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ORIGINAL RESEARCH ARTICLE

Population Pharmacokinetics and Pharmacodynamics of Linagliptin in Patients with Type 2 Diabetes Mellitus

Silke Retlich · Vincent Duval · Ulrike Graefe-Mody · Christian Friedrich · Sanjay Patel · Ulrich Jaehde · Alexander Staab

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Abstract

Background and Objectives Linagliptin is a dipeptidyl peptidase (DPP)-4 inhibitor, used to treat type 2 diabetes mellitus (T2DM). Population pharmacokinetic and pharmacodynamic analyses were performed to characterize the impact of clinically relevant intrinsic/extrinsic factors (covariates) on linagliptin exposure and DPP-4 inhibition in patients with T2DM.

Methods Linagliptin plasma concentrations and DPP-4 activities were obtained from four studies (two phase 1, two phase 2b). Non-linear mixed-effects modelling techniques were implemented using NONMEM software. The covariates that were studied comprised demographic information and laboratory values, including liver enzyme levels and creatinine clearance, as well as study-related factors such as metformin co-treatment. Covariate effects on parameters describing the pharmacokinetics and pharmacokinetic/pharmacodynamic relationship were investigated using stepwise forward inclusion/backward elimination.

Results The pharmacokinetic analysis included 6,907 measurements of plasma linagliptin concentrations from 462 patients; the pharmacokinetic/pharmacodynamic analysis included 9,674 measurements of plasma DPP-4 activity and linagliptin plasma concentrations from 607 patients. The non-linear pharmacokinetics were described by a target-mediated drug disposition model accounting for the concentration-dependent binding of linagliptin to its target, DPP-4. The difference in exposure between the 5th and 95th percentiles of the covariate distributions and median was <20 % for each single covariate. Likewise, the impact of the covariates on both the half-maximum effect (EC_{50}) and the concentration leading to 80 % DPP-4 inhibition was <20 %.

Conclusion These analyses show that the investigated factors do not alter the pharmacokinetics and DPP-4 inhibitory activity of linagliptin to a clinically relevant extent and that dose adjustment is not necessary on the basis of factors including age, sex and weight.

Key Points

These analyses, reporting previously unpublished data on the pharmacokinetic and pharmacodynamic profile of linagliptin, show that factors including age, sex and weight do not alter the pharmacokinetics and dipeptidyl peptidase-4 inhibitory activity of linagliptin to a clinically relevant extent.

These findings indicate there is no need for linagliptin dose adjustment on the basis of age, sex or weight, and they extend the findings of previous research that has shown that linagliptin does not require dose adjustment in patients with renal or hepatic impairment.

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S. Retlich (✉) · V. Duval · C. Friedrich · A. Staab
Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach, Germany
e-mail: silke.retlich@boehringer-ingelheim.com

U. Graefe-Mody
Boehringer Ingelheim Pharma GmbH & Co. KG, Ingelheim, Germany

S. Patel
Boehringer Ingelheim Ltd., Bracknell, Berkshire, UK

U. Jaehde
Institute of Pharmacy, Clinical Pharmacy, University of Bonn, Bonn, Germany

1 Introduction

Linagliptin (trade name: Trajenta®) is a dipeptidyl peptidase (DPP)-4 inhibitor, which is approved by the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) for treatment of type 2 diabetes mellitus (T2DM). Linagliptin has a unique pharmacokinetic and pharmacodynamic profile within the DPP-4 inhibitor class [1–4]. The pharmacokinetics and pharmacodynamics of linagliptin have been evaluated in healthy subjects [5–7] and in patients with T2DM [3]. Because of the high affinity and saturable binding of linagliptin to DPP-4, and the slow dissociation from the resulting enzyme–drug complex, linagliptin shows concentration-dependent protein binding in the therapeutic plasma concentration range, with the unbound fraction of linagliptin rising with increasing total linagliptin concentrations [4]. As a result, linagliptin shows non-linear pharmacokinetics after both oral and intravenous administration, with a less than dose-proportional increase in plasma concentrations in the dose range of 1–10 mg [1, 3, 5]. Unlike other DPP-4 inhibitors, linagliptin is predominantly excreted unchanged in the faeces, with renal excretion being only a minor elimination route [5, 7]. The excretion of linagliptin in the faeces is thought to result from both biliary excretion and direct P-glycoprotein-mediated efflux into the gut [8]. These pharmacological characteristics allow once-daily dosing of linagliptin 5 mg, with no requirement for dose adjustment in patients with renal impairment. At present, the linagliptin clinical trials programme includes more than 4,000 patients from over 40 countries worldwide. Linagliptin 5 mg once daily has been shown to improve glycaemic control, significantly reducing glycated haemoglobin (HbA_{1c}), fasting plasma glucose (FPG) and postprandial glucose (PPG) levels from baseline, compared with placebo [9–12].

To characterize the impact of clinically relevant covariates on the pharmacokinetics of linagliptin and its inhibition of plasma DPP-4 activity in patients with T2DM, two investigations were performed: (1) a population pharmacokinetic analysis; and (2) a population pharmacokinetic/pharmacodynamic analysis. The covariates weight, sex and age were of particular interest, as no dedicated phase 1 studies to investigate their effects on the pharmacokinetics/pharmacodynamics of linagliptin have been conducted.

2 Methods

2.1 Study Design

Data were obtained from four studies performed in patients with T2DM: two phase 1 studies (studies 1 and 2) and two phase 2b studies (studies 3 and 4) [13, 14] (Table 1). In the phase 1 studies, a full pharmacokinetic and DPP-4 activity profile was taken on the first and last days of treatment, with trough values measured during treatment, as indicated in Table 1. In the phase 2b studies, plasma concentrations and plasma DPP-4 activity were measured at trough and at about 1 and 2 h after linagliptin administration at four visits (at the visit when linagliptin was first administered, then at three subsequent visits 4–5 weeks apart) and at the follow-up visit (2–3 weeks after the final linagliptin administration).

2.2 Data Analysis

For both population analyses, non-linear mixed-effects modelling techniques were implemented using NONMEM software (version V, level 1.1; GloboMax LLC, Hanover, MD, USA). Investigation of the covariate effects on the parameters describing the pharmacokinetics and the pharmacokinetic/pharmacodynamic relationship was undertaken using the stepwise forward inclusion/backward elimination approach. Population modelling for the pharmacokinetic and pharmacokinetic/pharmacodynamic analyses are described separately below.

2.3 Population Pharmacokinetic Analysis

2.3.1 Base Model Development

A previously developed population pharmacokinetic model for linagliptin (based on the two phase 1 studies) was used as a starting point for the current analysis [15]. In the previous analysis, linagliptin plasma concentrations were best described by a two-compartment model, including concentration-dependent protein binding in the central and peripheral compartments. This prior model was tested and was found to be suitable for describing the linagliptin plasma concentrations obtained in the two phase 2b studies, which were not included in the previous analysis. The structural model used in the population pharmacokinetic analysis is shown in Fig. 1.

2.3.2 Covariate Model Development

The covariates that were investigated were age, weight, height, body surface area, sex, ethnic origin, smoking status, alcohol consumption status, creatinine clearance (CL_{CR}), metformin co-medication, formulation, DPP-4 activity at baseline and levels of serum creatinine, alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase, gamma-glutamyl transferase (GGT), total bilirubin, urea, creatine kinase, cholesterol, C-reactive protein, triglycerides (TG) and FPG (Table 2).

Table 1 Summary of important design characteristics of the included studies

	Study			
	1	2	3	4
Phase	1	1	2b	2b
Formulation	Powder in bottle	Tablet formulation 1	Tablet formulation 2	Tablet formulation 2
Linagliptin doses (mg)	1, 2.5, 5, 10	2.5, 5, 10	0.5, 2.5, 5	1, 5, 10
Duration	12 days	4 weeks	12 weeks	12 weeks
Number of patients on linagliptin	35	61	170	196
Add-on to metformin	No	No	No	Yes
Sampling schemes for linagliptin plasma concentrations and plasma DPP-4 activity				
Single-dose profile	Day 1		Before and 1 and 2 h after the first linagliptin administration	
	Before and 0.5, 1, 1.5, 2, 3, 4, 6, 8 and 12 h after the first administration			
C_{trough} during treatment	Days 2–11	Days 2, 6, 12, 19, 26 and 27	At three subsequent visits 4–5 weeks apart	
	Before linagliptin administration		Before linagliptin administration	
Overnight sample	Day 11	Day 28	–	
	18 h after drug administration on day 10	18 h after drug administration on day 27		
Steady-state profile	Day 12	Day 28	At three visits 4–5 weeks apart	
	Before and 0.5, 1, 1.5, 2, 3, 4, 6, 8 and 12 h after administration		1 and 2 h after linagliptin administration	
Samples after last dose	Days 13, 14, 16, 18 and 20	Days 29, 30, 33, 36, 39, 41 and 43	At one visit, one sample 2–3 weeks after the final linagliptin administration	
	In the morning			

C_{trough} trough plasma concentration, *DPP-4* dipeptidyl peptidase-4

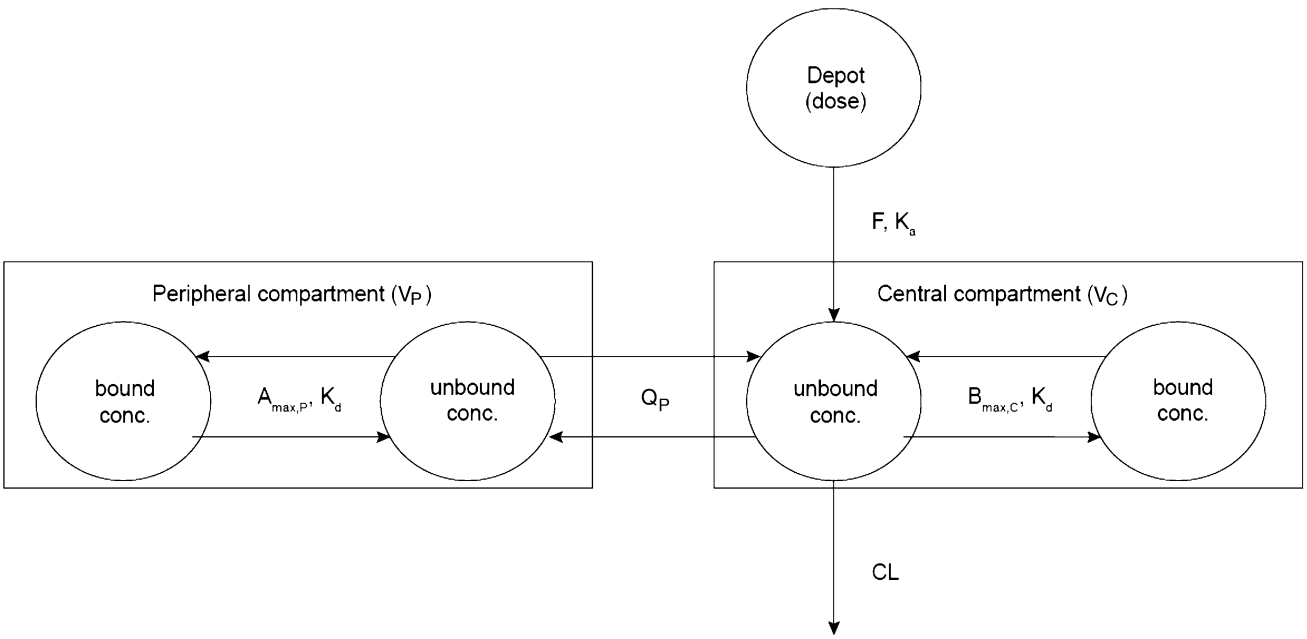

Fig. 1 Model structure of the population pharmacokinetic model. $A_{max,P}$ number of binding sites in the peripheral compartment, $B_{max,C}$ concentration of binding sites in the central compartment, *CL* clearance, *conc.* concentration, *F* bioavailability, K_a absorption rate constant, K_d affinity constant, Q_P inter-compartmental clearance between the central and peripheral compartments, V_C central volume of distribution, V_P peripheral volume of distribution

Table 2 Covariates investigated by graphical and generalized additive modelling (GAM) analysis to select those to be tested in the forward inclusion/backward elimination approach performed in NONMEM

Pharmacokinetic model parameter	Covariate
All model parameters with inter-individual variability	Demographic information: age, weight, height, body surface area, sex, ethnic origin, smoking status and alcohol consumption status
	Dose group
Absorption parameters with inter-individual variability	Formulation
Distribution and elimination parameters with inter-individual variability (including binding parameters)	Laboratory values: creatinine clearance and levels of serum creatinine, urea, alanine transaminase, aspartate transaminase, alkaline phosphatase, gamma-glutamyl transferase, total bilirubin, creatine kinase, cholesterol, C-reactive protein, triglyceride and fasting plasma glucose
	Metformin treatment, baseline DPP-4 activity in relative fluorescence units

DPP-4 dipeptidyl peptidase-4

A graphical and a generalized additive modelling (GAM) analysis was carried out using SPlus software (version 7; Insightful Corporation, Seattle, WA, USA) to select the covariates to be tested in the forward inclusion/backward elimination approach, performed in NONMEM (Fig. 2). Certain covariates were predefined on the basis of physiological considerations to be tested in the forward inclusion/backward elimination approach, independently of the results of the graphical and GAM analyses. The predefined covariates were age, sex and weight, since these were of special interest, as no dedicated phase 1 studies have yet been performed for these, as well as levels of liver enzymes (ALT and GGT) and CL_{CR}. Because of the extensive run times and high η-shrinkage (27 % bioavailability [F], 35 % clearance [CL], 22 % absorption rate constant [K_a], 24 % concentration of binding sites in the central compartment [$B_{max,C}$] and 58 % central volume of distribution [V_C]), the forward inclusion/backward elimination approach [16] was adapted: (1) a stricter inclusion criterion ($p = 0.01$ instead of 0.05, χ^2, 1 degree of freedom [df]) was applied; (2) major parts of the analysis were conducted separately per model parameter; i.e. only the typical pharmacokinetic parameter, its inter- and intra-individual variability (where applicable) and the covariate effects for the parameter of interest were estimated, all other parameters remained fixed to the base model values (for further detail on the covariate selection process, see Appendix Table 1 in the Electronic Supplementary Material). For the final step, the impact of the statistically significant covariates on the area under the plasma concentration–time curve (AUC) at steady state during one dosing interval ($AUC_{\tau,ss}$) with linagliptin 5 mg was investigated (using Berkeley Madonna software version 8.0.4; University of California, Berkeley, CA, USA) to evaluate the clinical relevance of the covariates.

2.3.3 Model Evaluation

Standard goodness-of-fit plots were performed to investigate the description of the plasma concentrations. Furthermore, the base model was evaluated by a visual predictive check and the final model by posterior predictive checks for the maximum and minimum plasma concentrations (C_{max} and C_{min}) of linagliptin. In the posterior predictive check, the inter-individual, intra-individual and residual variabilities were taken into account, but not the uncertainty in the parameter estimates.

2.4 Population Pharmacokinetic/Pharmacodynamic Analysis

2.4.1 Base Model Development

The initial plan was to simultaneously analyse linagliptin plasma concentrations and DPP-4 activity. However, this was not possible because of extensive run time (>5 days) of the population pharmacokinetic model. As linagliptin plasma concentrations and plasma DPP-4 activity were measured at the same time points, and because of the direct relationship between both measurements, with no hysteresis (data not shown), it was possible to correlate linagliptin plasma concentrations directly to DPP-4 activity without use of a pharmacokinetic model. To describe the correlation between linagliptin plasma concentrations and DPP-4 activity, a simple maximum effect (E_{max}) model and a sigmoid E_{max} model were tested. Inter-individual variability was investigated for all typical parameters.

2.4.2 Covariate Model Development

The covariates investigated on parameters with inter-individual variability were age, height, weight, body

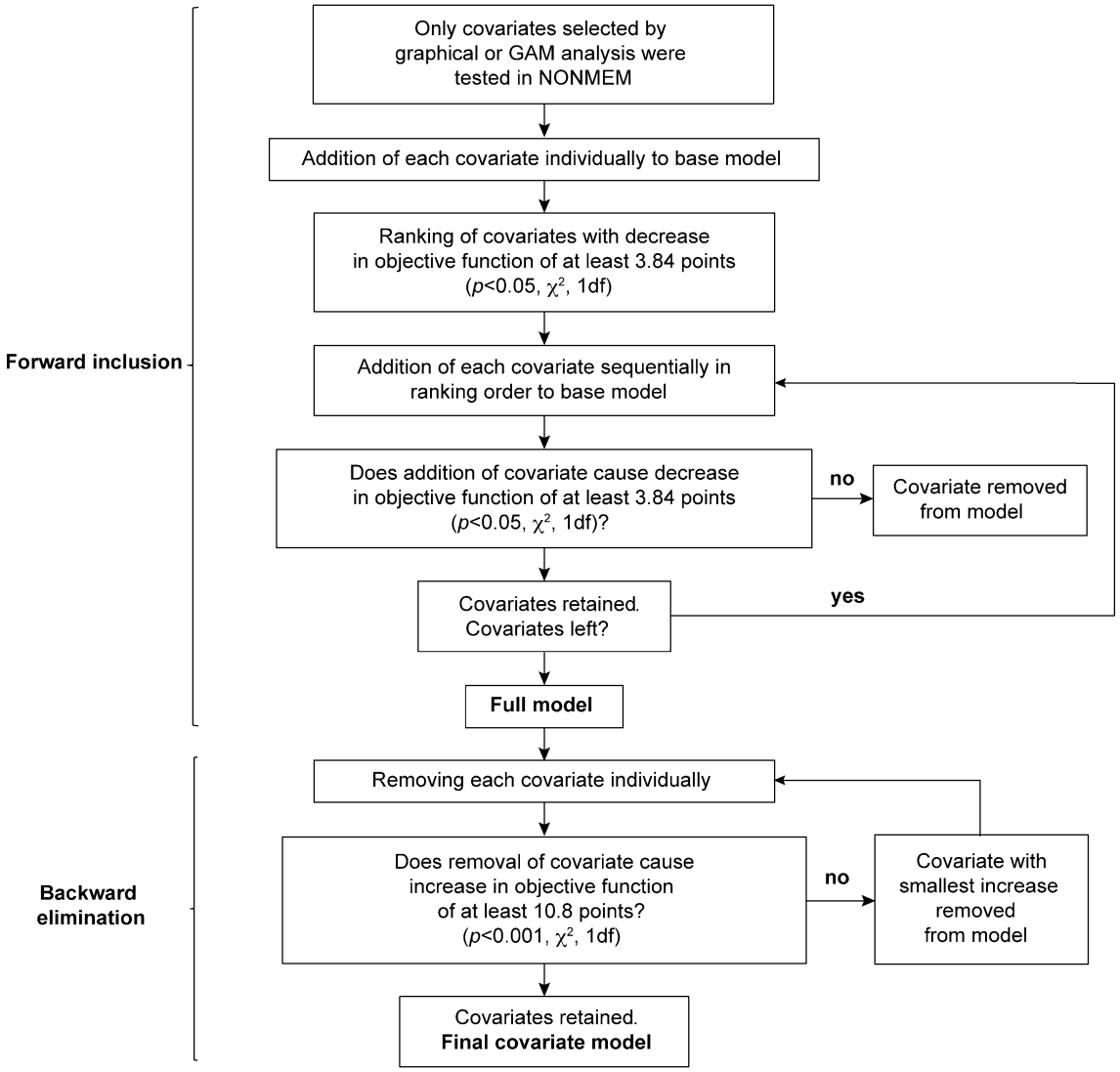

Fig. 2 Visual overview of the forward inclusion/backward elimination process. *df* degree of freedom, *GAM* generalized additive modelling

surface area, sex, metformin co-medication, dose, pre-dose DPP-4 activity, CL_{CR} and levels of ALT, AST, TG, GGT, C-reactive protein and alkaline phosphatase. First, a graphical analysis was performed, followed by a GAM analysis. Subsequently, the covariates suggested by both procedures were tested in NONMEM, using the forward inclusion/backward elimination procedure (Fig. 2). To further evaluate the impact of the covariates, their effect on the model parameter EC_{50} and on the concentration leading to 80 % inhibition ($EC_{80\ \%}$) of plasma DPP-4 was investigated. For continuous covariates, median and extreme covariate values (the 5th and 95th percentiles of the covariate distribution) were used; for categorical covariates, each category was evaluated. Appendix Table 2 in the Electronic Supplementary Material shows the covariates selected during the different steps of the analysis.

2.4.3 Model Evaluation

The final model was evaluated by a posterior predictive check, using the same approach that was used for the population pharmacokinetic model. The variables of interest were steady-state plasma DPP-4 inhibition 2 (±1) h and 24 (±4) h after linagliptin administration.

2.4.4 Pharmacokinetic and Pharmacodynamic Effects of a Missed Dose of Linagliptin

To determine the effect of a missed dose of linagliptin on linagliptin plasma concentrations and the degree of DPP-4 inhibition, simulations were performed using the final pharmacokinetic and pharmacokinetic/pharmacodynamic models. In the simulations, it was assumed that all patients who were included in the pharmacokinetic/

pharmacodynamic data set received 5 mg linagliptin once daily. After reaching steady state, the effect of a single missed dose at a certain time was simulated.

3 Results

3.1 Data Set Description

The final data set of the pharmacokinetic analysis included 6,907 measurements of plasma linagliptin concentrations from 462 patients with T2DM. In general, the plasma concentration–time profiles in the phase 2b studies showed higher variability than those in the phase 1 studies. In addition, the linagliptin plasma concentrations were apparently higher in study 4. The final data set of the population pharmacokinetic/pharmacodynamic analysis included 9,674 measurements of plasma DPP-4 activity and corresponding linagliptin plasma concentrations from 607 patients (receiving active treatment and placebo). Baseline demographic and laboratory data from the four included studies are shown in Table 3, as well as the numbers of patients from each trial included in the data set.

Table 3 Baseline demographic and laboratory information

	Pharmacokinetics ($n = 462$)	Pharmacokinetics/ pharmacodynamics ($n = 607$)
Number of patients in data set		
Study 1	35	47
Study 2	61	77
Study 3	170	216
Study 4	196	267
Male, *n* (%)	302 (65.4)	401 (66.1)
Ethnic origin, *n* (%)		
Caucasian	429 (92.9)	559 (92.1)
Black	8 (1.7)	15 (2.5)
Asian	7 (1.5)	11 (1.8)
Hispanic	18 (3.9)	22 (3.6)
Age, years	60 (30–78)	60 (30–78)
Weight, kg	89 (57–132)	89 (55–138)
Body mass index, kg/m^2	30.6 (20.4–42.2)	30.6 (20.4–42.2)
Fasting plasma glucose, mmol/L	9.9 (5.1–20.0)	9.9 (5.1–20.0)
Add-on to metformin		
No	266 (57.6)	340 (56.0)
Yes	196 (42.4)	267 (44.0)

The data are expressed as median (range [minimum–maximum]) unless specified otherwise

3.2 Population Pharmacokinetic Analysis

The linagliptin plasma concentrations in all included studies were adequately described by the initial model structure, which takes into account the binding of linagliptin to its target, DPP-4. The higher variability in the phase 2b studies was accounted for by different residual variability estimates dependent on the study type (ΔOBJF −2,257). The higher exposure in study 4 was best described by higher relative bioavailability in this study (ΔOBJF −65).

In addition to higher bioavailability estimated for the add-on to metformin study 4, the covariate analysis identified the following covariates as having a statistically significant impact on linagliptin pharmacokinetics (Table 4):

- The relative bioavailability was found to decrease with increasing weight (ΔOBJF 15).
- The rate of absorption was dependent on the dose (ΔOBJF 27) and study/formulation (ΔOBJF 34). The typical rate of absorption in study 1 (powder in the bottle formulation) was 0.933 1/h, compared with a lower rate with both tablet formulations (0.795 1/h [in study 2] and 0.441 1/h [in studies 3 and 4]).
- $B_{max,C}$, estimated by the model, which was likely to be a reflection of the plasma DPP-4 concentration, correlated with the pre-dose DPP-4 activity (ΔOBJF 68), dose (ΔOBJF 69) and age (ΔOBJF 14).

In comparison to the base model, the inter-individual variability decreased only for K_a (76.4 versus 87.6 %) and $B_{max,C}$ (15.0 versus 29.6 %) and was in the same range for bioavailability (F), CL and V_C, indicating that only a small part of the inter-individual variability was accounted for by the investigated covariates.

Apart from some slight model misspecification around the C_{max} value, the goodness-of-fit plots showed agreement between the observed plasma concentrations and the model predictions, indicating that the model performed adequately (see Appendix Fig. 1 in the Electronic Supplementary Material), as did the visual predictive checks for the base model (see Appendix Fig. 2 in the Electronic Supplementary Material). Posterior predictive checks showed that the final model provided an adequate description of the trough plasma concentration (C_{trough}) and C_{max} values (except for the C_{max} values of the lowest [0.5 mg] and highest [10 mg] dose groups, which were slightly outside the 90 % confidence interval, although showing only a small absolute difference; for further detail, see Appendix Fig. 3 in the Electronic Supplementary Material).

Table 4 Parameter estimates from final population pharmacokinetic model

Parameter	Value	Description
Typical parameter		
F (%)	100[a]	Typical relative bioavailability
F in study 4 (%)	169	Typical relative bioavailability in study 4
Weight_F[b]	−0.958	Percentage change per kg change from median weight of population
$K_{a,1}$ (1/h)	0.933	Typical absorption rate constant study 1 (powder in bottle formulation)
$K_{a,2}$ (1/h)	0.795	Typical absorption rate constant study 2 (tablet formulation 1)
$K_{a,3}$ (1/h)	0.441	Typical absorption rate constant studies 3/4 (tablet formulation 2)
Dose_K_a[c]	−6.51	Percentage change in K_a per dose unit change from the 5 mg dose group
V_C/F (L)	715	Typical central volume of distribution
Q_P/F (L/h)[d]	412	Typical inter-compartmental clearance between central compartment and peripheral compartment
V_P/F (L)[d]	1,650	Typical volume of distribution of the peripheral compartment
CL/F (L/h)	258	Typical clearance of the unbound concentration
GGT_CL[e,f]	−0.0339	Percentage change in CL/F per U/L change from the median GGT of the population
$B_{max,C}$ (nmol/L)	4.97	Typical concentration of binding sites in the central compartment (male)
DPP_$B_{max,C}$[g]	0.00332	Percentage change in $B_{max,C}$ per RFU change from the median DPP-4 activity of the population
Dose_$B_{max,C}$[g]	3.41	Percentage change in $B_{max,C}$ per dose unit change from the 5 mg dose group
Age_$B_{max,C}$[g]	0.561	Percentage change in $B_{max,C}$ per year from the median age of the population
Sex_$B_{max,C}$[e]	0.457	Absolute change in $B_{max,C}$ between males and females
K_d (nmol/L)[d]	0.0652	Typical affinity constant of the saturable binding
$A_{max,P}/F$ (nmol)[d]	1,650	Typical amount of binding partner in the peripheral compartment
Inter- and intra-individual variability		
ωF (CV %)	47.4	Inter-individual variability in relative bioavailability
Corr F_CL	−0.765	Correlation between ωF and ωCL
ωCL (CV %)	27.5	Inter-individual variability in clearance of the unbound concentration
ωK_a (CV %)	76.4	Inter-individual variability in the absorption rate constant
ωV_C (CV %)	24.4	Inter-individual variability in the central volume of distribution
ω$B_{max,C}$ (CV %)	15.0	Inter-individual variability in the concentration of the central binding partner
πF (CV %)	40.0	Intra-individual variability in relative bioavailability
Residual variability		
$\sigma_{prop,phase\ 2a}$ (%)[h]	13.6	Residual variability studies 1/2 (phase 1)
$\sigma_{prop,phase\ 2b}$ (%)[h]	38.3	Residual variability studies 3/4 (phase 2b)

CV coefficient of variation, *DPP-4* dipeptidyl peptidase-4, *RFU* relative fluorescence units

[a] Relative bioavailability fixed to 100 %

[b] $F_{i,o} = F \cdot (1 + \text{weight\_}F \cdot (\text{weight} - 88)) \cdot \exp(\eta F + \kappa F)$

[c] $K_{a,i} = K_a\ (1 + \text{dose\_}K_a \cdot (\text{dose} - 5)) \cdot \exp(\eta K_a)$

[d] Parameters not estimated, but fixed to estimates of the previous model

[e] During the backward elimination process, GGT on clearance and sex on the concentration of the binding partner in the central compartment ($B_{max,C}$) did not reach a statistically significant level. Nevertheless, these covariates were retained in the model, as the corresponding runs did not converge adequately and, therefore, could not be accepted as final models

[f] $CL_i = CL \cdot (1 + \text{GGT\_CL} \cdot (\text{GGT} - 33)) \cdot \exp(\eta \text{CL})$

[g] $B_{max,C,i} = (B_{max,C} + \text{sex\_}B_{max,C} \cdot \text{sex}) \cdot (1 + \text{DPP\_}B_{max,C} \cdot (\text{DPP} - 12{,}497)) \cdot (1 + \text{dose\_}B_{max,C} \cdot (\text{dose} - 5)) \cdot (1 + \text{age\_}B_{max,C} \cdot (\text{age} - 60)) \cdot \exp(\eta \cdot B_{max,C})$

[h] Coded as additive error for log transformed data

3.3 Impact of Significant Covariates on Exposure ($AUC_{\tau,ss}$)

The impact of the statistically significant covariates on the exposure to linagliptin ($AUC_{\tau,ss}$) after once-daily administration of linagliptin was <20 % for each covariate (Fig. 3). The impact of weight, age and sex, the covariates of most interest, was very small compared with the overall variability in the plasma concentration–time profiles (Fig. 4). Using the 5th and 95th percentiles of the covariate

distributions (numbers shown in parentheses), the data demonstrated that even a combination of covariate effects resulted in only a moderate impact on linagliptin exposure; i.e. the exposure changed by only +63 % or −26 %, respectively, for (a) an elderly (73-year-old) female patient of low weight (67 kg), with a high GGT level (158 U/L) and high pre-dose DPP-4 activity (18,623 relative fluorescence units [RFU]), on concomitant metformin therapy; or (b) a young (42-year-old), relatively heavy (117 kg) male patient, with a low GGT level (9.4 U/L) and low pre-dose DPP-4 activity (8,025 RFU).

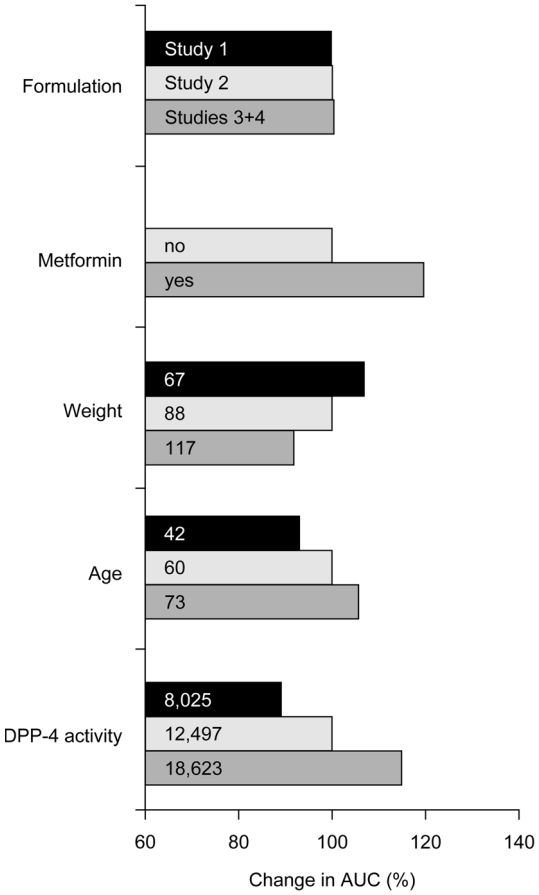

Fig. 3 Influence of statistically significant covariates (study/formulation, metformin co-medication, weight, age and baseline dipeptidyl peptidase [DPP]-4 activity) on the area under the plasma concentration–time curve (AUC) after administration of linagliptin 5 mg

3.4 Population Pharmacokinetic/Pharmacodynamic Analysis

DPP-4 activity correlated well with linagliptin plasma concentrations (Fig. 5). A sigmoid E_{max} model performed significantly better to describe this correlation than a simple E_{max} model (ΔOBJF −6,399).

The individual baseline DPP-4 activities and EC_{50} values were correlated, an observation that is physiologically plausible: the more DPP-4 molecules that are available, the higher the baseline DPP-4 activity is and the more linagliptin molecules are needed to reduce 50 % of the DPP-4 activity. Thus, the correlation between baseline DPP-4 activity and the concentration resulting in the half-maximum effect (EC_{50}) was implemented as follows:

$$EC_{50,i} = EC_{50} \cdot (1 + BSL\_EC_{50} \cdot (BSL_i - 11{,}600)) \cdot \exp(\eta EC_{50})$$

The individual EC_{50} parameter ($EC_{50,i}$) depends on the typical EC_{50} parameter, the typical covariate effect parameter BSL_EC_{50}, the difference between the individual predicted baseline estimate (BSL_i) and the median baseline value of 11,600 RFU, as well as the inter-individual variability of EC_{50} (ηEC_{50}). This implementation of the correlation was significantly superior to estimates of a correlation between BSL and EC_{50}, using the block option (ΔOBJF −76).

The final model took into account the covariate effects of GGT (ΔOBJF 108), ALT (ΔOBJF 25), FPG (ΔOBJF 250), TG (ΔOBJF 155), cholesterol (ΔOBJF 35) and sex

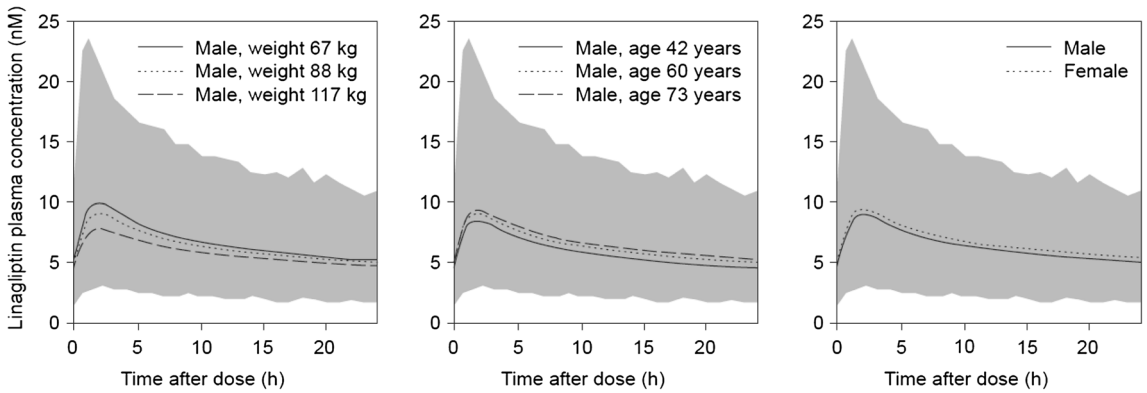

Fig. 4 Impact of weight, age and sex on linagliptin plasma concentration–time profiles after administration of linagliptin 5 mg. The overall variability was determined as the 90 % prediction interval of 1,000 simulated concentration–time profiles based on the base population pharmacokinetic model (*grey shaded area*)

(ΔOBJF 18) on the baseline DPP-4 activity, in addition to the effect of TG on the EC_{50} parameter (ΔOBJF 29) (Table 5). Compared with the base model, inter-individual variability for BSL and EC_{50} was reduced (16.9 versus 21.6 % and 15.4 versus 18.4 %, respectively). In addition, the residual variability of the final model was estimated to be smaller compared with the base model (14.8 versus 15.7 %, respectively). The goodness-of-fit plots indicated that the model provides an adequate description of the DPP-4 activity values (except for two extreme DPP-4 activity data points) (for further detail, see the Appendix and Appendix Fig. 4 in the Electronic Supplementary Material). The final model was evaluated by a posterior predictive check; the variables of interest were steady-state plasma DPP-4 inhibition at 2 and 24 h after linagliptin administration. The differences between the observed and predicted median DPP-4 inhibition were small (the maximum was a difference of 5 % in the 24 h DPP-4 inhibition in the 2.5 mg dose group) (see Appendix Table 3 in the Electronic Supplementary Material).

The statistically significant covariates were evaluated for their influence on the EC_{50} and $EC_{80\ \%}$ (Fig. 6). With use of the 5th and 95th percentiles of the covariate distributions (numbers shown in parentheses), the data demonstrated that the combination of covariate effects led to a maximum EC_{50} value of 4.13 nM and a minimum EC_{50} value of 2.49 nM ($EC_{80\ \%}$ maximum 7.38 nM and minimum 4.44 nM), respectively, for (a) female sex and high levels of GGT (124.8 U/L), ALT (75.9 U/L), FPG (13.4 mM), TG (422.1 mg/dL) and cholesterol (263.8 mg/dL); or (b) male sex and low levels of GGT (10.9 U/L), ALT (10.6 U/L), FPG (5.7 mM), TG (68.5 mg/dL) and cholesterol (98.6 mg/dL).

3.5 Pharmacokinetic and Pharmacodynamic Effects of a Missed Dose of Linagliptin

The simulations showed that when a linagliptin dose is missed, 48 h after the last dose, the median linagliptin plasma concentration is 4.21 nM (90 % prediction interval 2.65–6.86), compared with 5.58 nM (3.55–9.69) 24 h after dosing (Fig. 7a). The corresponding data for DPP-4 inhibition, following a missed dose, show that 48 h after the last linagliptin dose, enzyme inhibition remains at a median level of 69.6 % (27.0–87.1), compared with 81.7 % (47.5–90.5) 24 h after dosing. The simulations show that when a dose of linagliptin is taken after a missed dose, the linagliptin plasma concentration and the degree of DPP-4 inhibition are only slightly reduced after this dose compared with the dosing intervals before the missed dose, and the degree of DPP-4 inhibition remains above 80 % (median linagliptin C_{trough} 5.29 nM [3.38–8.82]; median trough DPP-4 inhibition 80.3 % [44.3–90.1]) (Fig. 7b).

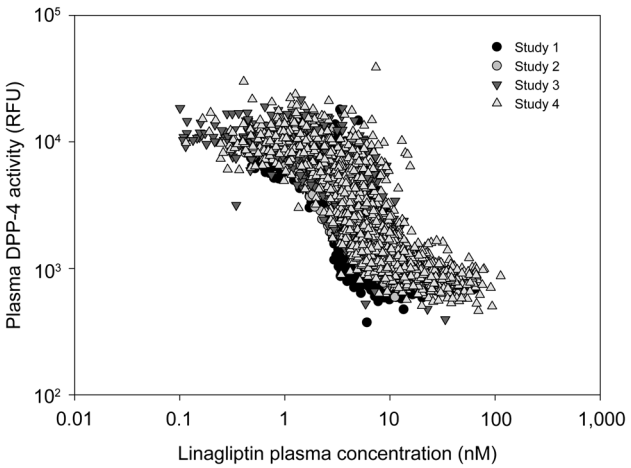

Fig. 5 Correlation of linagliptin plasma concentrations and plasma dipeptidyl peptidase (DPP)-4 activity in studies 1–4. Placebo and predose observations are not shown because of the logarithmic scale. *RFU* relative fluorescence units

4 Discussion

The current population analyses were performed to investigate the impact of clinically relevant covariates on the pharmacokinetics of linagliptin and their effects on DPP-4 inhibition. These analyses extend the findings of a previous population analysis, which showed that the non-linear pharmacokinetic profile of linagliptin is appropriately characterized by a target-mediated drug disposition model accounting for concentration-dependent binding of linagliptin to its target, DPP-4, in plasma and tissues [15]. Plasma DPP-4 activity was included in this model in a semi-mechanistic way by relating it to the model-calculated plasma DPP-4 occupancy with linagliptin. The description of DPP-4 inhibition by this semi-mechanistic occupancy model was comparable to a sigmoid E_{max} model, and thus the more mechanistic model was preferred. In the current analysis, however, the number of analysed data, together with the complexity of the pharmacokinetic model, led to extensively long run times of more than 5 days for the pharmacokinetic model alone. Therefore, pharmacokinetics and pharmacokinetics/pharmacodynamics were not analysed simultaneously, but two separate covariate analyses were performed, one for pharmacokinetics and one for pharmacokinetics/pharmacodynamics. For the population pharmacokinetic analysis, the target-mediated drug disposition model was used, and baseline DPP-4 activity was included as a covariate on the model parameter that reflects plasma DPP-4 concentrations (B_{max},C) (Fig. 3) to account for the close relationship between the pharmacokinetics of linagliptin and DPP-4 activity. In the population pharmacokinetic/pharmacodynamic analysis, linagliptin plasma concentrations were directly correlated with DPP-4 activity, using a descriptive sigmoid E_{max} model.

Table 5 Parameter estimates of the final population pharmacokinetic/pharmacodynamic model

Parameter	Value	RSE (%)	Description
Typical parameters			
BSL_{male} (RFU)	10,700	1.08	Typical baseline DPP-4 activity for males
BSL_{female} (RFU)[a]	11,565	20.5	Typical baseline DPP-4 activity for females
E_{max} (%)	92.4	0.12	Typical maximum decrease in DPP-4 activity
EC_{50} (nmol/L)	3.06	1.56	Typical linagliptin concentration that leads to half-maximum decrease in DPP-4 activity
HILL	3.22	1.82	Typical Hill coefficient
BSL_EC_{50}[c]	0.00792	7.98	Percentage change in EC_{50} per RFU change from median population baseline DPP-4 activity
GGT_BSL[b]	0.153	20.4	Percentage change in BSL per U/L change from median population baseline GGT, up to 175 U/L
GGT_BSL2[b] (%)	21.3	18.5	Percentage change in BSL if GGT > 175 U/L
ALT_BSL[b]	0.175	18.5	Percentage change in BSL per U/L change from population median baseline ALT
FPG_BSL[b]	1.46	12.3	Percentage change in BSL per mM change from population median baseline FPG
TRIG_BSL[b]	0.0294	13.9	Percentage change in BSL per mg/dL change from population median baseline triglyceride level
CHOL_BSL[b]	0.0261	43.7	Percentage change in BSL per mg/dL change from population median baseline cholesterol level
TRIG_EC50[c]	−0.0153	13.1	Percentage change in EC_{50} per mg/dL change from population median baseline triglyceride level
Inter-individual variability			
ωBSL (CV %)	16.9	7.61	Inter-individual variability in baseline DPP-4 activity
ωEC_{50} (CV %)	15.4	15.8	Inter-individual variability in EC_{50}
Residual variability			
σ_{prop} (%)	14.8	6.64	Residual variability

ALT alanine transaminase, *CV* coefficient of variation, *DPP-4* dipeptidyl peptidase-4, *FPG* fasting plasma glucose, *GGT* gamma-glutamyl transferase, *RFU* relative fluorescence units, *RSE* relative standard error

[a] Estimated as BSL_{male} + 865 RFU

[b] $BSL_i = BSL \cdot (1 + GGT\_BSL \cdot (GGT - 32.3)) \cdot (1 + ALT\_BSL \cdot (ALT - 28.8)) \cdot (1 + FPG\_BSL \cdot (FPG - 8.90)) \cdot (1 + TRIG\_BSL \cdot (TRIG - 160)) \cdot (1 + CHOL \cdot (CHOL - 183)) \cdot \exp(\eta BSL)$ if $GGT > 175$: $BSL_i = BSL \cdot (1 + GGT\_BSL2)) \cdot (1 + ALT\_BSL \cdot (ALT - 28.8)) \cdot (1 + FPG\_BSL \cdot (FPG - 8.90)) \cdot (1 + TRIG\_BSL \cdot (TRIG - 160)) \cdot (1 + CHOL \cdot (CHOL - 183)) \cdot \exp(\eta BSL)$

[c] $EC_{50,i} = EC_{50} \cdot (1 + BSL\_EC_{50} \cdot (BSL_i - 11{,}600)) \cdot (1 + TRIG\_EC_{50} \cdot (TRIG - 160)) \cdot \exp(\eta EC_{50})$

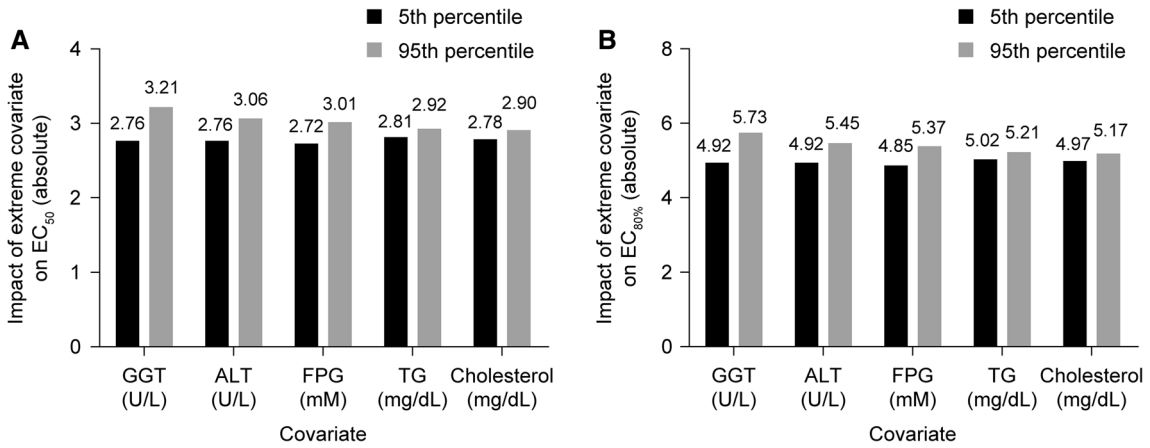

Fig. 6 Impact of statistically significant covariates on **a** half-maximum effect (EC_{50}) values and **b** concentration leading to 80 % inhibition ($EC_{80\ \%}$) values. 5th and 95th percentiles of laboratory values: gamma-glutamyl transferase (GGT) 10.9 and 124.8 U/L; alanine transaminase (ALT) 10.6 and 75.9 U/L; fasting plasma glucose (FPG) 5.7 and 13.4 mM; triglycerides (TG) 68.5 and 422.1 mg/dL; cholesterol 98.6 and 263.8 mg/dL

4.1 Population Pharmacokinetic Study

The previously developed target-mediated drug disposition model was found to also adequately describe the linagliptin plasma concentrations obtained in the two phase 2b studies, which were not included in the previous analysis. Some minor model misspecifications occurred during absorption. This may have been due to the fact that a first-order process

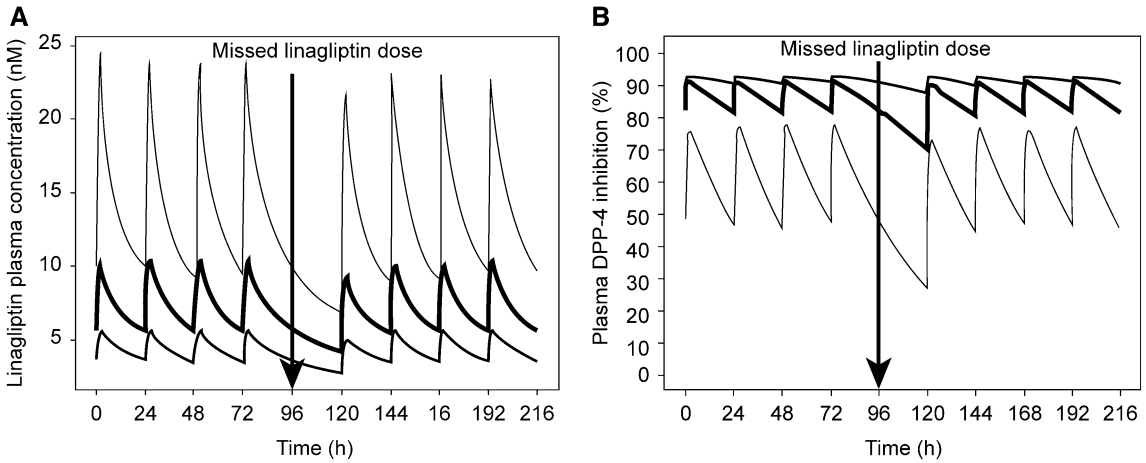

Fig. 7 **a** Steady-state pharmacokinetic–time profile after administration of linagliptin 5 mg. The plot shows four regular linagliptin dosings (time 0–72 h), followed by a missed dose at 96 h and four regular dosings at times between 120 and 216 h. **b** Steady-state pharmacodynamic–time profile after administration of four doses of linagliptin 5 mg (time 0–72 h), followed by a missed dose at 96 h and regular dosing at times between 120 and 216 h. The median profile is shown as the *bold line*, with the *lighter lines* either side indicating the 90 % prediction interval. *DPP-4* dipeptidyl peptidase-4

had not completely characterized the absorption of linagliptin, or it may have been a result of a factor that was not investigated during the covariate analysis. For most patients, only two measurements in the absorption phase per visit were available; thus, the pharmacokinetic model is more suitable for characterization of the overall exposure to linagliptin rather than its absorption.

The impact of the investigated covariates, including age, weight and sex, on the overall linagliptin exposure (AUC) was considered to be minor (all <20 %). These effects are well within the commonly used acceptance criteria for bioequivalence [17] and are therefore not considered to be clinically relevant.

The covariate analysis showed only a minor effect of age: for example, linagliptin exposure was increased by only 13.8 % in a 73-year-old patient compared with exposure in a 42-year-old patient (73 and 42 years were the 95th and 5th percentile of the age distribution in the current analysis). The minor impact of age on exposure to linagliptin is consistent with the known pharmacokinetic properties of linagliptin. Although age-dependent changes in distribution, metabolism and renal elimination have been reported for many drugs, these changes are not expected to have a clinically important effect on linagliptin, which is only slightly lipophilic; therefore, age-related changes in body composition are not likely to affect its pharmacokinetic characteristics [5, 7]. Weight showed only a small impact on linagliptin exposure (5.9 % increase and 8.7 % decrease at the 5th and 95th percentiles, respectively) and was found to be a covariate on the relative bioavailability of linagliptin (bioavailability decreased linearly by 0.96 % for every 1 kg increase in weight). However, because the data that were used were gathered following oral administration of linagliptin, this finding may have been due to differences in apparent clearance and/or volumes of distribution among obese individuals. A small sex-related difference in the pharmacokinetic profile of linagliptin was initially observed, which was no longer statistically significant in co-estimation of all covariate effects in the backward elimination.

In the add-on to metformin study (study 4), the exposure was around 20 % higher than that observed in the studies of linagliptin monotherapy (studies 1–3). A previous study of the drug–drug interaction of linagliptin and metformin showed a similar increase in linagliptin exposure when it was administered with metformin, with no significant change in the C_{max} or terminal half-life of linagliptin; therefore, these changes were not considered to be relevant [18]. Other covariates that underwent exploratory investigation, such as CL_{CR} and liver enzyme levels, showed no effect, or only a minor impact, on linagliptin exposure. These findings suggest that neither liver nor kidney impairment alter the pharmacokinetic profile of linagliptin; however, only patients with normal liver function and normal renal function or mild renal impairment were included, so conclusions about the effects of renal or liver impairment on the pharmacokinetic profile of linagliptin cannot be made from the current analyses. Meanwhile, dedicated studies have shown that neither renal impairment nor liver impairment affect the pharmacokinetic characteristics of linagliptin [19, 20].

Even when all covariates that statistically influenced the evaluated pharmacokinetic parameters were combined, in a worst-case scenario, this was not considered to have a clinically relevant effect on the safety and efficacy of linagliptin. With regard to the largest decrease in exposure

(−26 %) for the 5 mg dose, this would still be regarded as effective therapy, as the linagliptin exposure is within the range of the 2.5 mg dose group. The largest increase in exposure (+63 %) for the 5 mg dose can still be regarded as having acceptable safety on the basis of the currently available safety data, including a single rising-dose study of healthy volunteers, where a single dose of linagliptin was shown to be well tolerated up to a dose of 600 mg [5]. These findings indicate that the evaluated covariates do not affect the pharmacokinetic characteristics of linagliptin in a clinically important manner and that no dose adjustment is needed on the basis of age, sex or weight.

As a result of extensive run times and high η-shrinkage, the covariate selection process [16] was adapted (see the 'Covariate Model Development' section for details). In order to ensure that no covariate with a major impact on pharmacokinetics was missed, several covariates were predefined on the basis of physiological considerations to be tested in the forward inclusion/backward elimination approach; these included age, sex and weight, as well as levels of liver enzymes (ALT and GGT) and CL_{CR}. Moreover, graphical analysis of the correlation between the measured linagliptin concentrations and the investigated covariates for patients receiving linagliptin 5 mg (data not shown) confirmed the results of the model-based covariate analysis, demonstrating that none of the tested covariates had a major impact on the C_{trough} or C_{max} values.

4.2 Population Pharmacokinetic/Pharmacodynamic Study

The relationship between linagliptin plasma concentrations and plasma DPP-4 activity was best described by a sigmoid E_{max} model with typical EC_{50} values of 2.84 nM for males and 3.05 nM for females, and a typical Hill coefficient of 3.22. A correlation between EC_{50} and baseline DPP-4 activity was accounted for in the model. Thus, covariates affecting baseline DPP-4 activity also affect the EC_{50} value. The rationale for this is based on the premise that a higher DPP-4 plasma concentration, represented by higher DPP-4 baseline activity, necessitates a higher linagliptin concentration for half-maximum DPP-4 inhibition to be reached.

Individually, the investigated covariates, including age, sex and weight, had only a small impact on the baseline DPP-4 activity, the EC_{50} and the $EC_{80\ \%}$. Age had no impact on DPP-4 activity, an observation that is consistent with previous findings [21], although some studies have shown a correlation [22, 23], suggesting that the impact of age on DPP-4 activity, if it exists, is minor. Neither weight nor body mass index were shown to affect DPP-4 activity, which is in accordance with the findings of previous research [22, 24]. Baseline DPP-4 activity, and thus the EC_{50}, was slightly higher in females than in males in the present analysis (11,565 versus 10,700 RFU). In contrast, previous studies have suggested slightly lower DPP-4 activity in females than in males [24], or no influence of sex on DPP-4 activity [21]. Baseline DPP-4 activity was correlated with the levels of the liver enzymes GGT, ALT and AST, a finding that is consistent with previous observations [24]. Baseline DPP-4 activity was also correlated with FPG levels, a finding that is consistent with the knowledge that DPP-4 inhibition is linked to glucose-lowering efficacy [25] and is correlated with HbA_{1c} and FPG levels in patients with T2DM [22, 23].

Even the combined influence of all significant covariates, in a worst-case scenario, only changed the EC_{50} from a minimum of 2.49 nM to a maximum of 4.13 nM, and changed the $EC_{80\ \%}$ from a minimum of 4.44 nM to a maximum of 7.38 nM. In view of this finding, and the high variability in the DPP-4 inhibition/HbA_{1c} relationship, the investigated covariates, including weight, sex and age, are not considered to be clinically important. Moreover, a scenario leading to higher baseline DPP-4 activity, and thus higher EC_{50} or $EC_{80\ \%}$ values, would lead to an increase in linagliptin concentrations, since baseline DPP-4 activity was shown to be one of the main factors influencing linagliptin exposure.

The populations that were evaluated contained mostly Caucasian patients, with insufficient numbers of patients of black or Asian ethnicity to be included in the analyses. However, recent studies of linagliptin in Asian (Japanese) [26] and black subjects [27] reported that it exhibited pharmacokinetic/pharmacodynamic profiles similar to those observed in Caucasian subjects.

4.3 Simulation

The simulations of steady-state pharmacokinetic and pharmacodynamic–time profiles after administration of linagliptin showed that 24 h after a dose of linagliptin is missed, the median DPP-4 inhibition remains at about 70 %, suggesting that most patients will continue to experience glucose-lowering efficacy after a missed dose of linagliptin 5 mg. This pharmacological attribute may translate into clinical benefits for patients with poor adherence who might sometimes miss a dose, suggesting that this would have a minimal impact on the long-term efficacy of therapy. However, this has not been confirmed in longer-term prospective clinical studies in patients with T2DM.

5 Conclusion

The findings of these analyses, reporting previously unpublished data on the pharmacokinetic and

pharmacodynamic profile of linagliptin, show that factors including age, sex and weight do not significantly alter the pharmacokinetics and DPP-4 inhibitory activity of linagliptin. These findings are consistent with clinical data on the analysed covariates, which have shown that factors such as weight, sex, race and age do not significantly alter the efficacy and safety of linagliptin [28–31]. These results also indicate that there is no need for linagliptin dose adjustment on the basis of age, sex or weight, and they extend the findings of previous research showing that linagliptin does not require dose adjustment in patients with renal or hepatic impairment [19, 20].

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Conflicts of interest At the time of the analysis performed in this study, S. R. was a Ph.D. student at the University of Bonn and received a grant from Boehringer Ingelheim; she is now an employee of Boehringer Ingelheim. U. G. M., S. P. and A. S. are all employees of Boehringer Ingelheim. V. D. was an employee of Boehringer Ingelheim at the time of the analysis; he is now an employee of Novartis. C. F. was an employee of Boehringer Ingelheim while the study took place; he is now an employee of Bayer Healthcare. U. J. has received research Grants from Boehringer Ingelheim (between 2006 and 2011) and received a research Grant from Boehringer Ingelheim for the present study.

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