Population Pharmacokinetic/Pharmacodynamic Analysis of the DPP-4 Inhibitor Linagliptin in Japanese Patients with Type 2 Diabetes Mellitus

Yusuke Tadayasu[1,3], Akiko Sarashina[1], Yasuhiro Tsuda[1], Shinji Tatami[1], Christian Friedrich[2], Silke Retlich[2], Alexander Staab[2], Mikihisa Takano[4]

[1] Clinical PK/PD, Translational medicine, Nippon Boehringer Ingelheim Co., Ltd, Kobe, Hyogo, Japan. [2] Translational medicine, Boehringer Ingelheim GmbH & Co. KG, Biberach, Germany. [3] Graduate School of Biomedical Sciences, Hiroshima University, Hiroshima, Japan. [4] Graduate School of Biomedical & Health Sciences, Hiroshima University, Hiroshima, Japan.

ABSTRACT - [Objectives] Linagliptin is a novel, highly selective and long acting DPP-4 inhibitor for the treatment of type 2 diabetes mellitus (T2DM). Linagliptin exhibits non-linear pharmacokinetics (PK) due to saturable binding to plasma and tissue DPP-4. The aim of this study was to characterize the PK and PK/DPP-4 inhibition relationship of linagliptin in Japanese patients with T2DM using a population PK/DPP-4 model and to support the rationale for the therapeutic dose in Japanese patients by simulation. [Methods] Linagliptin plasma concentration and DPP-4 inhibition measurements from a placebo-controlled, parallel group multiple (28 days) dose trial that included 36 T2DM patients (18 patients each in 2.5 mg and 10 mg dose group) were used for analysis. Modeling was performed using FOCE INTERACTION estimation method implemented in NONMEM V. The linagliptin plasma concentration- and DPP-4 inhibition- time profiles were simulated for Japanese patients receiving 5 mg linagliptin once daily by the model established. [Results] Nonlinear PK of linagliptin in T2DM patients were well described by a 2-compartment model assuming concentration-dependent binding to DPP-4 in the central and peripheral compartment. Plasma DPP-4 inhibition was integrated in the model by relating the model-predicted DPP-4 occupancy with linagliptin linearly to DPP-4 inhibition. The simulation predicted that for the 5 mg dose group the trough DPP-4 inhibition at steady-state was 84.2%, which is higher than the target inhibition (≥80%) for an effective dose of DPP-4 inhibitor. In 2.5 mg dose group, steady-state DPP-4 inhibition of >80% was not maintained over 24 hours (observed and simulated). [Conclusions] The nonlinear PK of linagliptin and its plasma DPP-4 inhibition in patients were well characterized by a target-mediated drug disposition model relating DPP-4 occupancy with linagliptin to DPP-4 inhibition. Simulations of plasma DPP-4 inhibition suggest that 5 mg linagliptin once daily is an appropriate therapeutic dose for Japanese patients with T2DM.

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INTRODUCTION

Linagliptin is a dipeptidyl peptidase (DPP) -4 inhibitor which is licensed in the US, Europe, and Japan for the indication of long term improvement of glycaemic control in patients with type 2 diabetes mellitus (T2DM). The inhibition of DPP-4 leads to an increase of the incretins glucagon-like peptide 1 (GLP-1) and gastric inhibitory polypeptide (GIP), which are substrates of DPP-4. Incretins are secreted in the intestine in response to food intake. Active GLP-1 and GIP act by enhancing insulin secretion [1] and in the case of GLP-1 also by inhibiting glucagon secretion in a glucose dependent way [2]. Both mechanisms lead to lower plasma glucose levels. Moreover, the incretins have protective effects on pancreatic ß-cells by enhancing their proliferation and increasing their resistance to apoptosis in animals [3]. Other beneficial aspects of GLP-1 are the slowing of gastric emptying and the induction of satiety, supporting dietary goals in diabetes treatment. As DPP-4 inhibitors prevent the degradation of incretins by DPP-4, they can elevate the beneficial effects of these regulatory peptides for type 2 diabetic patients. Decreased incretin levels have in fact been reported for diabetic patients [4].

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Corresponding Author: Yusuke Tadayasu; Nippon Boehringer Ingelheim Co., Ltd. 6-7-5, Minatojima-Minamimachi; Chuo-ku, Kobe, Hyogo; Japan. E-mail: yusuke.tadayasu@boehringer-ingelheim.com.

DPP-4 inhibitors have been shown to alleviate this deficiency and reduce glucose and HbA1c successfully.

In clinical trial in non-Japanese patients [5], linagliptin exhibits nonlinear pharmacokinetics in the dose range of 1 to 10 mg once daily. The nonlinearity is characterized by a less than dose proportional increase in maximum plasma concentrations (C_{max}) and area under the curve (AUC) [5]. Apparent clearance and apparent volume of distribution increase with increasing dose [5]. Linagliptin exhibited a long-terminal half-life (> 100 hours at steady state after multiple oral doses of 5 mg) but the accumulation half-life is considered to be short (approximately 10 hours with 5 mg dosing), based on a low accumulation ratio (1.33 for AUC and C_{max}) [5]. Urinary excretion was low (<7% of dose) with 5 mg dosing. The urinary excretion of linagliptin increased with increasing dose [5].

Linagliptin treatment resulted in a rapid, potent and long-lasting inhibition of plasma DPP-4 in clinical trials, and linagliptin plasma concentrations correlated well with DPP-4 activity measured in plasma, without hysteresis. DPP-4 was effectively inhibited as shown by maximum DPP-4 inhibitions of 72.7 and 86.1% after a single dose of linagliptin of 2.5 and 5 mg, respectively, and >95% for doses of ≥25 mg [6]. At steady-state, inhibition ≥ 80% of plasma DPP-4 activity for at least 24 hours post dose was achieved with 5 mg and 10 mg linagliptin once daily dosing [5]. Generally, DPP-4 inhibition ≥80% over 24 h is thought to be therapeutically necessary, as this was shown to be related to maximum effects in terms of incretin response and glucose reduction [7, 8].

An *in vitro* protein binding study performed with radiolabelled linagliptin demonstrated that the bound fraction of linagliptin decreased from 99% at concentrations of ≤1 nmol/L to 70 to 80% at concentrations >100 nmol/L [9]. The binding of linagliptin to plasma protein was associated with very high affinity constants (in the order of 10^{10} L/mol). In contrast, protein binding was not concentration-dependent in plasma from DPP-4 deficient or knock-out animals, with approximately 70% of linagliptin bound to plasma protein [9]. In line with these observations, the DPP-4 deficient or knock-out animals showed approximately linear pharmacokinetics of linagliptin in the relevant dose range [10]. Based on these findings, the nonlinear pharmacokinetics of linagliptin can be explained predominantly by a high affinity, low capacity binding to its target enzyme DPP-4, which is readily saturated at therapeutically relevant concentration.

The target-mediated drug disposition has also been described for angiotensin I converting enzyme inhibitors such as trandolaprilat, which shows similar pharmacokinetic characteristics as linagliptin [11].

The approved dose of linagliptin is 5 mg once daily in US, Europe, and Japan. In a Phase III trial in Japanese with T2DM [12, 13], 5 and 10 mg once daily were tested. However, in the Phase II trial in Japanese patients with T2DM [14], linagliptin doses of 0.5, 2.5, 10 mg once daily, but not 5 mg, were administered. From the perspective of the practical efficiency, these 3 doses were selected to investigate the dose response in the wider range than in the precedent Caucasian Phase II trial where the doses from 2.5 mg to 10 mg were tested [5]. The first purpose of this analysis was to characterize the PK and PK/DPP-4 inhibition relationship of linagliptin in Japanese patients with T2DM using a population pharmacokinetic/pharmacodynamic (PK/PD) model. The second purpose of the model development was to simulate the plasma DPP-4 inhibition-time course for Japanese patients receiving 5 mg linagliptin once daily to support the linagliptin dose selection in Japanese patients with T2DM.

METHODS

Patient population

In a Phase II trial in Japanese patients with T2DM [14], randomized, double-blind, placebo-controlled multiple dose study, 72 Japanese patients with T2DM were assigned to receive oral doses of linagliptin 0.5, 2.5, or 10 mg or placebo (19 patients, 18 patients, 18 patients, or 17 patients) once daily for 28 days. The inclusion criteria of HbA1c were ≤8.5% for patients treated with ≤1 oral hypoglycemic agents or ≤8.0% for patients treated with 2 oral hypoglycemic agents. 5 clinical sites were involved in this study. In this analysis, all data were supposed to be included in the analysis. However, the data in 0.5 mg dose group were not fitted the assumption of quasi-equilibrium (for details refer to discussion). Therefore, the data from 2.5 mg dose group and 10 mg dose group were included but the data from 0.5 mg dose group were not included in the analysis. The majority of patients were males; only 9 female patients participated in this study. In the 2.5 mg and 10 mg dose group, the mean (±SD) fasting plasma glucose levels at baseline was 154.7±25.1 mg/dL and 158.4±28.6 mg/dL; the mean (±SD) HbA1c level at screening was 7.1±0.5% and 7.2±0.9%; the mean (range) age of patients was 60.2 (42-68) years and 59.1 (40-69)

years, the mean (range) body mass indices were 26.0 (19.7-33.3) kg/m^2 and 23.8 (18.4-34.4) kg/m^2, respectively. Patients were excluded if they have a relevant history of hepatic, renal, neurologic, cardiovascular, gastrointestinal, metabolic, or hormonal disorders; hyperlipidemia; or hypertension. Individual antidiabetic treatment was discontinued 14 days prior to the first drug administration.

In the result, the statistically significant changes of fasting plasma glucose from baseline of 13.6 (p<0.05) and 25.0 (p<0.01) mg/dL were observed for the 2.5, and 10 mg dose groups, respectively, whereas fasting plasma glucose level in the placebo group remained almost unchanged relative to baseline level (3.2 mg/dL).

Study design
This trial was a randomized, placebo-controlled double-blind multiple-dose study with a parallel group design. Patients received either placebo or 0.5, 2.5, or 10 mg of linagliptin as a tablet once daily for 28 days after an overnight fasting. Plasma samples were collected after the first and the last administration of linagliptin, and one trough sample was collected on day 14 (Table 1) for the linagliptin plasma concentration and DPP-4 inhibition. As linagliptin has a very long terminal half-life, plasma samples for concentration and for DPP-4 inhibition were collected until 15 days after the last administration of linagliptin.

The study protocol was reviewed and approved by the ethics committees or institutional review board at each study center. The study was conducted in compliance with the ethical standards established by the Declaration of Helsinki at the time the study was initiated [15] and in accordance with the International Conference on Harmonisation: Harmonised Tripartite Guideline for Good Clinical Practice and the Japanese Good Clinical Practice regulations [16].

Analytical assays
Linagliptin
Total (bound plus unbound) linagliptin plasma concentrations were determined by a validated high performance liquid chromatography coupled to tandem mass spectrometry (HPLC-MS/MS) at the Department of Bioanalytical Services, Covance Laboratories Ltd., Harrogate, UK as previously described [14]. In brief, plasma was obtained from EDTA blood and measured, undiluted, using [$^{13}C_3$] linglipitin as an internal standard. In this study the linear range of concentrations was 0.100 nmol/L to 100 nmol/L.

DPP-4 inhibition
DPP-4 activity was analysed by using a semi-quantitative enzyme activity assay with fluorescence detection at the Institut für Klinische Forschung und Entwicklung GmbH, Mainz, Germany as previously described [14]. In brief, alanine-proline-7-amido-4-trifluoro-methyl-coumarin was added to plasma samples, and DPP-4-induced cleavage of the substrate to the fluorescent product 7-amino-4-trifluoro-methylcoumarin was then measured as relative fluorescence units (RFU). DPP-4 inhibition was calculated as follows:

DPP-4 inhibition [%] = 100-(DPP-4 activity/DPP-4 activity at baseline)*100.

Population Pharmacokinetics and Pharmacodynamics analysis
For nonlinear mixed effects modeling NONMEM, version V level 1.1, NM-TRAN version III level 1.1, and PREDPP version IV level 1.1 (ICON Development solution, USA) [17] is the standard software. The first-order conditional estimation with interaction method (FOCE-INTERACTION) and the ADVAN 6 (general nonlinear kinetics) subroutine were used to estimate the typical population parameters, random inter-individual variability (IIV) on these parameters, and residual variability between observed and individually predicted plasma concentrations or DPP-4 inhibitions. Graphical visualization software (e.g., SAS 9.2 (SAS institute Inc., USA), Sigmaplot version 10.0 (Systat Software Inc., USA)) was used in addition to Xpose v4 http://xpose.sourceforge.net

Table 1. Plasma sampling points

Day	Time
1	Before and 0:30h, 1h, 1:30h, 2h, 3h, 4h, 6h, 8h, and 12h after the first administration
2, 14	Before linagliptin administration
28 to 43	Six h before the last administration , before, 0:30h, 1h, 1:30h, 2h, 3h, 4h, 6h, 8h, 12h, 24h, 48h, 120h, 168h, 240h, 312h, and 360h after the last administration

Throughout model development, log-transformed linagliptin concentration data were used. Drug concentrations below the limit of quantification were not implemented into the data set except when measured during the lag time, in which case they were implemented and set to zero.

Model selection was guided by the objective function value (OBJF), the graphical goodness-of-fit analysis, the precision of parameter estimates as reported by the relative standard error obtained from NONMEM. For nested models differing in 1 single parameter, a drop of at least 3.84 in the OBJF (corresponding to p < 0.05 for 1-parameter difference, assuming χ^2 distribution) was regarded as significant.

Pharmacokinetic model

As mentioned above, the nonlinear pharmacokinetics of linagliptin can be explained predominantly by a high affinity, low capacity binding to its target enzyme DPP-4, which is readily saturated at therapeutically relevant concentration. Therefore, in a previous analysis in non-Japanese patients with T2DM, this non-linear pharmacokinetics of linagliptin was described using a 2-compartment model with concentration-dependent protein binding in the central and the peripheral compartment (Figure 1) [18].

In this clinical Phase II trial in Japanese patients [14], the pharmacokinetic characteristics of linagliptin (i.e., nonlinear pharmacokinetics, a long terminal half-life, which is not dominant, and low urinary excretion) and the correlation between linagliptin plasma concentration and DPP-4 inhibition were consistent with those of the previous trials in non-Japanese patients [19].

Thus, the previous model structure developed for Caucasian patients accounting for the target-mediated drug disposition was used as starting point for the current analysis of the PK in Japanese patients. IIV was modeled using exponential random effect models, i.e. individual parameters were assumed to have a log-normal distribution around the typical parameter estimates (THETA, θ) and variance (OMEGA, ω^2). The residual variability was modeled using an additive residual variability model for the log-transformed linagliptin plasma concentrations which approximately corresponds to a proportional error model using untransformed data.

Pharmacokinetic/Pharmacodynamic model

DPP-4 inhibition was well correlated with total linagliptin plasma concentration by a sigmoid E_{max} model [14]. Therefore, a sigmoid E_{max} model using total linagliptin concentration was tested in this population analysis as well. In addition, a semi-mechanistic model was investigated relating the DPP-4 inhibition to the model calculated DPP-4 occupancy (i.e., percentage of DPP-4 molecules bound/inhibited by linagliptin). This model has already been used to describe the relationship between linagliptin pharmacokinetics and DPP-4 inhibition as previously described [18]. It is based on Clark's occupancy theory which states that occupancy is equal to the effect divided by the maximum effect. Thus, the model assumes a linear relationship between plasma DPP-4 inhibition and plasma DPP-4 occupancy with linagliptin. The more linagliptin is bound to DPP-4, the more DPP-4 activity is inhibited until all DPP-4 molecules are blocked by linagliptin and the maximal effect is reached. This is described by the following equation:
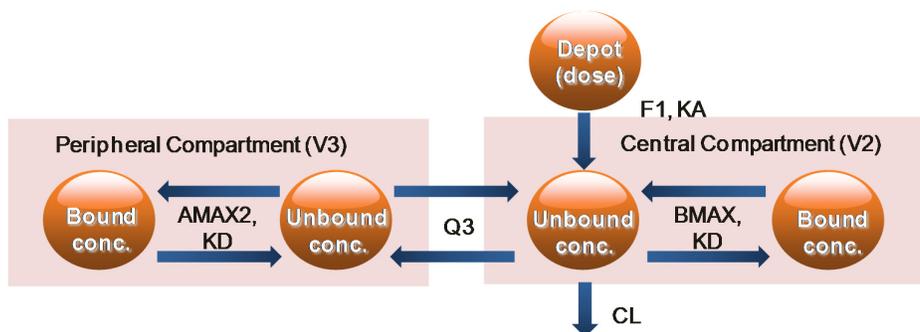
Figure 1. Structure of population PK model
F1: bioavailability, BMAX: concentration of binding sites in the central compartment, Q3: intercompartmental clearance between central and peripheral compartment, AMAX2: amount of binding sites in the peripheral compartment, KA: absorption rate constant, KD: affinity constant, V2: central volume of distribution, V3: peripheral volume of distribution, CL: clearance.

$$\text{DPP-4 inhibition} = E_{max} * \frac{C_b}{BMAX}$$

E_{max}: maximal effect
C_b/BMAX: percentage of DPP-4 molecules bound/inhibited by linagliptin calculated by PK model

IIV was modeled with an exponential random effect model, i.e., individual parameters were assumed to have log-normal distribution around the typical parameters estimates (THETA, θ) and variance (OMEGA, ω^2). DPP-4 inhibition data were untransformed and the residual variability was considered to be lower when DPP-4 inhibition was high than when DPP-4 inhibition was low. Therefore, the residual variability model was described using the following equation:

$$Y = \hat{Y} + (100 - \hat{Y}) * \varepsilon$$

where Y represents the observation (i.e. DPP-4 inhibition), $\hat{Y}$ represents the individual predicted concentration and ε are symmetrically distributed, zero-mean random variables with variance (σ^2).

Model evaluation
For the evaluation of the final population PK/PD model, a visual predictive check (VPC) [20, 21] was performed. For pharmacokinetics, the concentration-time profiles were simulated with 1000 subjects per dose group using the final model parameters. The median and the 5th and 95th percentiles of the simulated concentrations were calculated per dose group, and the observed concentrations were overlaid. The model describes the data sufficiently if most of the observed data are within the 5th to 95th percentiles interval and equally distributed around the median. The pharmacodynamic part of the population PK/PD model was evaluated accordingly.

Simulation
The objective of the simulation was to predict the DPP-4 inhibition-time profile of the following dose groups, 2.5 mg, 5 mg, and 10 mg linagliptin once daily, in Japanese patients with T2DM. The final PK/PD model was used for this simulation. The DPP-4 inhibition-time profiles at steady state were simulated 1000 times with 200 patients per dose group (2.5, 5, 10 mg once daily administration) using the final PK/PD model. The median profiles and 90% prediction intervals were calculated for the 1000 medians at each timepoints, which were calculated for each dose group in each simulated trial.

RESULTS

In the 2.5 mg and 10 mg dose group, 498 and 520 plasma concentrations were available for Pharmacokinetic evaluations, 520 and 538 DPP-4 inhibition observations for pharmacodynamic evaluation, respectively (Table 2).

Model development
Pharmacokinetic model
In a previous analysis in non-Japanese patients [18], the pharmacokinetics of linagliptin was described using a 2-compartment model with concentration-dependent protein binding in the central and the peripheral compartment. This structural model was applied to this population analysis of linagliptin in Japanese patients with T2DM.

Table 2. Number of data for PK/PD analysis

Linagliptin [mg]	Number of patients	Plasma concentrations			DPP-4 inhibition		
		Total sample [a]	Excluded [b]	Used for analysis	Total sample	Excluded [b]	Used for analysis
0.5	19	533	4	529	552	4	548
2.5	18	501	3	498	523	3	520
10	18	522	2	520	540	2	538
Total	55	1556	9	1547	1615	9	1606

a) BLQ samples not included in the table. 4 BLQ (1 patient in the 2. 5 mg dose group, >168 h after the last administration),

b) excluded due to the protocol violation (e.g., medication error, or incorrect plasma sampling).

Initially, all three available doses of linagliptin in Japanese patients, i.e. 0.5, 2.5 and 10 mg linagliptin were used for model building. However, applying the structural model of the previously developed target-mediated drug disposition model resulted in dose-dependent estimates for F1, V2/F1, BMAX. This may be an indication that the assumption of quasi-equilibrium cannot be applied for the 0.5 mg dose group (for details refer to discussion part). As the main aim of the analysis was to predict DPP-4 inhibition in the 5 mg linagliptin dose, a model with dose-dependent parameter estimates was inappropriate. When the data of the 0.5 mg dose group were excluded, the parameters of the target-mediated drug disposition model were well estimated without dose-dependency. To avoid dose dependence of model parameters, and as the main interest was the dose range between 2.5 and 10 mg, the 0.5 mg dose group data were excluded for further model development.

IIV was implemented on F1, KA, CL/F1 and BMAX. The IIV was estimated to be low to high (14.2 to 73.6%). OMEGA matrix was investigated using a full OMEGA block and a correlation was found between BMAX and CL/F1 (R=0.837). However, as the PK model with a OMEGA block between BMAX and CL/F1 overestimated the variability, the OMEGA block between BMAX and CL/F1 was not implemented in the final model. The residual variability, modeled using a proportional variability model, was moderate (27.0%). All typical parameters except Q3 and AMAX2/F1 were estimated with good precision (standard errors ranging from 10 to 30% (Table 3)).

The GOF plots of the final model showed that the model performed adequately, except for a slight under-prediction at higher concentrations (Figure 2).

Pharmacokinetic/Pharmacodynamic model

To describe the relationship between linagliptin pharmacokinetics and DPP-4 inhibition, a sigmoid E_{max} model using the total linagliptin plasma concentrations and a model relating the model-predicted DPP-4 occupancy with linagliptin to the DPP-4 inhibition (occupancy model) were tested.

As the sigmoid E_{max} model and the occupancy model were not nested, the OBJF could not be used for model comparison. The occupancy model was chosen as the final model because of the following reasons: (1) the occupancy model is more mechanistic and in line with the pharmacology effect of linagliptin, (2) population predictions and individual predictions in the occupancy model better predicted DPP-4 inhibition less than 40% compared

to the sigmoid E_{max} model, (3) based on a VPC, the occupancy model was superior to the sigmoid E_{max} model as the 90% prediction interval was wide in the sigmoid E_{max} model (not shown).

The residual variability was lower for near-maximal inhibitions, compared to lower inhibitions. This was reflected by using the residual variability model described in method parts. The residual variability was moderate (20.1%).

The IIV on E_{max} was tested but it was very low (< 3%). Therefore, only the IIV on BMAX was implemented into the final PK/PD model. E_{max} was estimated with a good precision (standard error was less than 1% (Table 3)).

In general, the GOF plots for DPP-4 inhibition in Figure 2 showed that the model performed adequately.

Model evaluation

For evaluation, a VPC was performed by simulating the data from this trial 1000 times. In the PK model, the C_{max} was slightly underpredicted for the 10 mg dose group and the variability of linagliptin plasma concentration was overpredicted for the 2.5 mg dose group after both the first dose and the last dose administration. However, the simulated median was close to the observed median (Figure 3A). Also in the PD model, the predicted DPP-4 inhibition showed good agreement with the observed DPP-4 inhibition for both doses (Figure 3B).

Simulation

The final population PK/PD model was used for simulating the steady-state DPP-4 inhibition-time profile after administration of 2.5, 5 and 10 mg linagliptin once daily. Figure 4 shows the median DPP-4 inhibition and the 90% prediction intervals of 1000 simulated studies with 200 patients per dose group for the three different dose groups. In the 5 mg and 10 mg dose groups, the simulated median DPP-4 inhibition was ≥80% over 24 hours at steady state (Figure 4). The median of DPP-4 inhibition at 24 hours after the last administration ($E_{24,ss}$) were 84.2% and 87.7% in the 5 mg and 10 mg dose groups, respectively. On the other hand, in the 2.5 mg dose group, ≥80% DPP-4 inhibition could not be maintained over 24 hours and $E_{24,ss}$ was less than 80% (79.2%).

DISCUSSION

This article describes a comprehensive investigation of the population PK/PD of linagliptin in Japanese patients with T2DM. PK/PD evaluation was

performed using the nonlinear mixed effect modeling program NONMEM, in conjunction with graphical visualization methods.

The nonlinear pharmacokinetics of linagliptin in Japanese patients was described by a 2-compartment model assuming concentration-dependent binding to DPP-4 in the central and the peripheral compartments. The model structure reflects the current knowledge about linagliptin's disposition and is in line with the result of in vitro and pre-clinical experiments [9, 10]. Previously, the PK model assuming concentration-dependent protein binding was successfully applied to describe linagliptin's nonlinear pharmacokinetics in non-Japanese patients [18].

Linagliptin shows an apparent nonlinear pharmacokinetics in the therapeutic dose range, although the other DPP-4 inhibitors (sitagliptin [22], saxagliptin [23], vildagliptin [24], alogliptin [25]) exhibit linear pharmacokinetics. This might be explained by the higher affinity of linagliptin to DPP-4 compared with other DPP-4 inhibitors. An *in vitro* study comparing the IC_{50} values to DPP-4 activity of several DPP-4 inhibitors showed that IC_{50} value of linagliptin was much lower than those of the other DPP-4 inhibitors (linagliptin: approximately 1 nmol/L, sitagliptin: 19 nmol/L, alogliptin: 24 nmol/L, saxagliptin:50 nmol/L, vildagliptin: 63 nmol/L) [26].

In addition, the dissociation rate from the DPP-4 was lower in linagliptin than in vildagliptin [26]. The higher affinity of linagliptin to its target (DPP-4) and slow dissociation from the target compared with the other DPP-4 inhibitors result in a unique target-mediated drug disposition.

Pharmacokinetics
The final model was based on linagliptin plasma concentrations after administration of 2.5 and 10 mg linagliptin. Including linagliptin plasma concentrations after administration of 0.5 mg linagliptin resulted in dose-dependent parameter estimates. As the main aim of the analysis was to predict DPP-4 inhibition in the 5 mg linagliptin dose, a model with dose-dependent parameter estimates was inappropriate.

A possible reason for the model misspecification in the 0.5 mg dose group might be that the applied model assumes quasi-equilibrium conditions for the protein binding process [27]. This assumption can only be made when the ligand concentration (i.e. linagliptin) exceeds the concentration of the binding partner (i.e. DPP-4). In the case of the 0.5 mg dose group, the plasma concentration was below the plasma DPP-4 concentration predicted (BMAX)

(Figure 5). This may be an indication that the assumption of quasi-equilibrium cannot be used for the 0.5 mg dose group. A target-mediated drug disposition model without the assumption of quasi-equilibrium was tested to investigate whether the linagliptin plasma concentration-time profile in the 0.5 mg dose group could be described well or not, however, this model did not converge successfully due to its complexity. Therefore, in this analysis, a target-mediated drug disposition model with the assumption of quasi-equilibrium was applied to the data in 2.5 and 10 mg dose group and the data of the 0.5 mg dose group were not used for analysis.

In the final population PK/PD model, C_{max} was under predicted in the 10 mg dose group. However, this underprediction was not important for the prediction of DPP-4 inhibition by the final PK/PD model, because the range around these peak concentrations (> 10 nmol/L) were still high enough to achieve the maximum effect in DPP-4 inhibition [14].

The model-estimated dissociation constant for the binding of linagliptin to DPP-4 was 0.108 nmol/L and is thus within the expected range from the dissociation constant obtained *in vitro* from plasma samples using equilibrium dialysis (1/Ki = 0.05 nmol/L) [9]. The DPP-4 concentration estimated by the model (BMAX), 6.07 nmol/L, was in the physiological range expected for the DPP-4 concentration in plasma [26, 28, 29, 30].

Simulation
The main objective of this analysis was to predict the DPP-4 inhibition-time profile of the 5 mg linagliptin dose group, because the approved dose of 5 mg once daily was not tested in a clinical Phase II trial in Japanese patients with T2DM [14]. The steady state DPP-4 inhibition after administration of 5 mg linagliptin once daily was simulated using the final population PK/PD model (Figure 4). The median values of $E_{24,ss}$ were predicted to be 79.2% for 2.5 mg, 84.2% for 5 mg, and 87.7% for 10 mg. The actual observed $E_{24,ss}$ values were 77.8% for 2.5 mg, and 89.7% for 10 mg in Japanese patients with T2DM [14] and 76.8% for 2.5 mg, 84.8% for 5 mg, and 89.1% for 10 mg in non-Japanese patients with T2DM [5].

Thus, no apparent difference was observed between the simulated and the actual $E_{24,ss}$ value.

The simulations suggest that the administration of 5 mg and 10 mg linagliptin lead to ≥80% DPP-4 inhibition throughout 24 hours at steady state in Japanese patients with T2DM. In contrast, the administration of 2.5 mg linagliptin could not

maintain ≥80% DPP-4 inhibition throughout 24 hours at steady state in both, the simulation in this analysis and the actual data in the clinical trial in Japanese and non-Japanese patients with T2DM [5, 14]. The literature data in rodent models showed that ≥80% DPP-4 inhibition is related to the near-maximum effect on glucose lowering [7, 8, 31]. Meanwhile there is also clinical data available that confirm this relationship:

In the current Phase II trial in Japanese patients with T2DM [14], the mean change from baseline of fasting plasma glucose (mean (±SD)) after 4 weeks was indeed lower in the 0.5 and 2.5 mg dose group than in the 10 mg dose group (0.5 mg: −11.5 (±8.3) mg/dL, 2.5 mg: −13.6 (±15.2) mg/dL, 10 mg: −25.0 (±12.3) mg/dL). And, in a Phase III trial in Japanese patients with T2DM [12], the administration of linagliptin 5 mg once daily, which is the approved dose, elicited reductions in HbA1c that were significantly greater than the changes achieved by either placebo at week 12 or voglibose at week 26.

The inclusion criteria of HbA1c in this study was determined to be 7.0–10.0% in the patients previously untreated with oral hypoglycemic agents or HbA1c 7.0–9.0% at screening and 7.0–10.0% after washout in those already receiving 1 or 2 oral hypoglycemic agents for ≥10 weeks. Also this reduction of HbA1c was confirmed for vildagliptin: in a Phase II trial in Japanese patients with T2DM [24], ≥ 80% DPP-4 inhibition throughout treatment period at steady state was consistently achieved by vildagliptin 50 mg twice daily but not by 25 mg twice daily. Consistent with these findings, in a clinical trial in the Japanese patients with T2DM [32], a clinically meaningful reduction of HbA1c was only shown by vildagliptin 50 mg twice daily, the approved dose of vildagliptin, but not by vildagliptin 25 mg twice daily.

Table 3. Parameter estimates from the final PK/PD model

	Parameter Estimate	SE (%)	Description
F1	1	FIX	Absolute bioavailability
KA [h^{-1}]	1.63	22.2	First order absorption rate constant
CL/F1 [L/h]	121	15.5	Apparent clearance
V2/F1 [L]	633	12.7	Apparent volume of distribution for central compartment
Q3/F1 [L/h]	73.0	68.8	Apparent inter-compartmental clearance between central and peripheral compartment
V3/F1 [L]	683	14.0	Apparent volume of distribution of the peripheral compartment
BMAX [nmol/L]	6.07	5.17	Concentration of binding sites in the central compartment
KD [nmol/L]	0.108	28.5	Affinity constant of the nonlinear binding
AMAX2/F1[nmol]	534	104	Apparent amount of binding sites in the peripheral compartment
EMAX [%]	92.5	0.48	Maximum inhibition in percentage based on baseline activity
Model parameters: IIV parameters			
IIV in F1 [CV%]	46.7	30.8 [a]	Inter-individual variability in the absolute bioavailability
IIV in KA [CV%]	73.6	32.5 [a]	Inter-individual variability in the absorption rate constant
IIV in CL [CV%]	68.8	25.7 [a]	Inter-individual variability in the clearance
IIV in BMAX [CV%]	14.2	30.5 [a]	Inter-individual variability in the concentration of binding sites in the central compartment
Model parameters: Residual variability			
Proportional residual variability PK [CV%]	27.0	9.96 [b]	
Proportional residual variability PD [CV%]	20.1	14.6	

a) SE is given on the variance scale

b) SE was derived by coding the residual error using THETAs
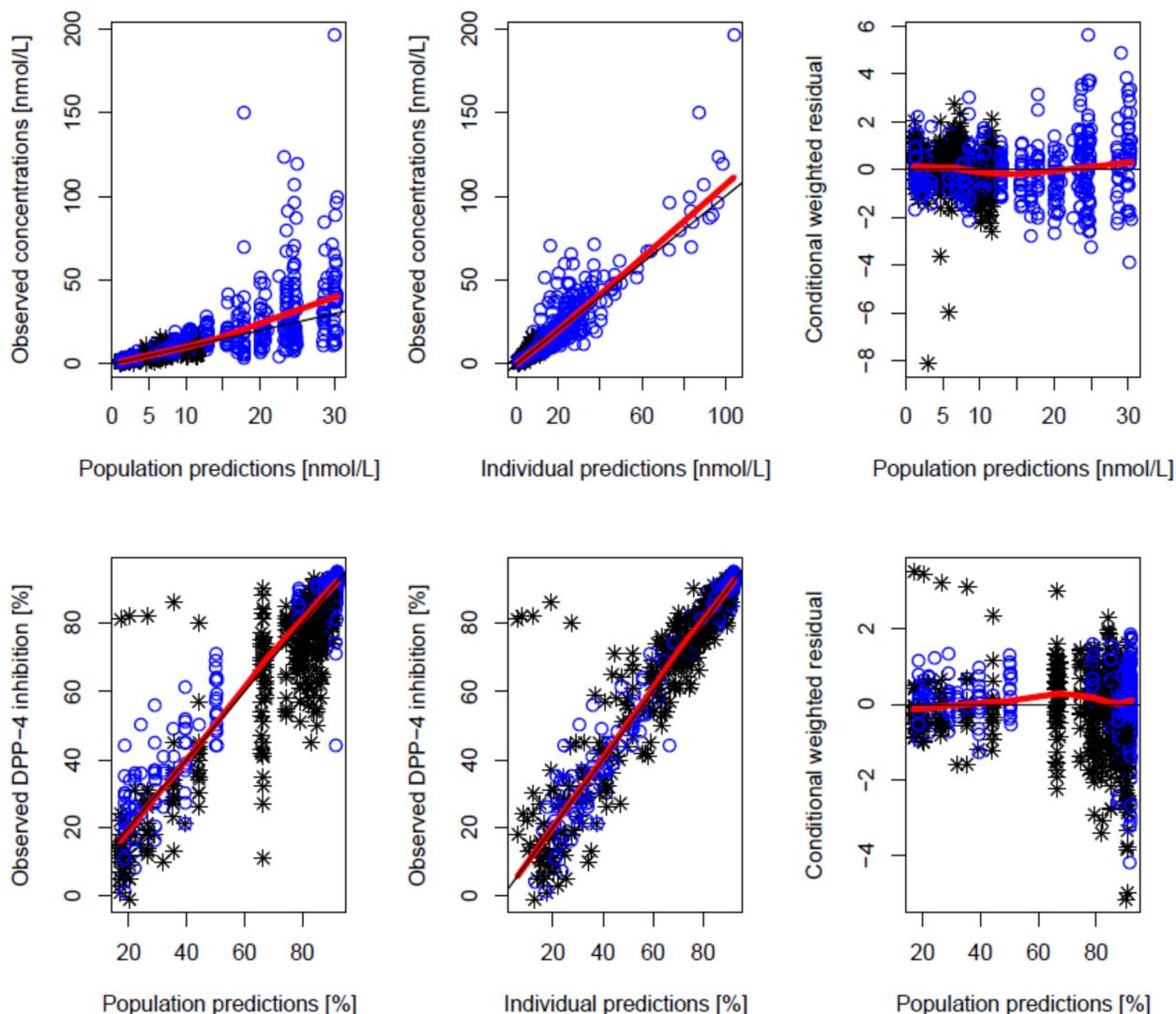
Figure 2. Goodness-of-fits plots with lines of identity (2.5 mg (∗), 10 mg (○)): for linagliptin plasma concentration (upper panels) and DPP-4 inhibition (lower panels). Observations versus population predictions (left panels), individual predictions (middle panels), population predictions versus conditional weighted residual (right panels)

For the other DPP-4 inhibitors (sitagliptin, alogliptin), the clinically meaningful reduction in HbA1c was shown by the approved dose which lead to ≥ 80% DPP-4 inhibition throughout the treatment period in clinical trial (sitagliptin [33, 34], and alogliptin [35, 36]). Taken together, ≥ 80% DPP-4 inhibition throughout treatment period at steady state is considered to be an appropriate pharmacodynamic criterion for full efficacy. Based on this criterion and our model, 5 mg linagliptin once daily would be the optimum dose in Japanese patients with T2DM.

CONCLUSION

Linagliptin's nonlinear pharmacokinetics and its plasma DPP-4 inhibition in Japanese patients with type 2 diabetes mellitus were well characterized by a target-mediated drug disposition model relating the DPP-4 occupancy with linagliptin to DPP-4 inhibition.

Simulations of the plasma DPP-4 inhibition-time profile suggest that 5 mg linagliptin once daily is the optimum therapeutic dose for Japanese patients with type 2 diabetes mellitus.
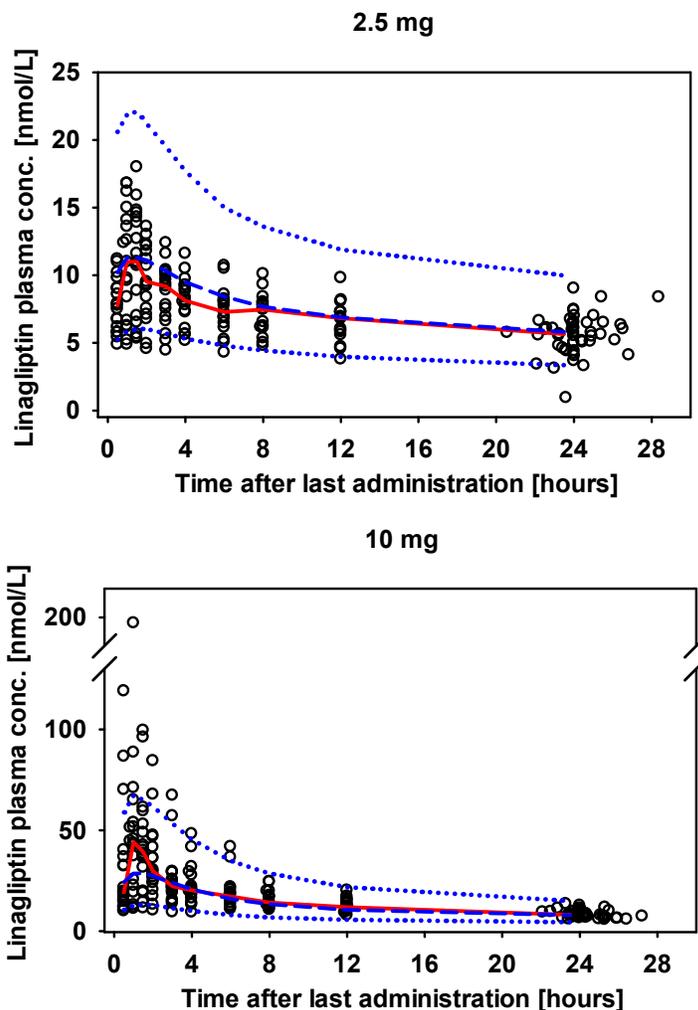
Figure 3A. Visual predictive check for linagliptin plasma concentration (only steady-state profiles shown) 1000 patients per dose group and study were simulated based on the final population PK/PD model. The open circles show the observations and the solid line shows the median of the observations. The dotted lines show the 90% prediction intervals and the median of the simulated profiles.

DECLARATION

Boehringer Ingelheim is the study sponsor and manufacturer of linagliptin. YuT, AS, YaT, ST, CF, SR, and AS are employees of Boehringer Ingelheim. MT has declared that he has no conflict of interest.

Abbreviations

AMAX2: amount of binding sites in the peripheral compartment, AUC: area under the curve, BMAX: concentration of binding sites in the central compartment, C_b: concentration of linagliptin bound to DPP-4 calculated by PK model, C_{max}: maximum plasma concentration, CL: clearance, DPP: dipeptidyl peptidase, E_{max}: maximal effect, FOCE-INTERACTION: first-order conditional estimation with interaction method, F1: bioavailability, GIP: gastric inhibitory polypeptide, GLP-1: glucagon-like peptide 1, GOF: goodness-of-fit, HbA1c: Hemoglobin A1c, HPLC-MS/MS: high performance liquid chromatography coupled to tandem mass spectrometry, IC_{50}: half maximal inhibition concentration, IIV: inter-individual variability, KD: affinity constant, NONMEM: non-linear mixed effect modeling, OBJF: objective function value, PK/PD: pharmacokinetic/pharmacodynamic, Q3: intercompartmental clearance between central and peripheral compartment, KA: absorption rate constant, RFU: relative fluorescence units, T2DM: type 2 diabetes mellitus, VPC: visual predictive check, V2: central volume of distribution, V3: peripheral volume of distribution.
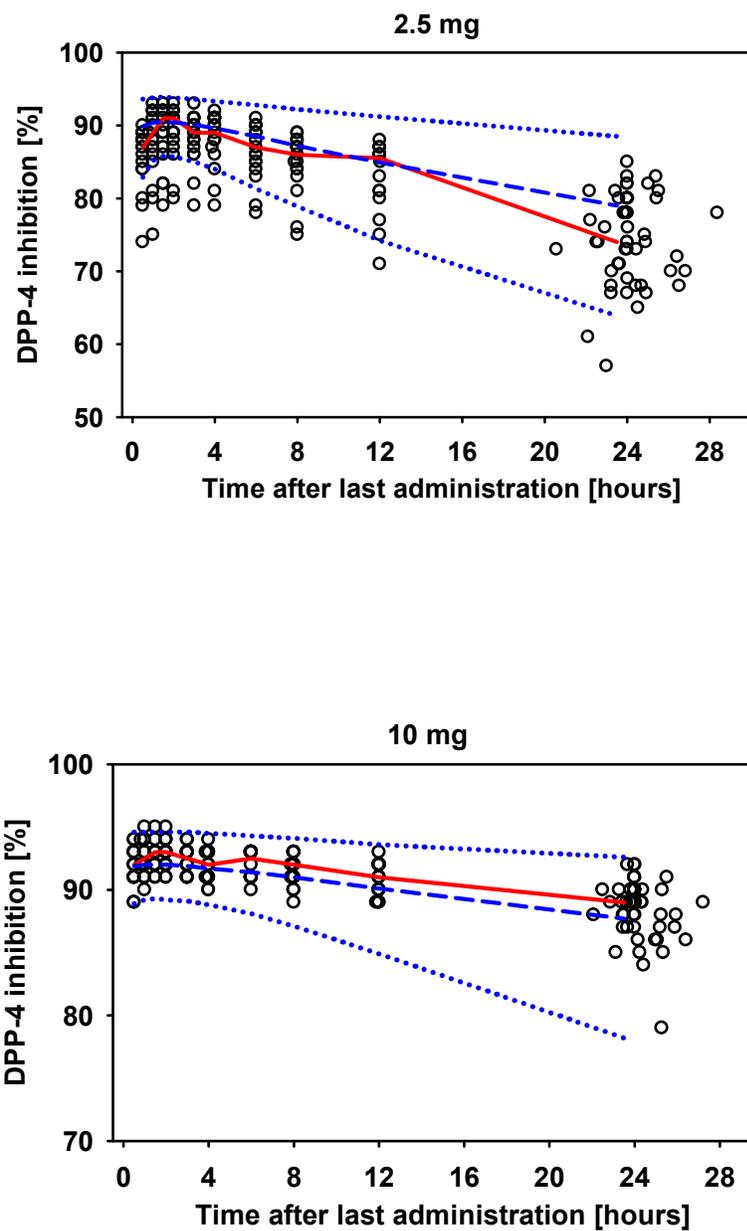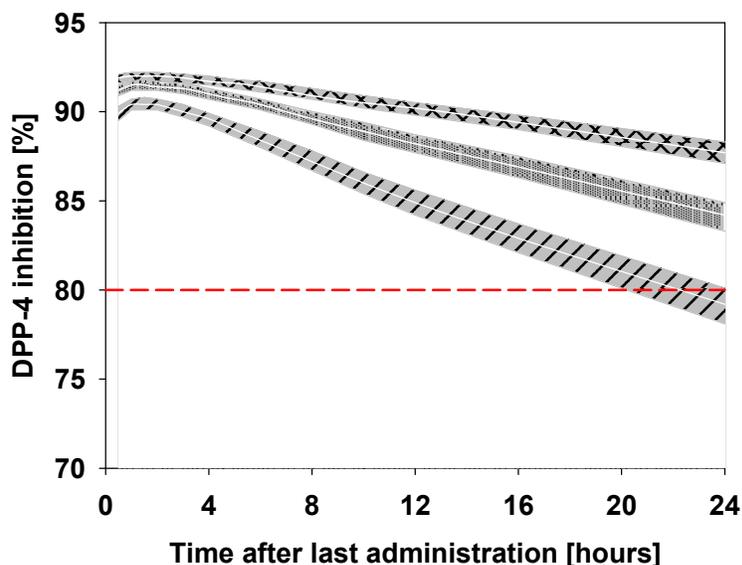
Figure 3B. Visual predictive check for DPP-4 inhibition (only steady-state profiles shown)

Figure 4. Predicted steady-state DPP-4 inhibition-time profile for patients receiving 2.5 mg (▨), 5 mg (▦) or 10 mg (▨) linagliptin once daily. For each dose group the predicted median inhibition (solid line) and the 90% prediction interval (shaded area) are depicted. 80% inhibition (red-line) is represented.
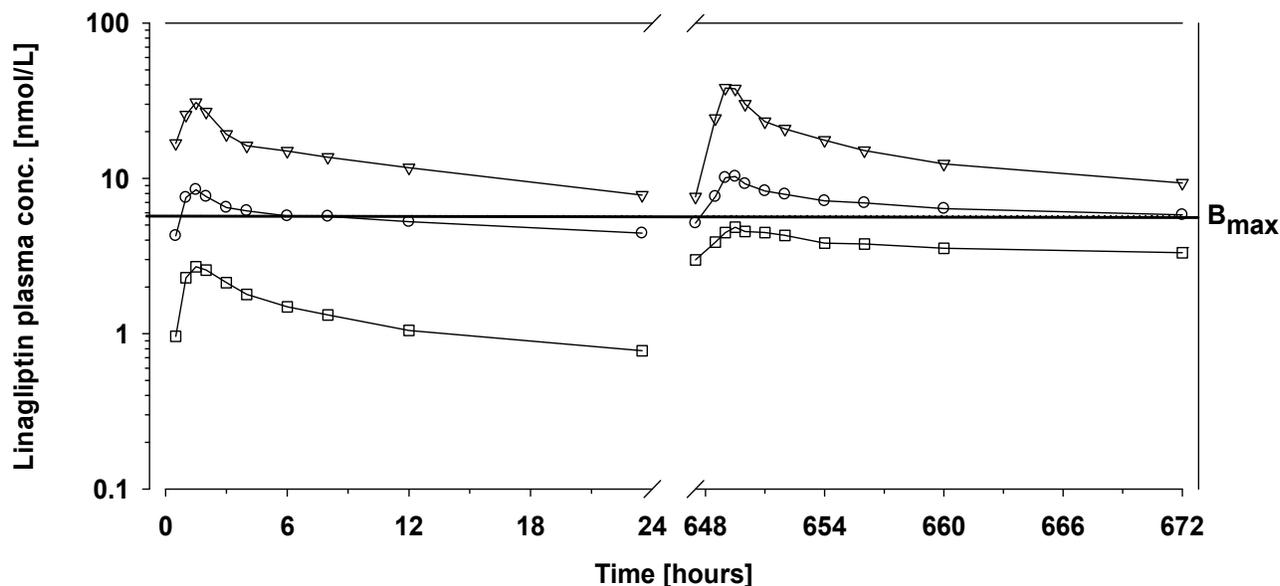
Figure 5. Geometric mean plasma concentration-time profiles of linagliptin (0.5 mg (N=19, □), 2.5 mg (N=18, ○), 10 mg (N=18, ▽)) with lines of B_{max} predicted by model.

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