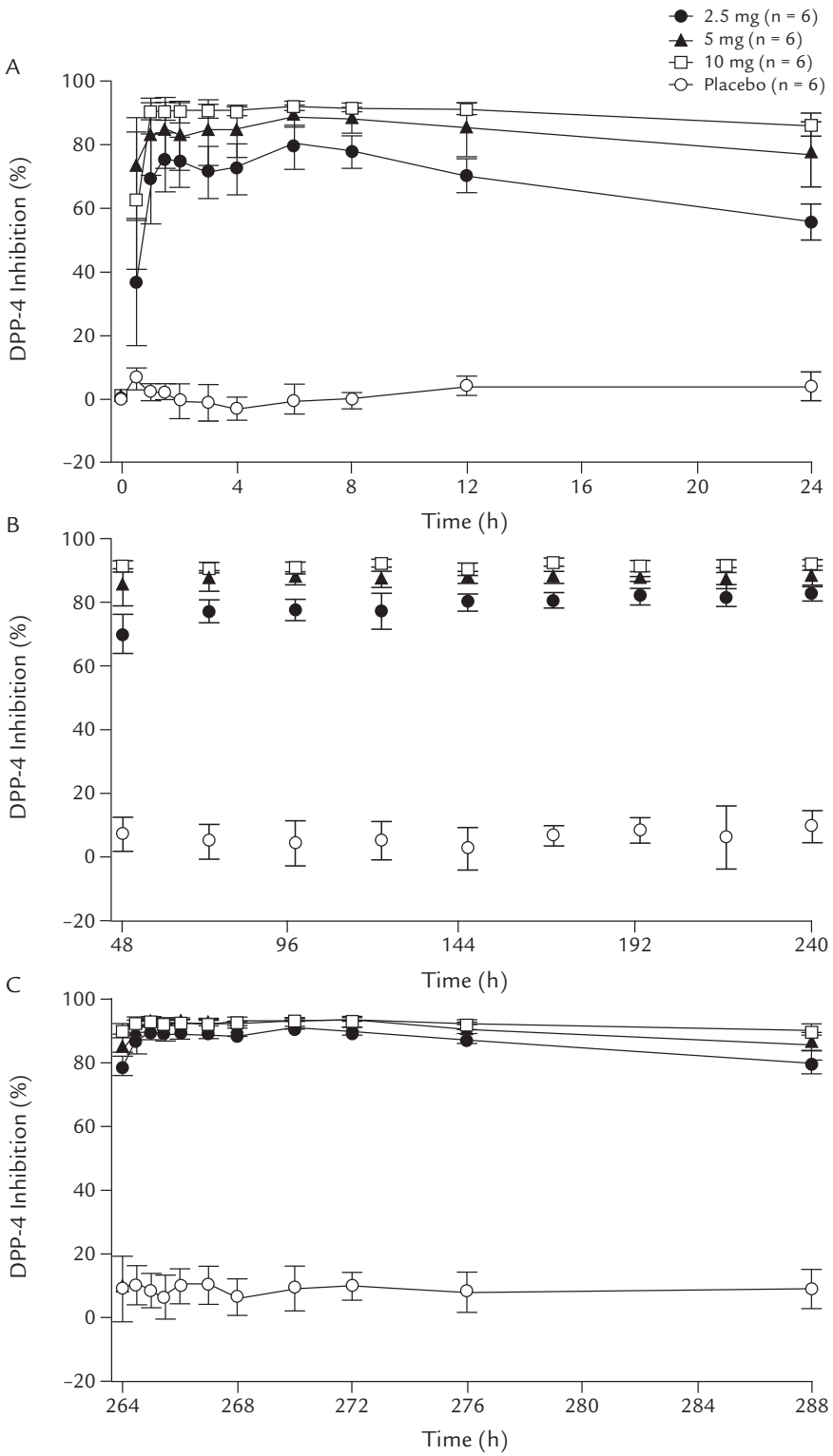

Figure 4. Mean (SD) percent inhibition of plasma dipeptidyl peptidase-4 (DPP-4) activity during administration of multiple oral doses of linagliptin or placebo in healthy adult male Japanese subjects on (A) day 1 (baseline and after dosing), (B) days 2 through 11 (trough), and (C) day 12 (trough and after dosing).

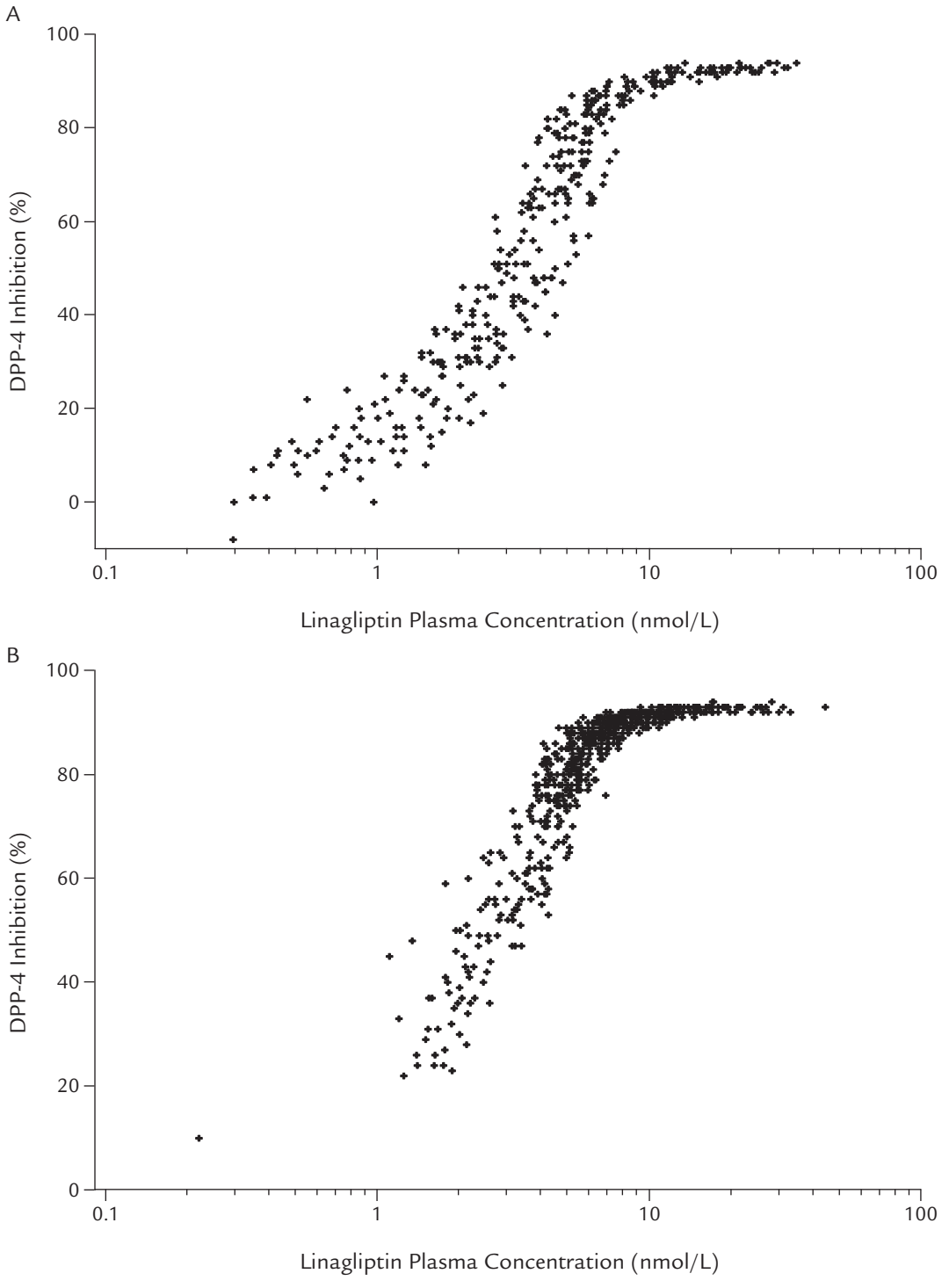

Figure 5. Scatter plots of the correlation between percent inhibition of plasma dipeptidyl peptidase-4 (DPP-4) and plasma concentrations of linagliptin after (A) single oral doses of linagliptin (1, 2.5, 5, and 10 mg) and (B) multiple oral doses of linagliptin (2.5, 5, and 10 mg once daily for 12 days) in healthy adult male Japanese subjects.

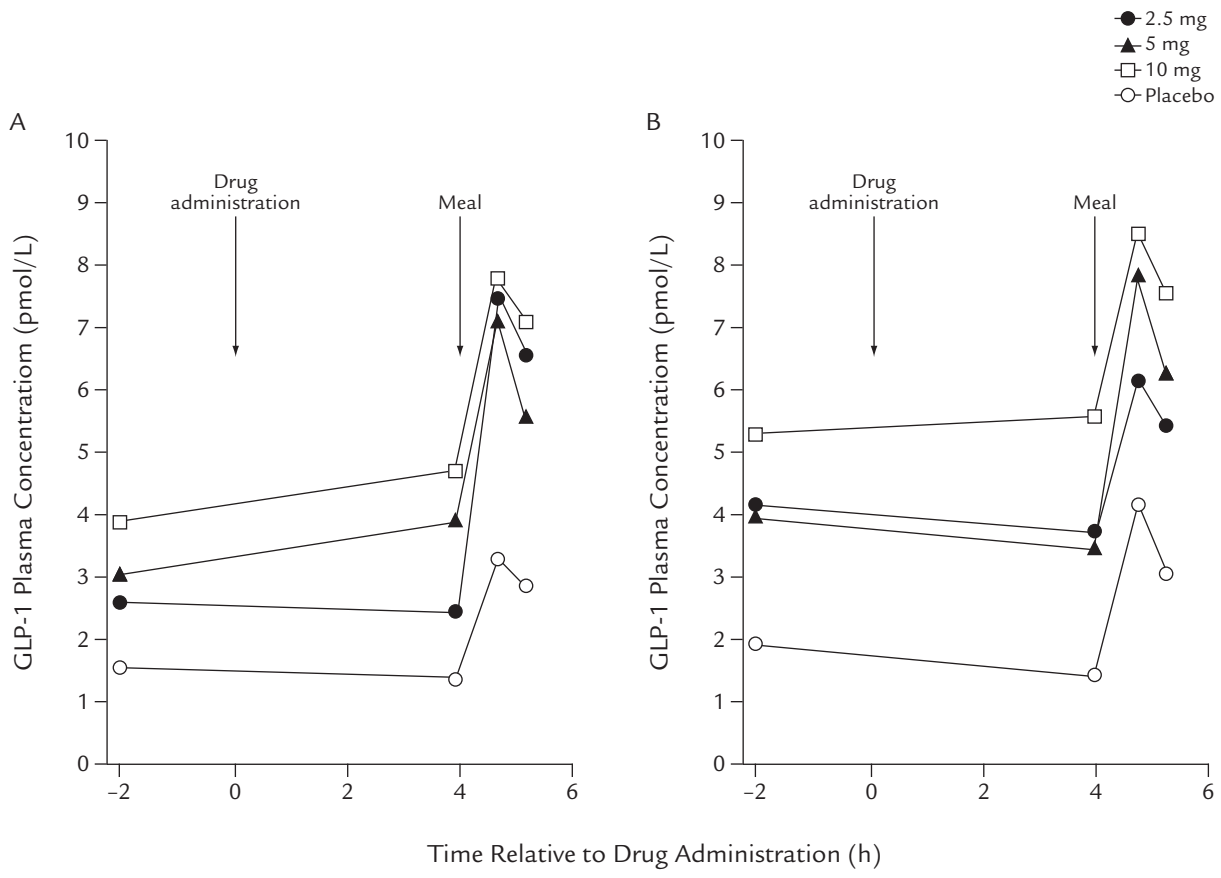

Figure 6. Mean preprandial and postprandial plasma concentrations of glucagon-like peptide-1 (GLP-1) on (A) day 1 and (B) day 12 with multiple oral doses of linagliptin (2.5, 5, and 10 mg) or placebo in healthy adult male Japanese subjects.

terminal $t_{1/2}$ that did not reflect the accumulation $t_{1/2}$, and low urinary excretion in these healthy Japanese subjects were consistent with previous observations in white subjects, as was its safety profile.[30–32] At doses ≥5 mg, >80% of Japanese subjects exhibited ≥80% DPP-4 inhibition at 24 hours after the last dose, also consistent with findings in white subjects.[31] The foregoing pharmacokinetic and pharmacodynamic characteristics support the use of a once-daily dosing regimen of linagliptin in the Japanese population.

Concentrations of tissue DPP-4 have been reported to be reduced in the nasal tissue of patients with chronic rhinosinusitis, and inhibition of DPP-4 has been found to exacerbate nasopharyngitis.[46] Based on these findings, patients treated with DPP-4 inhibitors should be monitored for the development of inflammatory conditions such as angioedema, rhinitis, and urticaria. Because this was the first trial of linagliptin in Japanese subjects, plasma histamine concentrations were measured as a marker of potential pseudoallergic AEs. The single instances of elevated histamine concentrations and pharyngitis in this study were not considered related to linagliptin.

The pharmacokinetic and pharmacodynamic profile of linagliptin in this study compares favorably with those of other DPP-4 inhibitors studied in healthy Japanese volunteers.[47,48] In a study of the tolerability, pharmacokinetics, and pharmacodynamics in 60 healthy male Japanese volunteers, multiple dosing of sitagliptin 50 mg once daily was associated with ≥80% weighted mean inhibition of plasma DPP-4 activity over 24 hours after dosing on days 1 and 10,[47] whereas multiple dosing of linagliptin at a 10-fold lower dose (5 mg once daily) in the present study was associated with ≥80%

mean DPP-4 inhibition after 12 days. In a single-dose study of alogliptin, doses of 25, 50, 100, or 200 mg once daily were required to achieve ≥80% DPP-4 inhibition at 24 hours after dosing, whereas doses of 6.25 and 12.5 mg were not adequate to achieve this degree of inhibition.[48] DPP-4 inhibition of ≥80% is expected to result in a clinically meaningful glucose-lowering effect in patients with DM.[49] Therefore, the results of the present study suggest that linagliptin 5 mg once daily may provide adequate DPP-4 inhibition to achieve this glucose-lowering effect over 24 hours in Japanese subjects.

In this study in healthy Japanese volunteers, the linagliptin fe remained low over time and with increasing doses (<7% at the 10-mg dose). Further investigation of linagliptin in patients with T2DM and impaired renal function may be helpful in determining whether dose adjustment is necessary in patients with renal impairment. The primarily nonrenal elimination of linagliptin, in combination with its low potential for accumulation[50] and wide safety margin,[30] may provide benefit in a patient population with a high prevalence of renal insufficiency.[2] In Japan, DM has been recognized as the leading cause of dialysis dependency since 1998.[51]

This pharmacokinetic and pharmacodynamic study had some limitations. As mentioned earlier, blinding of subjects' assignment to active drug or placebo was maintained within each dose group, although the current dose level was known to both subjects and investigators. This may have introduced bias into the assessment of the dose levels still to be studied and to the confounding of dose and time effects in statistical comparisons between dose levels. Nevertheless, this sequential-dose design had the advantage of minimizing the risk to subjects. All study participants were young, healthy male Japanese volunteers; therefore, the initial results cannot be extrapolated to Japanese patients with T2DM. Nonetheless, the findings provide a foundation for studies in such patients.[13]

CONCLUSIONS

In this randomized, double-blind, placebo-controlled study in healthy adult male Japanese volunteers, linagliptin's pharmacokinetic profile was characterized by nonlinear pharmacokinetics, a long terminal $t_{1/2}$ that did not reflect the accumulation $t_{1/2}$, and low urinary excretion. Linagliptin inhibited DPP-4 activity in a dose-dependent fashion for over 24 hours, with ≥80% inhibition in those receiving multiple doses of 5 and 10 mg and higher postprandial GLP-1 concentrations compared with placebo. Linagliptin was well tolerated, and there were no episodes of hypoglycemia during the study.

ACKNOWLEDGMENTS

This study was supported by Nippon Boehringer Ingelheim Co., Ltd., Tokyo, Japan. All authors except Drs. Sesoko and Nakashima are employees of Boehringer Ingelheim. The authors have indicated that they have no other conflict of interest with regard to the content of this article.

Medical writing and editorial support were provided by Patrick Foley of PHASE II International Ltd., Esher, United Kingdom, with financial support from Boehringer Ingelheim. All authors contributed to the writing and/or revision of the manuscript and reviewed and approved the final version of the manuscript. The authors thank the volunteers and staff who participated in the study.

REFERENCES

1. Wild S, Roglic G, Green A, et al. Global prevalence of diabetes: Estimates for the year 2000 and projections for 2030. *Diabetes Care*. 2004;27:1047–1053.
2. American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes Care*. 2009;32(Suppl 1): S62–S67.
3. Ministry of Health, Labour and Welfare. The National Health and Nutrition Survey 2007 [in Japanese]. http://www.mhlw.go.jp/houdou/2008/12/h1225-5a.html. Accessed March 31, 2010.
4. Kawamori R. Diabetes trends in Japan. *Diabetes Metab Res Rev*. 2002;18(Suppl 3):S9–S13.
5. Hirose T, Kawamori R. Diabetes in Japan. *Curr Diab Rep*. 2005;5:226–229.
6. Shibuya A, Yoshida A. Frequency of the atypical aldehyde dehydrogenase-2 gene (ALDH2(2)) in Japanese and Caucasians. *Am J Hum Genet*. 1988;43:741–743.
7. Matsuoka K. Genetic and environmental interaction in Japanese type 2 diabetics. *Diabetes Res Clin Pract*. 2000;50 (Suppl 2):S17–S22.
8. Saydah SH, Fradkin J, Cowie CC. Poor control of risk factors for vascular disease among adults with previously diagnosed diabetes. *JAMA*. 2004;291:335–342.
9. Rossi MC, Nicolucci A, Arcangeli A, et al, for the Associazione Medici Diabetologi Annals Study Group. Baseline quality-of-care data from a quality-improvement program implemented by a network of diabetes outpatient clinics. *Diabetes Care*. 2008;31:2166–2168.
10. Fleming BB, Greenfield S, Engelgau MM, et al. The Diabetes Quality Improvement Project: Moving science into health policy to gain an edge on the diabetes epidemic [published

correction appears in *Diabetes Care*. 2002;25:249]. *Diabetes Care*. 2001; 24:1815–1820.
11. DeFronzo RA. Current issues in the treatment of type 2 diabetes. Overview of newer agents: Where treatment is going. *Am J Med*. 2010;123 (Suppl 3):S38–S48.
12. Blonde L. Current antihyperglycemic treatment strategies for patients with type 2 diabetes mellitus. *Cleve Clin J Med*. 2009;76(Suppl 5): S4–S11.
13. Horie Y, Hayashi N, Dugi K, Takeuchi M. Design, statistical analysis and sample size calculation of a phase IIb/III study of linagliptin versus voglibose and placebo. *Trials*. 2009;10:82.
14. Pitocco D, Valle D, Rossi A, Gentilella R. Unmet needs among patients with type 2 diabetes and secondary failure to oral anti-diabetic agents. *J Endocrinol Invest*. 2008;31:371–379.
15. Richter B, Bandeira-Echtler E, Bergerhoff K, Lerch CL. Dipeptidyl peptidase-4 (DPP-4) inhibitors for type 2 diabetes mellitus. *Cochrane Database Syst Rev*. 2008;(2):CD006739.
16. Deacon CF. Therapeutic strategies based on glucagon-like peptide 1. *Diabetes*. 2004;53:2181–2189.
17. Ahrén B, Gomis R, Standl E, et al. Twelve- and 52-week efficacy of the dipeptidyl peptidase IV inhibitor LAF237 in metformin-treated patients with type 2 diabetes. *Diabetes Care*. 2004;27:2874–2880.
18. Pratley RE, Salsali A. Inhibition of DPP-4: A new therapeutic approach for the treatment of type 2 diabetes. *Curr Med Res Opin*. 2007;23:919–931.
19. Inzucchi SE. Oral antihyperglycemic therapy for type 2 diabetes: Scientific review. *JAMA*. 2002;287:360–372.
20. Mu J, Woods J, Zhou YP, et al. Chronic inhibition of dipeptidyl peptidase-4 with a sitagliptin analog preserves pancreatic beta-cell mass and function in a rodent model of type 2 diabetes. *Diabetes*. 2006; 55:1695–1704.
21. Pospisilik JA, Stafford SG, Demuth HU, et al. Long-term treatment with the dipeptidyl peptidase IV inhibitor P32/98 causes sustained improvements in glucose tolerance, insulin sensitivity, hyperinsulinemia, and beta-cell glucose responsiveness in VDF (fa/fa) Zucker rats. *Diabetes*. 2002;51:943–950.
22. Moritoh Y, Takeuchi K, Asakawa T, et al. Chronic administration of alogliptin, a novel, potent, and highly selective dipeptidyl peptidase-4 inhibitor, improves glycemic control and beta-cell function in obese diabetic ob/ob mice. *Eur J Pharmacol*. 2008;588:325–332.
23. Ahrén B, Pacini G, Foley JE, Schweizer A. Improved meal-related beta-cell function and insulin sensitivity by the dipeptidyl peptidase-IV inhibitor vildagliptin in metformin-treated patients with type 2 diabetes over 1 year. *Diabetes Care*. 2005;28: 1936–1940.
24. Mari A, Sallas WM, He YL, et al. Vildagliptin, a dipeptidyl peptidase-IV inhibitor, improves model-assessed beta-cell function in patients with type 2 diabetes. *J Clin Endocrinol Metab*. 2005;90:4888–4894.
25. Drucker DJ. Enhancing incretin action for the treatment of type 2 diabetes. *Diabetes Care*. 2003;26:2929–2940.
26. Buse JB, Henry RR, Han J, et al, for the Exenatide-113 Clinical Study Group. Effect of exenatide (exendin-4) on glycemic control over 30 weeks in sulfonylurea-treated patients with type 2 diabetes. *Diabetes Care*. 2004; 27:2628–2635.
27. Koh N, Sakamoto S, Chino F. Improvement in medication compliance and glycemic control with voglibose oral disintegrating tablet. *Tohoku J Exp Med*. 2008;216:249–257.
28. 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.
29. 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.
30. 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.
31. 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.
32. 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.
33. 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.
34. 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.
35. Ehm MG, Karnoub MC, Sakul H, et al, for the American Diabetes Association GENNID Study Group, Genetics of NIDDM. Genomewide search

for type 2 diabetes susceptibility genes in four American populations [published correction appears in *Am J Hum Genet.* 2002;70:284]. *Am J Hum Genet*. 2000;66:1871–1881.
36. Brunetti P. The lean patient with type 2 diabetes: Characteristics and therapy challenge [published correction appears in *Int J Clin Pract Suppl.* 2007;61:1776]. *Int J Clin Pract Suppl*. 2007;61:3–9.
37. Fukushima M, Usami M, Ikeda M, et al. Insulin secretion and insulin sensitivity at different stages of glucose tolerance: A cross-sectional study of Japanese type 2 diabetes. *Metabolism*. 2004;53:831–835.
38. Torréns JI, Skurnick J, Davidow AL, et al, for the Study of Women's Health Across the Nation (SWAN). Ethnic differences in insulin sensitivity and beta-cell function in premenopausal or early perimenopausal women without diabetes: The Study of Women's Health Across the Nation (SWAN). *Diabetes Care*. 2004;27:354–361.
39. Ko GT, Chan JC, Cockram CS, Woo J. Prediction of hypertension, diabetes, dyslipidaemia or albuminuria using simple anthropometric indexes in Hong Kong Chinese. *Int J Obes Relat Metab Disord*. 1999;23:1136–1142.
40. Deurenberg-Yap M, Yian TB, Kai CS, et al. Manifestation of cardiovascular risk factors at low levels of body mass index and waist-to-hip ratio in Singaporean Chinese. *Asia Pacific J Clin Nutr*. 1999;8:177–183.
41. Pharmaceuticals & Medical Devices Agency (PMDA). Points to be considered by the review staff involved in the evaluation process of new drug. http://www.pmda.go.jp/english/service/pdf/points.pdf. Accessed March 31, 2010.
42. Rowland M, Tozer TN. *Clinical Pharmacokinetics: Concepts and Applications*. 3rd ed. Baltimore, Md: Williams & Wilkins; 1995.
43. Senn S. *Statistical Issues in Drug Development.* New York, NY: John Wiley; 1997.
44. Hoffman D, Kringle R, Lockwood G, et al. Nonlinear mixed effects modelling for estimation of steady state attainment. *Pharm Stat*. 2005;4:15–24.
45. 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.
46. Richter B, Bandeira-Echtler E, Bergerhoff K, Lerch C. Emerging role of dipeptidyl peptidase-4 inhibitors in the management of type 2 diabetes. *Vasc Health Risk Manag*. 2008;4:753–768.
47. Narita H, Nonaka K, Stevens C, et al. Multiple dose administration of sitagliptin, a dipeptidyl peptidase-4 inhibitor, in healthy Japanese subjects. Presented at: 66th Scientific Sessions of the American Diabetes Association; June 9–13, 2006; Washington, DC. http://professional.diabetes.org/Content/Posters/2006/p533-P.pdf. Accessed March 31, 2010.
48. Hirayama M, Matsuno K, Fujita T, et al. Pharmacokinetics, pharmacodynamics, and tolerability of single and multiple doses of the dipeptidyl peptidase-4 inhibitor alogliptin in Japanese healthy male subjects. Presented at: 68th Scientific Sessions of the American Diabetes Association; June 9–13, 2008; San Francisco, Calif. http://professional.diabetes.org/Abstracts_Display.aspx?TYP=1&CID=69829. Accessed March 31, 2010.
49. Herman GA, Stevens C, Van Dyck K, et al. 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. *Clin Pharmacol Ther*. 2005;78:675–688.
50. 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.
51. Watanabe Y, Fujii H, Aoki K, et al. A cross-sectional survey of chronic kidney disease and diabetic kidney disease in Japanese type 2 diabetic patients at four urban diabetes clinics. *Intern Med*. 2009;48:411–414.

Address correspondence to: Akiko Sarashina, MSc, Pharmacokinetics and Non-clinical Safety Department, Nippon Boehringer Ingelheim Co., Ltd., 6-7-5, Minatojima-Minamimachi, Chuo-ku, Kobe, Hyogo, Japan. E-mail: sarasina@kaw.boehringeringelheim.com