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ORIGINAL ARTICLE

The oral DPP-4 inhibitor linagliptin significantly lowers HbA1c after 4 weeks of treatment in patients with type 2 diabetes mellitus

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Aim: To investigate the safety, tolerability, pharmacokinetics and pharmacodynamics of linagliptin in patients with type 2 diabetes mellitus (T2DM).
Methods: After screening and a 14-day washout, subjects received linagliptin 2.5, 5 or 10 mg or placebo once-daily for 28 days in this randomized, double-blind, parallel, placebo-controlled within-dose groups study.
Results: Seventy-seven patients entered the study (linagliptin: 61; placebo: 16). Four patients withdrew prematurely. There was little evidence of linagliptin accumulation. Exposure, maximum and trough plasma concentrations of linagliptin increased less than dose-proportionally. Rapid and sustained inhibition of dipeptidyl peptidase-4 reached 91–93% across linagliptin doses at steady state. At the end of the 24-h dosing interval, inhibition was still high (82–90%). There were marked increases in plasma glucagon-like peptide-1 after 28 days of dosing. Compared to placebo, all linagliptin doses resulted in statistically significant decreases of the area under the glucose curve following a meal tolerance test on day 29, that is, 24 h after the last study drug intake. After 28 days of treatment with linagliptin the placebo-corrected mean change in haemoglobin A1c (HbA1c) (median baseline 7.0%) was −0.31% (2.5-mg dose), −0.37% (5-mg dose) and −0.28% (10-mg dose). The frequency of adverse events was similar for linagliptin (31%) and placebo (34%). There were no notable safety concerns.
Conclusions: Linagliptin administration led to attenuation of postprandial glucose excursions and, despite a low HbA1c at baseline, statistically significant reductions in HbA1c after only 4 weeks of treatment. Linagliptin had a safety and tolerability profile similar to placebo in T2DM patients.
Keywords: DPP-4 inhibitor, glycaemic control, type 2 diabetes

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Introduction

Inhibition of the dipeptidyl peptidase-4 (DPP-4) enzyme represents a recent development in the therapeutic options for the treatment of type 2 diabetes mellitus (T2DM). Under normal physiological conditions, DPP-4 rapidly degrades the incretin hormones glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP). Following food intake, GLP-1 and GIP regulate the actions of insulin. Glucagon secretion is also decreased by GLP-1, which in turn reduces hepatic gluconeogenesis [1]. Consequently, prolonging the half-life of these two incretins augments glucose-dependent insulin secretion, inhibits endogenous glucose production and lowers blood glucose [2].

Physiological rebalancing of the insulin-glucose response axis resulting in improved glycaemic control because of inhibition of the DPP-4 catalyzed breakdown of incretins has been shown using several DPP-4 inhibitors [3]. The possibility of additional benefits beyond the acute effect on glycaemic control has also been proposed. Studies conducted in animal models suggest that exposure to DPP-4 inhibitors and GLP-1 analogues increases survival of β-cells [2,4]. Furthermore, data from clinical studies suggest improved β-cell function [5,6]. If this property is confirmed, it would represent the first treatment paradigm for T2DM that is associated with disease modification as well as improved glycaemic control.

Linagliptin (BI 1356) is a xanthine-based, orally available, potent and long-acting non-peptidomimetic DPP-4 inhibitor that is being developed for the treatment of T2DM [7]. Linagliptin has been shown to inhibit DPP-4 (50% inhibition concentration, IC_{50}, 1 nM) more potently than sitagliptin (19 nM), alogliptin (24 nM), saxagliptin (50 nM) and vildagliptin (62 nM) *in vitro*. *In vivo* animal studies showed inhibition of DPP-4 with linagliptin after 24 h to be greater than that seen with alogliptin, saxagliptin, sitagliptin or vildagliptin [8]. After absorption, linagliptin binds in a concentration-dependent manner to plasma protein, giving the

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drug a nonlinear pharmacokinetic profile [9]. Tissue binding studies have shown that binding capacity is saturated at low doses with only minor accumulation occurring [10]. The fraction of linagliptin which does not bind to DPP-4 is rapidly cleared from the body [11]. In healthy adult volunteers, single doses of 5 mg linagliptin have shown >80% DPP-4 inhibition for over 24 h, with a terminal half-life of up to 69.7 h [12]. Unlike other DPP-4 inhibitors so far characterized, linagliptin is excreted mainly through the faeces rather than by the renal route [13–15]. A safety margin of >100-fold in excess of the proposed therapeutic dose of 5 mg has been observed for linagliptin [12], along with a safety profile comparable with that of placebo [12,13,16]. These properties support a once-daily dosing regime in T2DM patients, with no expected requirement for dose-adjustment in patients with renal impairment [15].

The current parallel group study was designed to investigate the safety and tolerability of three dose levels of linagliptin over 4 weeks of treatment in patients with T2DM. In addition, the pharmacokinetic and pharmacodynamic effects of linagliptin were explored, including assessment of glycosylated haemoglobin A1c (HbA1c) and GLP-1 concentrations.

Methods

Study Design

This Phase IIa study (European Clinical Trials Database registration number: 2005-001265-34) followed a randomized, double-blind within-dose level, parallel, placebo-controlled design. Patients attended a screening visit 15–35 days before first drug administration, underwent a 14-day washout period and then entered a 28-day treatment period. Patients stayed at the study unit from the day before first dose (day −1) to day 6 of dosing and from day 26 of dosing to day 30 after the start of dosing. During the study, patients also attended walk-in visits on days 12, 19, 33, 36, 39, 41 and 43, with a final follow-up visit during days 43–50. A fasting blood glucose test was performed on each study day and the patient entered the results into their diary when living outside the study unit.

For each dose level (2.5, 5 or 10 mg), patients were randomly assigned in a 4 : 1 ratio to receive linagliptin or placebo. Hence, randomization was stratified by dose level. The centres were advised to randomize five patients per dose level consecutively to ensure that the placebo treatment was randomized in each block. Randomization involved a pseudo-random number generator and a supplied seed number so that the resulting allocation of medication numbers to treatment was both reproducible and non-predictable. The allocation process was performed on day −1 of each treatment period. Study drug was administered orally in the morning 1 h before breakfast for 28 consecutive days.

Study Population

Men aged 21–70 years, inclusive, and postmenopausal women aged 60–70 years, inclusive, with T2DM, managed by diet and exercise only or treated with up to two oral hypoglycaemic agents (besides glitazones), were enrolled in the study. Patients were to have a body mass index of 18.5–35 kg/m^2. Glycosylated HbA1c was to be ≤8.5% at screening for patients treated with diet and exercise and/or one oral hypoglycaemic agent or ≤8.0% at screening for patients treated with two oral hypoglycaemic agents. Concomitant medications were restricted to antihypertensive therapy, acetyl salicylic acid and statins. Individual antidiabetic therapies (ADTs) were to be discontinued 14 days prior to first study drug administration.

The study was conducted at three centres in Germany, one in The Netherlands and one in the UK. Every subject provided written informed consent to participate in the study. Local ethics committees reviewed and approved the study protocol and the study was conducted within the ethical standards established by the Declaration of Helsinki and in accordance with applicable regulatory requirements.

Pharmacokinetic Methods

For quantification of linagliptin plasma concentrations, blood (2.7–3 ml) was drawn from a forearm vein into an ethylenediaminetetraacetic acid (EDTA)-anticoagulant blood-drawing tube predose and at 30 min, 1, 1.5, 2, 3, 4, 6, 8, 12 and 23.5 h postdose. Blood samples were centrifuged immediately for 10 min at 2000–4000 *g* at 4–8 °C. Plasma was removed and stored at −20 °C or below. Plasma concentrations of linagliptin were analysed by a fully validated method using high performance liquid chromatography coupled with tandem mass spectrometry as described previously [17].

Pharmacokinetic analysis of linagliptin was carried out by non-compartmental analysis of the plasma concentration-time data using the WinNonlin® software program (Professional, version 5.0.1; Pharsight, Mountain View, CA, USA) as described previously [13].

Pharmacodynamic Methods

All pharmacodynamic measurements were performed by the Institut für Klinische Forschung und Entwicklung GmbH, Mainz, Germany, using validated assays. In this study, plasma was obtained from all blood samples using standard methods of centrifuging (2500 *g* for 10 min at 4 °C) and storage (−20 °C or below).

For the analysis of DPP-4, blood samples (3.0 ml) were taken in EDTA plasma tubes at the same time points as pharmacokinetic samples and plasma was isolated. Blood samples (4.5 ml) for the determination of active GLP-1 were taken in ice-cooled EDTA plasma tubes on days −1, 1 and 29 before intake of a standardized meal for the meal tolerance test (MTT). The second sample for GLP-1 assay on these days was taken 30 min after the intake of the meal. An appropriate amount of DPP-4 inhibitor was added within 30 s of blood collection and plasma was isolated. To provide plasma for the determination of HbA1c, blood samples (1.2 ml) were taken in EDTA tubes in the morning of days −1 and 29 and at the end-of-study examination. Plasma samples for the determination of glucagon were obtained from blood samples drawn on days −1, 1 and 29 before the intake of a standardized meal (Ensure® plus, Abbott, Wiesbaden-Delkenheim, Germany) for the MTT. The second sample of glucagon on these days was taken 30 min after the intake of the meal.

Approximately 4.9 ml of blood was collected in EDTA-plasma monovettes containing 250 KIU Trasylol® (Aprotinin, Bayer, Leverkusen, Germany) per millilitre of whole blood to protect glucagon from proteolysis during sample storage and assay procedure. Plasma for the determination of fructosamine was obtained from blood samples (2.7 ml) drawn in the morning of days −1, 12 and 29 in EDTA plasma tubes.

Plasma glucose was determined as part of the MTT, a seven-point glucose measurement (1-, 2-, 4-, 5-, 8-, 9-and 13-h postdrug administration) and at several time points in a fasted condition. For the MTT, patients drank 200 ml of Ensure® Plus diet, after which blood was drawn for the determination of fasting plasma glucose (FPG). At indicated time points, after the intake, an additional 2.0 ml blood sample was taken in sodium fluoride tubes for plasma glucose testing. Plasma was isolated and stored using standard methods. Blood (2.0 ml) was also taken 24 h after the administration of Ensure® Plus (fasted state) to determine the area under the curve (AUC) of glucose after 24 h.

Additional blood samples were taken before and 1 h after the intake of Ensure® Plus (taken for breakfast), before and 1 h after lunch and dinner and at bedtime. On day 28, an additional blood sample was taken at approximately 03:00 hours. For these time points, blood (2.0 ml) was collected in sodium fluoride tubes for determination of plasma glucose concentration.

Safety Methods

Medical examinations were performed at screening and within 15–22 days of the last drug administration. Adverse events and concomitant therapies were monitored and assessed throughout the study. Assessment of clinical laboratory parameters (chemistry, haematology and urinalysis), 12-lead electrocardiograms (ECGs) and vital signs were performed at screening, at set time points throughout the study and the end-of-study examination.

Statistical Analyses

For the analysis of HbA1c, an analysis of covariance fixed-effects model was used for the change from baseline on day 29. This model factored treatment, the number of previous ADTs and baseline of HbA1c as covariates. Treatment contrasts (linagliptin doses vs. placebo) on HbA1c were derived based on this model and their two-sided confidence intervals (CIs) were computed. Hence, the nominal significance level for all effects was ±5%, without adjustments for multiplicity. This analysis was also performed for the other biomarker endpoints, with the addition of the baseline of that biomarker in the model.

For the MTT, a fasted measurement for the determination of FPG was taken on each day it was conducted. The time course of the glucose concentration, with emphasis on the postprandial glucose as 2-h postmeal (2-h PPG), the AUC of plasma glucose levels until 3 h after administration of a standardized meal relative to baseline area under the effect curve ($AUEC_{(0-3)}$), was also investigated. The seven-point glucose was derived as a weighted mean of the seven blood glucose concentrations within 24 h [18,19].

Also, the GLP-1 levels and the relationship between DPP-4 activity and reduction of the plasma glucose $AUEC_{(0-3)}$ after the MTT were investigated.

The dose proportionality of linagliptin pharmacokinetics was explored using a power model that described the functional relationship between the dose and the pharmacokinetic endpoints maximum plasma concentration ($C_{max,ss}$), $AUC_{\tau,ss}$ and predose concentration at steady state ($C_{pre,ss}$).

DPP-4 inhibition was expressed as a percentage of the baseline activity, where baseline was the arithmetic mean of two predose measurements obtained on days −1 and 1.

The planned sample size was not based on a power calculation, as all statistical analyses were exploratory in nature. Group sizes were based on feasibility and were considered sufficient for the exploratory evaluation of safety, pharmacokinetics and pharmacodynamics of multiple doses of linagliptin. It was planned to randomize at least four blocks of 5 patients for each dose level, leading to 16 patients in the active medication groups and 4 patients in the placebo group. Overall, 75 patients were planned to be randomized, with overall at least 12 patients on placebo to achieve an almost balanced comparison between all treatment groups.

Results

Subject Disposition and Demographics

Seventy-seven Caucasian patients entered the study: 16 patients received placebo and 61 patients received different doses of linagliptin (figure 1). Four patients withdrew from the study prematurely. One patient in the linagliptin 5-mg group was withdrawn on the first day of study drug administration after one dose of linagliptin 5 mg because ventricular extrasystoles were noted on an ECG. The patient returned to the end-of-study examination a few weeks later. The other patients (two who received placebo and one who received linagliptin 2.5 mg) were withdrawn from the study after they reached the prespecified stopping criterion for elevated FPG (>240 mg/dl or >13.3 mmol/l on two consecutive occasions). In all three cases, the patients had already completed the treatment phase of the study and returned for the end-of-study examination. There were no notable differences between treatment groups in demographic characteristics (Table 1).

Upon analysing the data, an imbalance in the number of ADTs taken by the patients prior to the start of study was noted, because there was no stratification for ADTs during randomization. We noted that a higher percentage of patients, who washed out two ADTs, was being randomized to the linagliptin 10-mg dose group compared with the other dose groups and placebo (Table 1). Therefore, the number of previous ADTs was taken into account as a factor in the statistical analysis of the pharmacodynamic endpoints.

Safety and Tolerability Results

One subject experienced myocardial infarction 23 days after the last intake of linagliptin. This event was rated as serious and it was not considered to be related to the study drug. Thirty patients (30/77, 39.0%) experienced at least one adverse

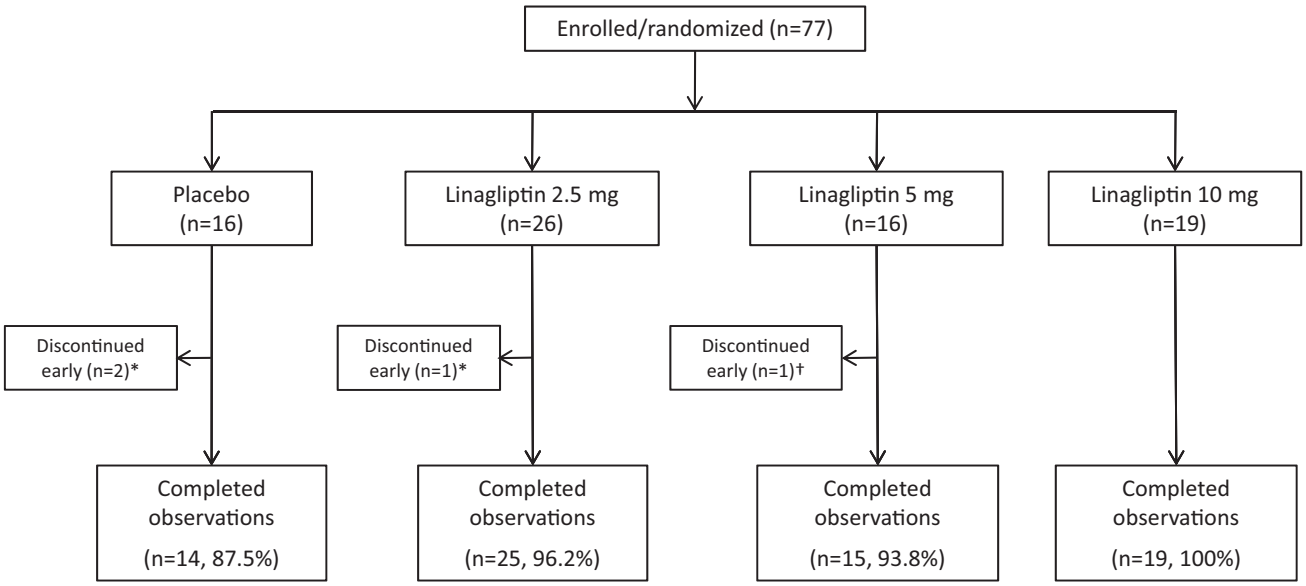

Figure 1. Patients' disposition. *Three patients were withdrawn from the study after completing the treatment phase but prior to completion of the observation phase. †One patient was withdrawn after receiving only one dose of study medication and did not complete the treatment phase.

event during the study (including screening and poststudy). Five patients (5/16, 31.3%) treated with placebo reported six events and 21 patients (21/61, 34.4%) treated with linagliptin reported 35 adverse events. Most events were rated as mild in intensity, with four subjects experiencing events rated as moderate.

No subjects showed signs or symptoms indicative of hypoglycaemia. The most frequently reported adverse events were nasopharyngitis (five patients) and back pain (four patients). The incidence of nasopharyngitis and back pain was comparable between placebo- and linagliptin-treated patients. There was no notable difference in the frequency of investigator-defined drug-related adverse events: two patients each in the placebo and 5-mg linagliptin groups and one patient each in the 2.5- and 10-mg linagliptin groups. In general, clinical laboratory parameters remained stable relative to baseline. Minor deviations from the reference range were noted in several clinical laboratory parameters, but these were not considered to be clinically relevant with the exception of one patient in the 5-mg dose group, who showed a clinically relevant increase in blood uric acid. In addition, one placebo-treated patient with normal baseline values of liver enzymes experienced an elevation to more than twice the upper limit of normal in aspartate transaminase [to 187 U/l on day 19 (normal range, 0–53 U/l)]. No clinically relevant changes were observed in routine blood tests and vital signs, including 12-lead ECG. Relative to prestudy baseline values, mean body weight decreased in all treatment groups with a reduction of

Table 1. Demographic and baseline characteristics.

	Placebo	Linagliptin 2.5 mg	Linagliptin 5 mg	Linagliptin 10 mg	Total
Subjects randomized and treated	16	26	16	19	77
Gender					
Male, n (%)	16 (100)	22 (85)	15 (94)	19 (100)	72 (94)
Age (years)					
Median (range)	62 (49–69)	62 (40–68)	64 (48–69)	62 (40–68)	62 (40–69)
Weight (kg)					
Median (range)	89.5 (74–112)	86.5 (66–119)	87.5 (69–114)	93.0 (64–115)	88.0 (64–119)
Body mass index (kg/m^2)					
Median (range)	28.8 (24.9–35.0)	29.0 (21.1–34.9)	29.1 (21.3–33.4)	29.0 (23.2–34.9)	29.0 (21.1–35.0)
HbA1c (%)					
Median (range)	7.5 (5.4–8.5)	7.0 (5.9–8.0)	6.7 (5.6–8.0)	7.2 (5.5–9.0)	7.0 (5.4–9.0)
Patients with previous ADT					
No ADT, n (%)	5 (31)	11 (42)	6 (37)	3 (16)	25 (32)
One ADT, n (%)	8 (50)	12 (46)	7 (44)	9 (47)	36 (47)
Two ADT, n (%)	3 (19)	3 (12)	3 (19)	7 (37)	16 (21)

Three subjects completed the treatment period but did not perform all study investigations. ADT, antidiabetic therapy; HbA1c, haemoglobin A1c.

Table 2. Single dose and steady-state pharmacokinetic parameters of linagliptin.

	Geometric mean (geometric coefficient of variation)		
	Linagliptin 2.5 mg (N = 26)	Linagliptin 5 mg (N = 15)	Linagliptin 10 mg (N = 19)
Single dose (day 1)			
$AUC_{(0-24)}$ (nM·h)	93.1 (27.5)	124 (20.4)	188 (32.5)
C_{max} (nM)	6.09 (42.0)	9.55 (39.3)	18.8 (64.5)
t_{max} (h)*	1.50 (0.50–8.00)	2.00 (0.983–6.20)	1.50 (1.00–8.00)
Steady state (day 28)			
$AUC_{\tau,ss}$ (nM·h)	116 (20.7)	148 (19.1)	207 (26.8)
$C_{max,ss}$ (nM)	7.41 (27.9)	12.3 (40.4)	18.6 (56.3)
$t_{max,ss}$ (h)*	1.00 (0.500–3.00)	1.00 (0.500–4.02)	1.00 (0.45–6.00)
$t_{½,ss}$ (h)	183 (20.9)	194 (15.1)	203 (16.4)
CL/F_{ss} (ml/min)	785 (20.7)	1190 (19.1)	1700 (26.8)
$R_{A,Cmax}$	1.22 (34.1)	1.29 (40.5)	0.991 (87.3)
$R_{A,AUC}$	1.25 (19.2)	1.20 (19.9)	1.10 (29.6)
Acc $t_{½}$ (h)	10.8 (41.2)	9.46 (56.3)	8.60 (79.6)

Acc $t_{½}$, accumulation half-life; $AUC_{(0-24)}$, area under the plasma concentration time curve from time 0- to 24-h postdose; CL/F_{ss}, apparent clearance; C_{max}, maximum plasma concentration; $R_{A,AUC}$, accumulation ratio over a dosing interval determined using AUC; $R_{A,Cmax}$, accumulation ratio over a dosing interval determined using C_{max}; ss, steady state; t_{max}, time to C_{max}; $t_{½}$, terminal half-life.
*Presented as median (range).

1.8 kg in the placebo group and mean reductions of 0.9–1.6 kg for the linagliptin-treated groups.

Pharmacokinetic Results

Following oral dosing, linagliptin was rapidly absorbed (median t_{max} 1.5–2.0 h) and exhibited nonlinear pharmacokinetics over the dose range investigated, with a less than dose-proportional increase in systemic exposure in terms of C_{max} and AUC (Table 2). After multiple dosing, C_{max} and $AUC_{(0-24)}$ showed only a small increase compared with day 1. Accumulation ratios for C_{max} and AUC were below 1.3 for the linagliptin 2.5- and 5-mg doses. There was almost no accumulation at the linagliptin 10-mg dose, as apparent clearance (CL/F_{ss}) increased with dose. Accumulation half-lives decreased slightly with dose from 10.8 h in the 2.5-mg dose to 8.60 h in the 10-mg dose. Estimates for terminal half-life, which mainly represent the binding/dissociation kinetics of the linagliptin/DPP-4 complex, were around 200 h, irrespective of dose.

Assessment of dose proportionality showed that exposure, maximum and trough plasma concentrations of linagliptin increased in a less than dose-proportional manner. Slope point estimates (95% CI) were 0.41 (0.32, 0.51) for $AUC_{\tau,ss}$, 0.67 (0.50, 0.84) for $C_{max,ss}$ and 0.25 (0.16, 0.34) for $C_{pre,ss}$. Trough plasma concentrations taken on days 2, 6, 12, 19, 26, 27 and 28 indicated that steady state for linagliptin was reached within 6 days. At steady state, trough plasma concentrations ranged from ~4 to ~7 nM and correlated with the linagliptin doses (data not shown).

Pharmacodynamic Results

Dipeptidyl Peptidase-4 Inhibition. Rapid and sustained inhibition of DPP-4 was observed after the first dose of linagliptin 2.5, 5 and 10 mg (figure 2). There was no notable inhibition of DPP-4 for placebo. Mean maximum inhibition of DPP-4 ranged from approximately 86% for linagliptin 2.5 mg to 93% for linagliptin 10 mg after a single dose. At the end of the 24-h dosing interval, DPP-4 inhibition ranged from approximately 65% for linagliptin 2.5 mg to 88% for linagliptin 10 mg 24 h after a single dose.

Similar findings of large and sustained inhibition of DPP-4 were also seen at steady state. Mean maximum inhibition of DPP-4 was approximately 91–93% across all linagliptin doses. At the end of a dosing interval at steady state, after subjects had not received a dose for 24 h, inhibition of DPP-4 was still high, ranging from approximately 82–90%.

Assessing the interaction of pharmacokinetic and pharmacodynamic endpoints showed that increases in linagliptin dose were accompanied by increasing linagliptin $AUC_{(0-24)}$ and C_{max} values (Table 2). With increasing linagliptin dose, inhibition of DPP-4 indicated dose- and concentration-dependent behaviour.

Plasma Glucagon-like Peptide-1. Mean plasma levels of active GLP-1 measured 30 min after an MTT showed marked increases after 28 days of dosing with linagliptin at all dose levels (2.5–10 mg; Table 3), although there was no significant difference between the different doses and placebo, because of the large inter-individual variability of this endpoint. There were no notable changes in plasma GLP-1 concentrations for subjects who received placebo. Day 29 values for adjusted mean plasma GLP-1 concentration ranged from 7.3 to 13.9 pmol/l for linagliptin doses compared with 5.1 pmol/l for placebo (Table 3). Values for GLP-1 measured 30 min after the MTT at day −1 were similar for all groups, ranging from 3.5 to 5.0 pmol/l.

The majority of GLP-1 concentration measurements in blood taken prior to the MTT were below the limit of quantification of 2 pmol/l. For several patients, GLP-1 concentrations were still below the limit of quantification 30 min after the MTT and could not be determined. In order to estimate changes in GLP-1 concentrations (before and after the MTT) all GLP-1 concentrations falling below the lower limit of quantification of 2 pmol/l were replaced by half the lower limit of quantification (1 pmol/l).

Fasting Plasma Glucose. FPG levels decreased significantly at day 29 for all doses of linagliptin, relative to the baseline measurement taken at day −1. Adjusted mean reductions in FPG on day 29 were statistically significant for all linagliptin doses, varying from −16.6 to −21.4 mg/dl compared with a mean reduction of 3.2 mg/dl for placebo (Table 3).

Seven-Point Mean Glucose. Plasma glucose levels were also determined by seven-point glucose measurements, collected on days −1, 1 and 29. At day 29, the reduction of the seven-point glucose for all linagliptin doses was statistically significant compared with placebo, the greatest reduction in plasma glucose levels at day 29 was detected in the 5-mg dose group, followed by the 2.5-mg group.

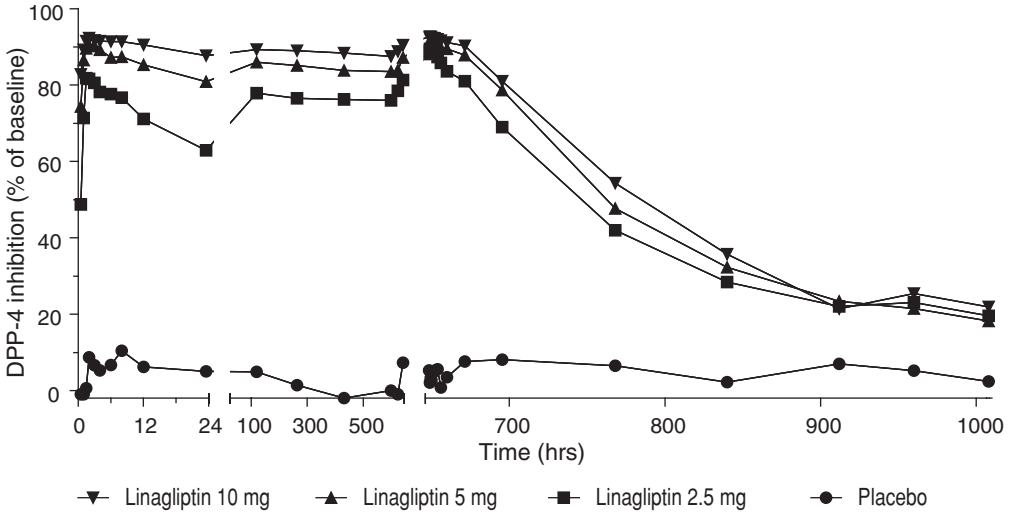

Figure 2. Arithmetic mean dipeptidyl peptidase-4 (DPP-4) inhibition (percentage of baseline) after administration of linagliptin 2.5, 5, 10 mg and placebo. Condensed graph presenting DPP-4 activity from predose to approximately day 43.

Plasma Glucose Following the Meal Tolerance Test. Patients with linagliptin treatment showed a small reduction in peak plasma glucose concentration on days 1 and 29, compared with day −1.

Statistically significant reductions from baseline in 2-h PPG and plasma glucose $AUEC_{(0-3)}$ were observed on day 29 for all three linagliptin doses, compared with placebo (Table 3). At day 1, the decrease compared to placebo was not statistically significant; however, numerical reductions were observed for all linagliptin doses.

Glycosylated HbA1c. Statistically significant decreases from baseline in mean HbA1c were observed on day 29 for all linagliptin groups compared with placebo (Table 3). The placebo-corrected mean change in HbA1c was −0.31% for linagliptin 2.5 mg, −0.37% for linagliptin 5 mg and −0.28% for linagliptin 10 mg ($p = 0.046$).

Plasma Fructosamine and Glucagon. After 28 days of dosing, there were no statistically significant changes in plasma fructosamine levels between the linagliptin and placebo groups (Table 4). A general trend towards a reduction in fructosamine levels with increased dose was observed, but significance could not be confirmed because of small sample size. After 28 days, reductions in plasma glucagon concentration from 30 min before to 30 min after meal intake in the MTT were observed, but changes were not significant.

Discussion

This study examined the pharmacokinetic and pharmacodynamic properties of the oral DPP-4 inhibitor linagliptin in patients with T2DM after 4 weeks of treatment. The effects of linagliptin on DPP-4 activity as well as the consequences of DPP-4 inhibition (plasma GLP-1 concentrations, plasma glucose concentrations, postprandial glucose excursions and HbA1c concentrations) were assessed to determine the potential of once-daily treatment with linagliptin to improve glycaemic control.

After single oral dose administration and at steady state, linagliptin showed less than dose-proportional pharmacokinetics. This nonlinear pharmacokinetic profile is related to concentration-dependent binding to the target DPP-4 in plasma [20]. In this study, there was little evidence of linagliptin accumulation after repeated dosing over 4 weeks. Although linagliptin is also known to undergo high affinity binding to DPP-4 in tissues, particularly in the kidney, this binding capacity is limited and readily saturated at low doses, so tissue accumulation is therefore restricted [10]. In this study, at the proposed therapeutic dose (5 mg) of linagliptin, mean trough plasma concentrations at steady state were approximately 4–5 nM. Previously, it has been estimated that linagliptin concentrations of approximately 2–4 nM and 4–6 nM would lead to 50 and 80% inhibition of the DPP-4 enzyme, respectively [12]. This agrees with the high level of inhibition (≥80%) observed at steady state 24-h postdosing in the present study for this dose and the other doses evaluated (2.5 and 10 mg). The sustained inhibition of DPP-4 activity over 24 h, together with the low potential for accumulation, supports once-daily administration [12]. In a clinical setting, once-daily dosing for an oral ADT may be more convenient for patients and can result in significantly better adherence rates than two or three times daily regimens [21].

The inhibition of DPP-4 activity by linagliptin resulted in marked increases in plasma GLP-1 concentrations after 28 days of dosing and was further reflected in the improvements in glycaemic control observed in the patients treated. Compared with placebo, all linagliptin doses resulted in statistically significant decreases of the AUC glucose following an MTT on day 29, that is, 24 h after the last study drug intake. FPG levels were also reduced significantly in all linagliptin-treated groups on day 29. Chronic hyperglycaemia is a recognized risk factor for microvascular and macrovascular disease in T2DM and observational studies indicate that

Table 3. Single dose and steady-state pharmacodynamic biomarker results: mean by treatment and placebo-corrected adjusted mean CFB; adjusted for HbA1c at baseline, use of ADTs and baseline of endpoint.

	Placebo (N = 15)	Linagliptin 2.5 mg (N = 26)		Linagliptin 5 mg (N = 15)		Linagliptin 10 mg (N = 19)	
	Mean (s.e.)	Mean (s.e.)	Pbo corr. adj. mean CFB (95% CI)	Mean (s.e.)	Pbo corr. adj. mean CFB (95% CI)	Mean (s.e.)	Pbo corr. adj. mean CFB (95% CI)
7-point glucose (mg/dl)							
Day−1	190.6 (10.9)	168.8 (7.5)		166.1 (12.9)		180.2 (9.4)	
Day 1	182.5 (10.0)	157.9 (7.2)	−4.4 (−11.6, 2.8)	155.8 (12.4)	−4.6 (−12.8, 3.6)	172.1 (9.7)	−2.1 (−9.8, 5.6)
Day 29	188.0 (11.7)	151.6 (7.8)	−19.3 (−34.1, −4.5)*	146.3 (10.5)	−24.4 (−41.2, −7.6)*	167.6 (9.6)	−11.7 (−32.0, −0.3)*
FPG (mg/dl)							
Day−1	168.7 (8.1)	156.2 (7.5)		151.9 (11.9)		158.9 (7.7)	
Day 2	160.8 (8.2)	148.2 (6.9)	−1.0 (−9.9, 7.9)	146.4 (11.6)	0.3 (−9.8, 10.4)	154.5 (8.2)	0.6 (−8.9, 10.2)
Day 12	168.5 (10.1)	143.1 (6.1)	−14.4 (−26.8, −2.1)*	139.5 (9.5)	−16.1 (−30.2, −2.1)*	155.2 (7.9)	−9.2 (−22.5, 4.0)*
Day 28	162.6 (8.7)	136.6 (6.7)	−14.7 (−28.7, −0.7)*	136.4 (10.3)	−13.8 (−29.7, 2.1)	152.4 (8.7)	−9.1 (−24.1, 5.9)
Day 29	165.5 (10.4)	135.2 (7.1)	−19.2 (−34.2, −4.2)*	131.9 (9.1)	−21.4 (−38.5, −4.4)*	148.2 (8.7)	−16.6 (−32.7, −0.5)*
Day 30	162.1† (9.1)	138.5 (7.3)	−15.2 (−32.4, 2.9)	138.7 (10.9)	−13.8 (−34.4, 6.8)	158.2 (10.8)	−5.7 (−25.4, 14.0)
2-h PPG (mg/dl)							
Day−1	225.3 (18.9)	196.9 (12.1)		204.1 (21.5)		214.2 (12.8)	
Day 1	220.5 (17.9)	187.9 (10.8)	−4.7 (−25.4, 16.0)	182.5 (18.4)	−10.9 (−34.6, 12.9)	207.1 (12.7)	−4.4 (−26.6, 17.7)
Day 29	230.3 (17.1)	173.1 (11.7)	−32.4 (−56.3, −8.6)*	156.6 (15.7)	−52.5 (−79.8, −25.3)*	200.9 (13.5)	−27.2 (−52.6, −1.7)*
Glucose $AUEC_{(0-3)}$ (mg·h/dl)							
Day−1	667.4 (42.8)	595.0 (29.3)		590.0 (51.1)		633.7 (31.5)	
Day 1	632.2 (41.0)	543.3 (27.3)	−20.8 (−51.4, 9.7)	542.7 (48.9)	−18.2 (−53.0, 16.6)	596.3 (30.6)	−11.8 (−44.5, 20.9)
Day 29	648.8 (40.0)	517.4 (29.1)	−71.5 (−121.1, −18.8)*	484.7 (38.8)	−105.6 (−165.6, −45.6)*	575.9 (34.3)	−65.4 (−121.7, −9.1)*
GLP-1 (pmol/l)							
Day−1	3.5 (0.5)	5.0 (0.7)		4.4 (1.3)		4.0 (0.6)	
Day 1	3.1 (0.4)	8.3 (1.1)	4.3 (−0.2, 8.8)	7.4 (1.4)	4.0 (−1.0, 9.1)	10.3 (2.7)	7.0 (2.3, 11.7)*
Day 29	5.1 (1.8)	13.7 (2.7)	7.3 (−0.8, 15.4)	13.9 (4.7)	8.39 (−0.9, 17.5)	11.1 (2.2)	5.6 (−3.0, 14.3)
HbA1c (%)							
Day−1	7.34 (0.25)	6.97 (0.12)		6.80 (0.17)		7.11 (0.19)	
Day 29	7.62 (0.28)	6.90 (0.15)	−0.31 (−0.56, −0.05)*	6.72 (0.20)	−0.37 (−0.67, −0.08)*	7.25 (0.22)	−0.28 (−0.56, −0.01)*
Day 43	7.51† (0.30)	6.86‡ (0.18)	−0.34 (−0.73, 0.05)	6.77 (0.22)	−0.39 (−0.83, 0.05)	7.27 (0.25)	−0.36 (−0.78, 0.06)

ADT, antidiabetic therapy; AUEC, area under the effect curve; CFB, change from baseline; CI, confidence interval; FPG, fasting plasma glucose; GLP-1, glucagon-like peptide-1; HbA1c, haemoglobin A1c; PPG, postprandial glucose.

*$p < 0.025$.

†N = 14.

‡N = 25.

Table 4. Plasma fructosamine concentrations at day −1, 12 and 29.

	Geometric mean (geometric coefficient of variation)			
	Placebo (N = 16)	Linagliptin 2.5 mg (N = 26)	Linagliptin 5 mg (N = 15)	Linagliptin 10 mg (N = 19)
Fructosamine concentration (µmol/l)				
Day −1	263 (16.2)	246 (18.2)	232 (19.5)	251 (16.2)
Day 12	275 (15.1)	250 (16.2)	239 (16.6)	265 (15.5)
Day 29	267 (18.0)	238 (15.3)	228 (18.5)	253 (19.1)

isolated postprandial hyperglycaemia increases cardiovascular mortality in these patients [22]. Control of both fasting and postprandial hyperglycaemia is therefore needed to obtain optimal HbA1c control. DPP-4 inhibitors such as linagliptin, which mechanistically address postprandial hyperglycaemia, while also having a low propensity for hypoglycaemia or weight gain, may provide an attractive option when considering the available therapeutic options for T2DM patients.

Despite the short duration of the study period (4 weeks), a reduction in HbA1c levels was observed after dosing with linagliptin for 29 days at all dose levels. Generally, determination of the change in HbA1c consequent to introduction of glycaemia-modifying therapies is made after sufficient time to allow turnover of the red blood cell stock, at least 3 months. It is worth noting that, at 7.0%, the median baseline HbA1c of the subjects in this trial is lower than may be expected for T2DM patients who are failing to reach glycaemic targets and require treatment with an oral ADT [23]. As a lower baseline glycaemia reduces the apparent efficacy of an oral glucose-lowering agent [24], patients with a higher baseline HbA1c than those in the present study may show greater reductions when treated with linagliptin. Therefore, it is most probable that the approximate 0.3% reduction seen in this 4-week study would translate into a more marked effect with long-term linagliptin treatment. Indeed, initial results from a Phase III study in which 503 T2DM patients were randomized to monotherapy with linagliptin 5 mg or placebo show that after 24 weeks the linagliptin-treated patients had a mean placebo-adjusted change in HbA1c from baseline of −0.69% ($p < 0.0001$) [25].

It is notable that, for most parameters, the numeric changes seen with the 10-mg dose were lower than those with the 5-mg dose. The difference was less for HbA1c than for the other parameters, because the HbA1c change was adjusted for previous use of ADTs which differed between the treatment arms. In general, the numerically lower efficacy for glucose parameters and biomarkers seen with the 10-mg dose is most probably because of chance in this trial of low sample size.

Despite the low sample size, the statistically significant reduction in both FPG and AUC glucose after an MTT, measured 24 h after the last study drug intake, provides clinical proof of concept with regard to glucose-lowering for all doses tested in this trial. To identify the optimal dose of linagliptin for the treatment of T2DM, subsequent trials explored a wider dose range. Doses <2.5 mg were tested in the Phase II programme of the drug in order to identify the minimally effective dose.

Linagliptin exhibited a tolerability profile comparable with that of placebo across all active dosing groups in the present study. Importantly, administration of linagliptin did not appear to result in signs or symptoms of hypoglycaemia. This is consistent with the observation that the effects of DPP-4 inhibitors on glucose-lowering are glucose-dependent [1].

In conclusion, this study showed the effect of the potent and selective DPP-4 inhibitor linagliptin on glycaemic control in patients with T2DM without any major or minor hypoglycaemic episodes. In addition, the data suggest that in future studies dedicated to identifying the optimal dose for the treatment of patients with T2DM, the dose range should include, but not be limited to, 2.5–10 mg linagliptin, as tested in the present study. Data from long-term studies will be needed to support the long-term effects of linagliptin as well as to fully understand the mechanism of the effects and determine whether its unique non-renal clearance pathway translates into clinical benefit over the other DPP-4 inhibitors launched to date. Finally, in the present study, linagliptin showed a safety and tolerability profile similar to placebo in its target population of patients with T2DM.

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Conflict of Interest

This study was sponsored by Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach, Germany. With the exception of Prof. T. Forst, whose involvement was carried out under contract, all the authors are employees of Boehringer Ingelheim Pharma. The study protocol was designed by the authors who were also responsible for data collection, analysis and reporting of results. All authors contributed to the writing or critical revision of the manuscript and saw and approved the submitted version of the manuscript.

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