ORIGINAL ARTICLE doi: 10.1111/j.1463-1326.2009.01046.x

Pharmacokinetics, pharmacodynamics and tolerability of multiple oral doses of linagliptin, a dipeptidyl peptidase-4 inhibitor in male type 2 diabetes patients

T. Heise,[1] E. U. Graefe-Mody,[2] S. Hüttner,[3] A. Ring,[4] D. Trommeshauser[2] and K. A. Dugi[5]

[1]Profil Institut für Stoffwechselforschung GmbH, Hellersbergstraße, Neuss, Germany
[2]Department of Drug Metabolism and Pharmacokinetics, Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach, Germany
[3]Department of Clinical Research, Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach, Germany
[4]Department of Medical Data Services, Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach, Germany
[5]Corporate Department Medical Affairs, Boehringer Ingelheim GmbH, Ingelheim, Germany

Aims: To investigate the safety, tolerability, pharmacokinetic and pharmacodynamic properties of multiple oral doses of the dipeptidyl peptidase-4 (DPP-4) inhibitor linagliptin (BI 1356) in patients with type 2 diabetes mellitus.
Methods: Forty-seven male type 2 diabetic patients received linagliptin 1, 2.5, 5 or 10 mg, or placebo, once daily for 12 days.
Results: Linagliptin exposure [area under the plasma concentration–time curve and maximum plasma concentration (C_{max})] increased less than proportionally with dose. Accumulation half-life was short (8.6–23.9 h), resulting in rapid attainment of steady state (2–5 days) and little accumulation (range: 1.18–2.03). The long terminal half-life (113–131 h) led to a sustained inhibition of DPP-4 activity. Renal excretion was below 1% on day 1 in all dose groups. Inhibition of plasma DPP-4 activity correlated well with linagliptin plasma concentrations, resulting in DPP-4 inhibition >90% in the two highest dose groups; even 24 h postdose, DPP-4 inhibition was >80%. Following an oral glucose tolerance test, 24 h after the last dose, statistically significant reductions of glucose excursions were observed with linagliptin (2.5, 5 and 10 mg doses) compared with placebo. Linagliptin was well tolerated. The frequency of adverse events (AEs) was not higher with linagliptin (54%) than with placebo (75%). No serious AEs and no episodes of hypoglycaemia were reported.
Conclusions: In type 2 diabetic patients, multiple rising doses of linagliptin were well tolerated and resulted in significant improvements of glucose parameters. Together with the favourable pharmacokinetics, these results confirm the unique profile of linagliptin in the DPP-4 inhibitor class.

Keywords: DPP-4, glycaemic control, linagliptin, pharmacokinetics, safety
Received 13 November 2008; returned for revision 12 January 2009; revised version accepted 16 January 2009

Introduction

Dipeptidyl peptidase-4 (DPP-4) inhibitors are a new class of oral antihyperglycaemic agents for the treatment of type 2 diabetes mellitus [1,2]. These agents act by increasing active (intact) levels of incretin peptides, including glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide. GLP-1 and the other incretins

Correspondence:
Dr Klaus A. Dugi, MD, Corporate Department Medical Affairs, Boehringer Ingelheim GmbH, Binger Str. 173, D-55216 Ingelheim, Germany.
E-mail:
klaus.dugi@boehringer-ingelheim.com

Declaration of Competing Interests:
This study was sponsored by Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach, Germany. With the exception of T. H. 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.

augment insulin secretion in a glucose-dependent manner [3], whereas DPP-4 rapidly inactivates these peptides, thus attenuating their beneficial effects on glucose homeostasis [4]. The DPP-4 inhibitors delay the breakdown of incretins, thereby prolonging and enhancing their action. In type 2 diabetic patients, treatment with DPP-4 inhibitors is associated with improved glycaemic control and postprandial glucose excursions [5,6].

Inhibition of DPP-4 as a treatment paradigm for type 2 diabetes offers several advantages. Because incretin stimulation of insulin release is glucose dependent [3], the risk of hypoglycaemic episodes with DPP-4 inhibitors is low. The DPP-4 inhibitors also appear to have a neutral effect on body weight [7,8], compared with the weight gain found with insulin, sulphonylurea or thiazolidinedione treatments [9]. Beneficial effects have also been observed on β-cell mass and function after long-term treatment of rats and mice with DPP-4 inhibitors [1,10]. Similar benefits on β-cell function have been demonstrated in people with type 2 diabetes [11,12] leading to substantial improvements in glycaemic control and insulin sensitivity [11]. Finally, unlike GLP-1 analogues [13], DPP-4 inhibitors can be administered orally and may not cause the gastrointestinal adverse effects that have been noted with pharmacological GLP-1 levels [14].

Linagliptin (BI 1356) (8-(3-(R)-aminopiperidin-1-yl)-7-but-2-ynyl-3-methyl-1-(4-methyl-quinazolin-2-ylmethyl)-3,7-dihydropurine-2,6-dione) is an orally active DPP-4 inhibitor in phase III development for the treatment of type 2 diabetes. Linagliptin exhibited a high potency and selectivity for DPP-4 inhibition, increased the half-life of circulating incretin hormones and improved glucose homeostasis in preclinical studies [15]. Linagliptin demonstrated a safety and tolerability profile similar to placebo and a true 24 h duration of action in a phase I trial in healthy subjects [16]. The aim of the current trial was to explore the safety, tolerability, pharmacokinetics and pharmacodynamics of linagliptin after multiple daily doses in the primary target population, that is patients with type 2 diabetes.

Patients and Methods

Study Design

This randomized, placebo-controlled, double-blind, two-centre, multiple rising dose study was carried out in four sequential groups, each comprising 12 male type 2 diabetic patients, nine receiving linagliptin (in rising doses per cohort of 1, 2.5, 5 or 10 mg) and three receiving placebo. Written informed consent was obtained from all patients, and the study was conducted in full compliance with the Declaration of Helsinki [17] following approval from the local ethics committees.

Patients were washed off their previous antidiabetic medication, if any, during a washout period of 3–12 days. Patients whose fasted blood glucose levels did not exceed 240 mg/dl (13.3 mmol/l) on two consecutive days during the washout period were admitted to the in-house part of the study (days −2 to day 13) during which they received linagliptin once daily (i.e. before breakfast) or matching placebo from days 1–12. Pharmacokinetic assessments of linagliptin concentrations in plasma and urine and assessments of DPP-4-activity were carried out throughout the treatment phase with more intensive sampling on days 1 (i.e. at single dose conditions) and 12 (steady-state conditions) and in the morning of days 13, 14, 16, 18 and 20. Pharmacodynamic measurements comprised assay of blood glucose concentrations during an oral glucose tolerance test (OGTT; 75 g of glucose dissolved in 200 ml water) conducted in the morning of day −1, 1 and 13 (i.e. 24 h after the last linagliptin dose). In addition, fructosamine and GLP-1 concentrations were determined on days −1 and 13. During the whole in-house period, patients received standardized meals and a snack prior to bedtime. Safety and tolerability assessments were based on adverse events (AEs), regular checks of routine blood chemistry, haematology and urine values, ECGs, vital signs and physical examinations. All subjects who received one dose of the study drug were included in the safety evaluation.

Patients

Male Caucasian patients with type 2 diabetes, aged 21–65 years with a body mass index (BMI) of 18.5–35 kg/m^2, were eligible for this study, as long as they were not taking an oral antihyperglycaemic agent (other than glitazones) or if they could safely be washed off from one oral antihyperglycaemic agent or a dual oral combination therapy in low doses. Key exclusion criteria included significant hepatic, renal, neurological, cardiovascular, gastrointestinal, metabolic or hormonal disorders, hyperlipidaemia and hypertension.

Pharmacokinetic Assessments

Linagliptin concentrations in plasma and urine were analysed by high-performance liquid chromatography coupled to tandem mass spectrometry (HPLC-MS/MS) using [$^{13}C_3$]-labelled linagliptin as internal standard as described previously [16]. Pharmacokinetic analysis of linagliptin was carried out by non-compartmental analysis of the plasma/urine concentration–time data using

the WinNonlin™ software program (Professional, version 5.0.1; Pharsight, Mountain View, CA, USA). Actual sampling times were used for pharmacokinetic analysis. The apparent terminal rate constant (λ) at steady state was estimated by regression of the terminal log-linear portion (determined by inspection) of the plasma concentration–time profile using the last three available data points; $t_{1/2}$ was calculated as the quotient of ln(2) and λ. Area under the plasma concentration–time curve (AUC) was calculated using the linear trapezoidal method for ascending concentrations and the log trapezoidal method for descending concentrations. Accumulation ratios R_A (day 12/day 1) for AUC and maximum plasma concentration (C_{max}) were calculated for each subject. Accumulation half-life ($t_{1/2}$) was calculated from the AUC accumulation ratio by the following formula:

$$\text{Accumulation } t_{1/2} = \left[\mathrm{t} \cdot \ln(2)\right] / \ln\left[R_A/(R_A - 1)\right].$$

The fraction of dose excreted unchanged in urine over the 24 h interval on day 1 (fe_{0-24}) and on day 12 ($fe_{\tau,ss}$) was calculated from the sum of the amounts of linagliptin collected in the urine in each collection interval. Renal clearance was determined as the quotient of amount excreted unchanged in urine and AUC over the respective interval.

Bioanalytical Methods

DPP-4 Enzyme Assay

DPP-4 activity in plasma was analysed by a validated method at the Institute for Clinical Research and Development (ikfe GmbH), Mainz, Germany, using a semi-quantitative enzyme activity assay with fluorescence detection (substrate: H-Ala-Pro-7-aminoamido-4-trifluoromethylcoumarin). Fluorescence was detected at 535 nm (emission) using 405 nm excitation wavelength after 10 min of incubation at amplification/gain 60 using a GENios FL fluorescence reader (Tecan, Durham, NC, USA).

Glucose, Active GLP-1 and Fructosamine

Plasma glucose was quantitatively determined using an electrochemical, enzymatic-amperometric measuring principle (SuperGL Hitado Diagnostic Systems GmbH, Möhnesee, Germany). For the calculation of the area under the plasma glucose concentration–time curve, the linear trapezoidal method was used.

Active GLP-1 in plasma was measured at the Institute for Clinical Research and Development (ikfe GmbH), Mainz, Germany using a GLP-1 active [GLP-1-(7-36) and -(7-37)] enzyme-linked immunosorbent assay kit (Linco Research, St Charles, IL, USA) with a lower limit of quantification of 2.0 pmol/l.

Fructosamine was analysed on an Olympus AU 600 by a colour test from Roche Diagnostics (Basel, Switzerland).

Statistical Analysis

An exploratory analysis of drug dose proportionality was carried out by an analysis of variance regression model for the pharmacokinetic parameters C_{max} and $AUC_{\tau,1}$ after the first dose and steady-state maximum plasma concentration ($C_{max,ss}$) and area under the steady-state plasma concentration–time curve to the dosing interval ($AUC_{\tau,ss}$) after the last dose. These parameters were dose normalized and compared graphically.

The statistical analysis of the attainment of steady state using the trough concentrations (C_{pre}) between day 2 and day 12 and the concentrations taken directly at the end of the first and the last dosing interval was performed using a parametric approach [18]. A monoexponential increase of the trough level (until the individual steady-state concentration was reached) was modelled within each dose group.

Results

Subject Disposition and Demographics

Forty-eight patients with type 2 diabetes [age 56.0 (7.6) years, mean (s.d.); BMI 28.6 (3.0) kg/m^2; mean haemoglobin A1c (HbA1c) at baseline, 7.0%] entered the study. Forty-two patients were on oral antidiabetic drug (OAD) monotherapy (mostly metformin) at the time of screening. One patient randomized to the linagliptin 5 mg dose group was withdrawn before drug administration on day 1 because of elevated blood glucose during the OGTT on day −1. There were no other discontinuations.

Pharmacokinetic Results

Mean plasma concentration–time profiles of linagliptin after a single oral dose on day 1 and at steady state on day 12 are given in figure 1. Linagliptin was rapidly absorbed in all patients with a median time to first occurrence of maximum plasma concentration (t_{max}) around 1.5 h (range: 1–3 h) after drug intake, both after single and multiple dosing. Linagliptin AUC and C_{max}

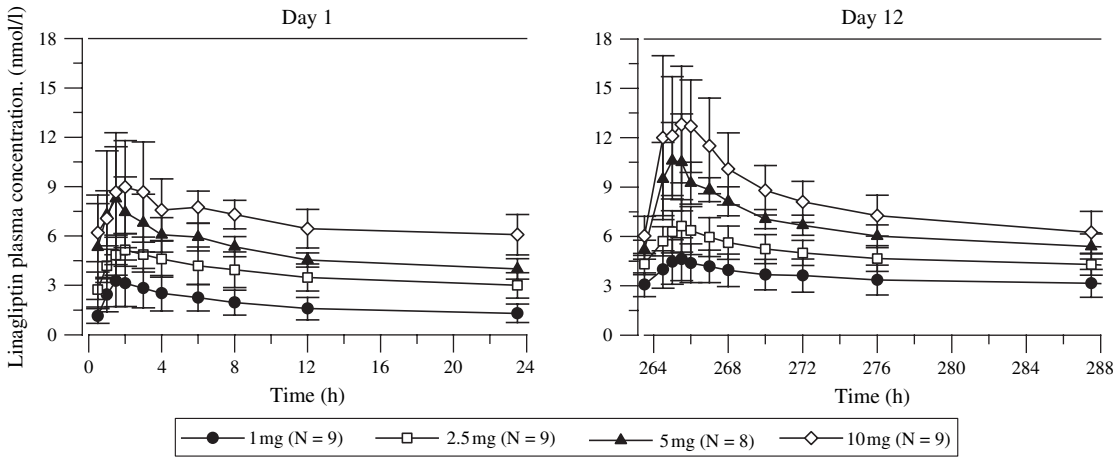

Fig. 1 Arithmetic mean drug plasma concentration–time profiles of linagliptin after oral administration of linagliptin (left panel: day 1; right panel: day 12).

values increased dose dependently, but less than dose proportionally, within the dose range tested (table 1).

Moderate accumulation was observed after once-daily dosing of linagliptin (table 1). The $R_{A,AUC}$ decreased with increasing doses from 2.0-fold for the 1 mg dose group to 1.2-fold for the 10 mg dose group. This also indicated that the calculated terminal half-life of linagliptin (110–130 h) was not the dominant half-life of the compound. The accumulation half-life decreased accordingly with dose from about 24 h for the 1 mg dose group to about 9 h for the 10 mg dose group.

Time to attain steady-state linagliptin plasma concentrations decreased dose dependently. Following 1, 2.5 and 5 mg, steady-state conditions for pharmacokinetics were reached between days 4 and 6, but following 10 mg the predose plasma concentrations were similar on day 2 and on day 13. More than 90% of the predose plasma concentration on day 13 was reached between days 2 and 5 in all dose groups.

Renal excretion of the parent compound seemed to be only a minor route of elimination. For all dose groups, the cumulative amount of parent compound excreted in urine

Table 1 gMean and gCV of pharmacokinetic parameters of linagliptin at steady state (day 12)

Parameter	1 mg, gMean (gCV)	2.5 mg, gMean (gCV)	5 mg, gMean (gCV)	10 mg, gMean (gCV)
AUC_{0-24} (nmol h/l)	40.2 (39.7)	85.3 (22.7)	118 (16.0)	161 (15.7)
$AUC_{\tau,ss}$ (nmol h/l)	81.7 (28.3)	117 (16.3)	158 (10.1)	190 (17.4)
C_{max} (nmol/l)	3.13 (43.2)	5.25 (24.5)	8.32 (42.4)	9.69 (29.8)
$C_{max,ss}$ (nmol/l)	4.53 (29.0)	6.58 (23.0)	11.1 (21.7)	13.6 (29.6)
t_{max} (h)*	1.50 [1.00–3.00]	2.00 [1.00–3.00]	1.75 [0.92–6.02]	2.00 [1.50–6.00]
$t_{max,ss}$ (h)*	1.48 [1.00–3.00]	1.42 [1.00–3.00]	1.53 [1.00–3.00]	1.34 [0.50–3.00]
$t_{1/2,\ ss}$ (h)	121 (21.3)	113 (10.2)	131 (17.4)	130 (11.7)
Accumulation $t_{1/2,}$ (h)	23.9 (44.0)	12.5 (18.2)	11.4 (37.4)	8.59 (81.2)
$R_{AC_{max}}$	1.44 (25.6)	1.25 (10.6)	1.33 (30.0)	1.40 (47.7)
$R_{A,AUC}$	2.03 (30.7)	1.37 (8.2)	1.33 (15.0)	1.18 (23.4)
fe_{0-24} (%)	NC	0.139 (51.2)	0.453 (125)	0.919 (115)
$fe_{\tau,ss}$ (%)	3.34 (38.3)	3.06 (45.1)	6.27 (42.2)	3.22 (34.2)
$CL_{R,ss}$ (ml/min)	14.0 (24.2)	23.1 (39.3)	70 (35.0)	59.5 (22.5)

AUC, area under the plasma concentration–time curve; $AUC_{t,ss}$, area under the steady-state plasma concentration–time curve to the dosing interval; C_{max}, maximum plasma concentration; $C_{max,ss}$, steady-state maximum plasma concentration; $fe_{0-24,}$ fraction of dose excreted unchanged in urine over the 24 h interval on day 1; $fe_{\tau,ss}$, fraction of dose excreted unchanged in urine over the 24 h interval on day 12; gCV, geometric coefficient of variation; gMean, geometric mean; $R_{A,AUC}$, accumulation ratio AUC; $R_{A,C_{max}}$, accumulation ratio C_{max}; $t_{1/2}$, elimination half-life; t_{max}, time to first occurrence of maximum plasma concentration; $t_{max,ss}$, time to first occurrence of maximum plasma concentration at steady state; NC, not calculated as most values below lower limit of quantification.

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

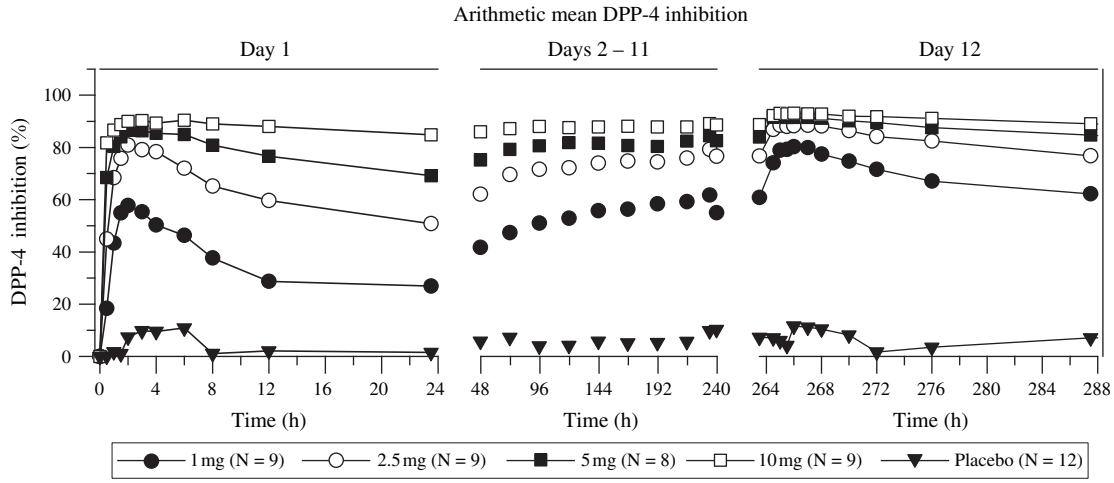

Fig. 2 Arithmetic mean dipeptidyl peptidase-4 inhibition (% of baseline) after oral administration of linagliptin or placebo on day 1 and on day 12 throughout the 12-day treatment period.

on day 1 (0–24 h) was below 1% of the administered dose and increased to no more than 3–6% on day 12 (table 1).

Pharmacodynamic Results

Plasma DPP-4 activity was not inhibited in placebo-treated patients, but was dose dependently inhibited in patients administered linagliptin doses from 1 to 10 mg (figure 2; table 2). Plasma DPP-4 activity was inhibited by more than 80% in one of the nine patients in the 2.5 mg dose group, in seven of the eight patients in the 5 mg dose group and all nine patients in the 10 mg dose group. In fact, DPP-4 inhibition was 85.3% 24 h after the first 10 mg dose of linagliptin. Maximum DPP-4 inhibition within one dosing interval at steady state [maximum pharmacodynamic effect at steady state ($E_{max,ss}$)] was 92.3 and 93.7% for the 5 and 10 mg dose groups, respectively. DPP-4 inhibition correlated well with linagliptin plasma concentration (figure 3).

Linagliptin considerably reduced the area under the glucose concentration curve ($AUEC_{0-2}$) during the OGTT, both on day 1 and more markedly on day 13 (figure 4). The reduction in plasma glucose excursions was dose dependent and reached statistical significance ($p < 0.05$) for the 2.5, 5 and 10 mg groups compared with placebo. A decrease of about 10% in fructosamine concentrations was observed with 10 mg of linagliptin, but not with the lower doses. Considerably higher (in the range of 12.4–14.0 pmol/l) GLP-1 increases from baseline (predose) were observed with linagliptin 2.5, 5

Table 2 Mean (s.d.) maximum, plasma dipeptidyl peptidase-4 inhibition (maximum pharmacodynamic effect and maximum pharmacodynamic effect at steady state) and the plasma dipeptidyl peptidase-4 inhibition 24 h after dosing (pharmacodynamic effect 24 h postdose and maximum effect at steady state) on day 1 and day 12

Dose	E_{max} (%)	E_{24} (%)	$E_{max,ss}$ (%)	$E_{\tau,ss}$ (%)
Placebo	12.7 (8.94)	1.50 (8.66)	18.2 (11.3)	7.08 (14.1)
1 mg	60.6 (14.3)	26.9 (9.55)	81.7 (5.32)	62.2 (4.84)
2.5 mg	82.4 (5.29)	50.8 (10.1)	89.3 (1.87)	76.8 (2.77)
5 mg	88.3 (5.55)	69.3 (11.9)	92.3 (1.04)	84.8 (3.20)
10 mg	91.4 (2.19)	84.8 (4.58)	93.6 (1.33)	89.1 (2.67)

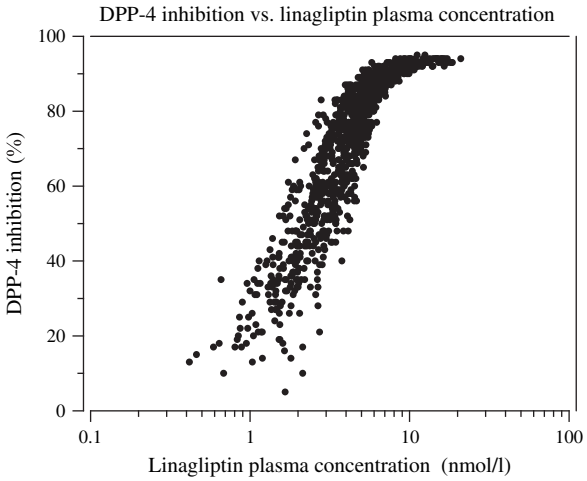

Fig. 3 Correlation of plasma dipeptidyl peptidase-4 inhibition and linagliptin plasma concentration.

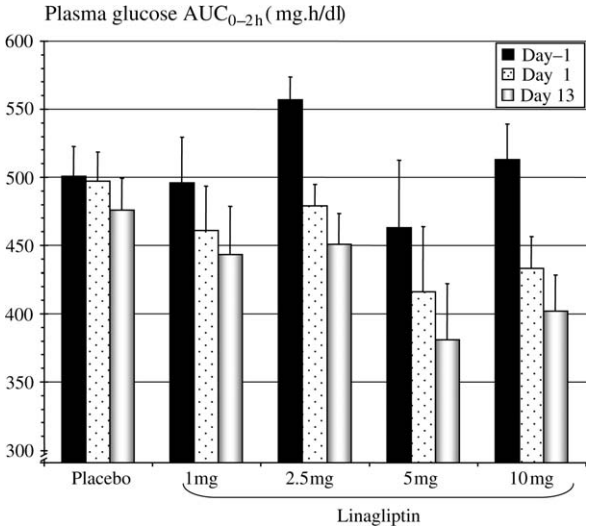

Fig. 4 Effect of multiple oral doses of linagliptin on 2 h postprandial glucose levels after an oral glucose tolerance test (area under the glucose concentration–time curve over 2 h) on days −1, 1 and 13. Baseline plasma glucose concentrations are given as mean (s.d.).

and 10 mg on day 13 compared with day −1 (1.43–3.83 pmol/l), but the interpretation of this finding was restricted by the fact that altogether 36% of GLP-1 plasma concentrations were below the limit of quantification.

Safety and Tolerability Results

Multiple oral doses of linagliptin were well tolerated during this trial. No serious AEs, deaths or significant AEs were reported, and there were no discontinuations because of AEs. Nineteen of the 35 patients (54.3%) administered linagliptin reported 31 AE episodes and nine of the 12 patients (75.0%) administered placebo reported 18 AE episodes. Apart from three moderate AEs, all AE episodes were considered mild in intensity. No dose effect was discerned. No clinically relevant changes in laboratory or ECGs parameters occurred during the study. In addition, no episodes of hypoglycaemia were observed in any of the patients.

Discussion

This first investigation of the multiple dose pharmacokinetic (PK) and pharmacodynamic properties of linagliptin in the primary target population established that linagliptin showed a dose-dependent increase in PK levels, reached steady-state plasma levels between the second and fifth day of treatment, showed little accumulation with an accumulation ratio of $\leq$1.4 with doses above 1 mg and had a long-lasting effect on DPP-4 inhibition with almost complete DPP-4 inhibition at the 5 and 10 mg dose levels (92.3 and 93.7% inhibition at steady state, respectively, and more than 80% inhibition over a 24 h interval after drug intake). These properties resulted in a considerable increase in GLP-1 levels during the treatment period and a significant reduction in blood glucose levels during an OGTT after the first intake of linagliptin at doses of 2.5 mg or higher, which was even more pronounced on day 13, that is 24 h after the last drug intake. Linagliptin showed a clean safety profile in this small study with an AE rate similar to that of placebo. Only a minor portion (<7%) of linagliptin was eliminated through the kidneys.

These properties compare favourably with those reported for other DPP-4 inhibitors. Vildagliptin shows a shorter duration of action with an increase of DPP-4 activity to more than 75% of baseline 24 h after a single administration of a therapeutic dose of 50 mg [19] and to only about 50% with 100 mg [20]. Therefore, vildagliptin needs twice-daily administration to achieve a sufficient DPP-4 inhibition over 24 h and a significant reduction in postprandial blood glucose levels [21]. Other DPP-4 inhibitors in development such as PHX1149 did not show sustained DPP-4 inhibition (>80%) under steady-state conditions in doses below 400 mg [22].

A longer effect on DPP-4 inhibition has been described for sitagliptin (used once daily) [23]. Nevertheless, DPP-4 inhibition decreased to about 80% with 200 mg sitagliptin at 24 h after drug administration in a study in people with type 2 diabetes [24]. This is in line with previously published animal data suggesting that linagliptin may have an even longer-lasting effect [15]. Clinical studies directly comparing various DPP-4 inhibitors have not been conducted to date.

It has to be stressed that the present study was an early, short-term investigation of the pharmacological properties of linagliptin under multiple dose (steady-state) conditions. Other DPP-4 inhibitors that are more advanced in their development showed improvements in β-cell function and insulin sensitivity [11] as well as reductions in HbA1c of 0.3–0.7% vs. placebo [25–28] up to 2 years of treatment. These improvements were comparable with those achieved with a sulphonylurea [29]. Similar clinical benefits are still to be shown with linagliptin. Nevertheless, the first indications observed in this study are quite promising: treatment with linagliptin increased postprandial excursions of active GLP-1 to levels several fold higher than those observed with placebo. This led to a significant decrease in

postprandial blood glucose excursions by about 80 mg h/dl already on day 1 in doses of 2.5 mg and higher which improved further as treatment progressed. Again, these results compare favourably with previous observations with other DPP-4 inhibitors. Sitagliptin in doses of 50 and 200 mg increased active GLP-1 levels by approximately twofold and decreased blood glucose excursions by 9–18% during an OGTT performed 24 h after last drug administration [24]. The decrease in blood glucose concentrations was significant for the 200 mg dose, but not for 50 mg sitagliptin. Similarly, vildagliptin in a dose range of 10–400 mg reduced blood glucose excursions by about 10% after an oral glucose challenge administered 30 min after a single administration of the compound [19]. However, comparisons across studies always are difficult to interpret, and the heterogeneity of people with type 2 diabetes and the high variability in post-OGTT blood glucose excursions has to be taken into account. Therefore, no firm conclusions should be drawn from these data before clinical comparative data of the different DPP-4 inhibitors will become available.

Nevertheless, the elimination pathway of linagliptin may be an advantage in patients with renal impairment. In contrast to other DPP-4 inhibitors [23,30–33] only a minor fraction of linagliptin is eliminated through the kidneys. This fraction increased only very slightly over time and with increasing dose, so that there will likely be no need to modify the dose of linagliptin based on the patients' renal function. The non-renal elimination of linagliptin in combination with its low accumulation potential and broad safety margin may be of significant benefit in a patient population that has a high prevalence of renal insufficiency [34] and diabetic nephropathy [35].

In addition to the elimination pathway, the pharmacological properties of linagliptin differed from that of other DPP-4 inhibitors with regard to the observed non-linearity in the pharmacokinetics of linagliptin. This finding is related to a concentration-dependent change in plasma protein binding. In plasma protein–binding studies, the binding of linagliptin to its target enzyme DPP-4 is characterized by high affinity, but low capacity. Once binding to DPP-4 is saturated, the free fraction of linagliptin increases, with a resulting increase in drug elimination [36]. Interindividual variability in steady-state exposure of linagliptin was low (about 10% geometric coefficient of variation for the 5 mg dose group, and below 30% for all dose groups). The change in plasma protein binding adds to the favourable pharmacokinetic profile of linagliptin, with rapid attainment of steady state and little accumulation while preserving a long-lasting effect on DPP-4 inhibition. Thus, with low doses of about 5 mg, linagliptin acts as a true once-daily drug with full 24 h duration of DPP-4 inhibition.

Finally, the results from this multiple dose study in patients with type 2 diabetes confirm the good safety and tolerability profile that was observed in a single-dose study in healthy volunteers [16]. Multiple doses of linagliptin over 12 days in patients with type 2 diabetes were well tolerated irrespective of dose and not associated with any incidence or signs or symptoms suggestive of hypoglycaemia. In a study in healthy subjects, daily exposures of up to and including 600 mg (120-fold the expected therapeutic dose) were well tolerated [16]. This large therapeutic window may, at least in part, be related to the >10 000-fold selectivity of linagliptin for DPP-4 vs. DPP-8 and DPP-9 [15].

In conclusion, in this first investigation in people with type 2 diabetes, linagliptin showed a placebo-like safety and tolerability and a long-lasting effect on DPP-4 inhibition. In addition, a significant improvement of glucose excursions was observed 24 h after the last administration in doses of up to 10 mg. With doses of 5 mg and higher, linagliptin was demonstrated to be a true once-daily DPP-4 inhibitor. Further investigations are needed to confirm that these unique pharmacological properties of linagliptin, together with the predominantly non-renal elimination route, lead to clinical improvements beyond those already observed with other compounds of the DPP-4 inhibitor class.

Acknowledgements

The authors thank the volunteers and staff who participated in this study, and Dr Barbara Withopf for bioanalytical support and Elke Krug for data analysis.

Data from this study have previously been published, in part, at the International Diabetes Federation (IDF) Conference 2006 in Cape Town, South Africa and at the American Diabetes Association (ADA) 2007 in Chicago, Illinois.

References

1 Ahrén B. Gut peptides and type 2 diabetes mellitus treatment. Curr Diab Rep 2003; **3:** 365–372.
2 Drucker DJ. Therapeutic potential of dipeptidyl peptidase IV inhibitors for the treatment of type 2 diabetes. Expert Opin Investig Drugs 2003; **12:** 87–100.
3 Deacon CF. Therapeutic strategies based on glucagon-like peptide 1. Diabetes 2004; **53:** 2181–2189.
4 Deacon CF. What do we know about the secretion and degradation of incretin hormones? Regul Pept 2005; **128:** 117–124.

5 Idris I, Donnelly R. Dipeptidyl peptidase-IV inhibitors: a major new class of oral antidiabetic drug. Diabetes Obes Metab 2007; **9:** 153–165.
6 Ahrén B, Landin-Olsson M, Jansson PA, Svensson M, Holmes D, Schweizer A. Inhibition of dipeptidyl peptidase-4 reduces glycemia, sustains insulin levels, and reduces glucagon levels in type 2 diabetes. J Clin Endocrinol Metab 2004; **89:** 2078–2084.
7 Ahrén B, Gomis R, Standl E, Mills D, Schweizer A. 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.
8 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.
9 Inzucchi SE. Oral antihyperglycemic therapy for type 2 diabetes: scientific review. JAMA 2002; **287:** 360–372.
10 Reimer MK, Hoist JJ, Ahrén B. Long-term inhibition of dipeptidyl peptidase IV improves glucose tolerance and preserves islet function in mice. Eur J Endocrinol 2002; **146:** 717–727.
11 Ahrén B, Pacini G, Foley JE, Schweizer A. Improved meal-related β-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.
12 Mari A, Sallas WM, He YL *et al.* Vildagliptin, a dipeptidyl peptidase-IV inhibitor, improves model-assessed β-Cell function in patients with type 2 diabetes. J Clin Endocrinol Metab 2005; **90:** 4888–4894.
13 Drucker DJ. Enhancing incretin action for the treatment of type 2 diabetes. Diabetes Care 2003; **26:** 2929–2940.
14 Buse JB, Henry RR, Han J, Kim DD, Fineman MS, Baron AD. 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.
15 Thomas L, Eckhardt M, Langkopf E, Tadayyon M, Himmelsbach F, Mark M. (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.
16 Hüttner S, Graefe-Mody EU, Withopf B, Ring A, Dugi KA. 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.
17 World Medical Association. Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Patients. 59th WMA General Assembly, Seoul, October 2008. World Medical Association. France.
18 Hoffman D, Kringle R, Lockwoos G, Turpault S, Yow E, Mathieu G. Nonlinear mixed effects modelling for estimation of steady state attainment. Pharm Stat 2005; **4:** 15–24.
19 He YL, Wang Y, Bullock JM *et al.* Pharmacodynamics of vildagliptin in patients with type 2 diabetes during OGTT. J Clin Pharmacol 2007; **47:** 633–641.
20 He YL, Sabo R, Campestrini J *et al.* The effect of age, gender, and body mass index on the pharmacokinetics and pharmacodynamics of vildagliptin in healthy volunteers. Br J Clin Pharmacol 2008; **65:** 338–346.
21 He YL, Serra D, Wang Y *et al.* Pharmacokinetics and pharmacodynamics of vildagliptin in patients with type 2 diabetes mellitus. Clin Pharmacokinet 2007; **46:** 577–588.
22 O'Farrell AM, van Vliet A, Abou Farha K *et al.* Pharmacokinetic and pharmacodynamic assessments of the dipeptidyl peptidase-4 inhibitor PHX1149: double-blind, placebo-controlled, single- and multiple-dose studies in healthy subjects. Clin Ther 2007; **29:** 1692–1705.
23 Bergman AJ, Stevens C, Zhou Y *et al.* Pharmacokinetic and pharmacodynamic properties of multiple oral doses of sitagliptin, a dipeptidyl peptidase-IV inhibitor: a double-blind, randomized, placebo-controlled study in healthy male volunteers. Clin Ther 2006; **28:** 55–72.
24 Herman GA, Bergman A, Stevens C *et al.* Effect of single oral doses of sitagliptin, a dipeptidyl peptidase-4 inhibitor, on incretin and plasma glucose levels after an oral glucose tolerance test in patients with type 2 diabetes. J Clin Endocrinol Metab 2006; **91:** 4612–4619.
25 Bosi E, Camisasca RP, Collober C, Rochotte E, Garber AJ. Effects of vildagliptin on glucose control over 24 weeks in patients with type 2 diabetes inadequately controlled with metformin. Diabetes Care 2007; **30:** 890–895.
26 Scherbaum WA, Schweizer A, Mari A *et al.* Efficacy and tolerability of vildagliptin in drug-naïve patients with type 2 diabetes and mild hyperglycaemia. Diabetes Obes Metab 2008; **10:** 675–682.
27 Charbonnel B, Karasik A, Liu J, Wu M, Meininger G. Efficacy and safety of the dipeptidyl peptidase-4 inhibitor sitagliptin added to ongoing metformin therapy in patients with type 2 diabetes inadequately controlled with metformin alone. Diabetes Care 2006; **29:** 2638–2643.
28 Aschner P, Kipnes MS, Lunceford JK, Sanchez M, Mickel C, Williams-Herman DE. Effect of the dipeptidyl peptidase-4 inhibitor sitagliptin as monotherapy on glycemic control in patients with type 2 diabetes. Diabetes Care 2006; **29:** 2632–2637.
29 Nauck MA, Meininger G, Sheng D, Terranella L, Stein PP. Efficacy and safety of the dipeptidyl peptidase-4 inhibitor, sitagliptin, compared with the sulfonylurea, glipizide, in patients with type 2 diabetes inadequately controlled on metformin alone: a randomized, double-blind, non-inferiority trial. Diabetes Obes Metab 2007; **9:** 194–205.
30 He YL, Sadler BM, Sabo R *et al.* The absolute oral bioavailability and population-based pharmacokinetic

modelling of a novel dipeptidyl peptidase-IV inhibitor, vildagliptin, in healthy volunteers. Clin Pharmacokinet 2007; **46:** 787–802.

31 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.

32 Christopher R, Covington P, Davenport M *et al*. Pharmacokinetics, pharmacodynamics, and tolerability of single increasing doses of the dipeptidyl peptidase-4 inhibitor alogliptin in healthy male subjects. Clin Ther 2008; **30:** 513–527.

33 Covington P, Christopher R, Davenport M *et al*. Pharmacokinetic, pharmacodynamic, and tolerability profiles of the dipeptidyl peptidase-4 inhibitor alogliptin: a randomized, double-blind, placebo-controlled, multiple-dose study in adult patients with type 2 diabetes. Clin Ther 2008; **30:** 499–512.

34 Ritz E, Rychlik I, Locatelli F, Halimi S. End-stage renal failure in type 2 diabetes: a medical catastrophe of worldwide dimensions. Am J Kidney Dis 1999; **34:** 795–808.

35 Kramer HJ, Nguyen QD, Curhan G, Hsu CY. Renal insufficiency in the absence of albuminuria and retinopathy among adults with type 2 diabetes mellitus. JAMA 2003; **289:** 3273–3277.

36 Fuchs H, Tillement JP, Urien S, Greischel A, Roth W. 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.

