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Original Article

Bioequivalence Studies of Two Brands of Linagliptin Tablets in Healthy Adults Under Fed and Fasted Conditions

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Abstract

Background and objectives: This study aimed to summarize the clinical pharmacokinetics and bioequivalence of generic and branded linagliptin tablets during fasting and fed conditions, and the influence of food on the pharmacokinetics (PK) of linagliptin tablets was also explored in healthy Chinese subjects.

Methods: An open-label, randomized, single-center, two-period, and single-dose crossover bioequivalence study was performed in this research. Healthy subjects in fasting (n = 32) and fed (n = 32) conditions received 5 mg of generic (test) linagliptin or a commercial (reference) capsule, respectively. Blood sample collection was conducted at the baseline and post-dose. Plasma concentrations of linagliptin were detected by a a high-performance liquid chromatography with tandem mass spectrometry method. A non-compartmental method was performed to analyze pharmacokinetic parameters, and safety was monitored.

Results: A total of 64 subjects completed the study, 32 for the fasting and 32 for the fed study. The major PK parameters of linagliptin, including C_{max} and area under the concentration-time curve from time 0 to 72 hours (AUC0–72), were similar between the preparations under fasting and fed conditions. Under fasting conditions, the 90% confidence intervals (CI) of the test/reference ratios (T/R) of C_{max} and $AUC_{0–72}$ were 95.9~110.9% and 96.8~101.9%, respectively. Under fed conditions, the 90% CI of T/R of C_{max} and $AUC_{0–72}$ were 98.2~103.4% and 97.7~103.5%, respectively. None of the volunteers had a severe adverse event.

Conclusions: Generic linagliptin tablet is bioequivalent to the reference drug under both fasting and feeding conditions. Food delays the absorption of linagliptin. Chinese subjects taking a single dose of linagliptin of 5 mg have good tolerance to the drug.

Keywords: Linagliptin; DPP-4 inhibitor; Bioequivalence; Pharmacokinetics.

Abbreviations: AEs, adverse events; AUC, area under the plasma concentration-time curve; AUC_{0-72}, AUC from zero to time 72h; CI, confidence intervals; C_{max}, maximum plasma drug concentration; DM, diabetes mellitus; DM2, type 2 diabetes mellitus; DPP4, dipeptidyl deptidase-4; GLP-1, glucagon-like Peptide-1; GIP, glucose-dependent insulinotropic peptide; IR, insulin resistance; PK, pharmacokinetics; QC, quality control; SAS, statistical analysis system; T_{max}, time to reach maximum plasma concentration.

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Introduction

Diabetes mellitus (DM) is a metabolic disorder characterized by an abnormal glucose and lipid metabolism causing persistent hyperglycemia.[1,2] About 95 percent of diabetes cases belong to type 2 diabetes mellitus (DM2), characterized by insulin resistance (IR).[3] Diabetes, an epidemic connected with the combination of social, behavioral, fetal, and genetic factors, is one of the greatest health problems of the 21st century.[4] Recent statistics from the International Diabetes Federation show that 425 million adults worldwide had diabetes in 2017. A total of 629 million people are expected to suffer from diabetes by 2045.[5] There have been many advances in treating DM2; however, reaching optimal glycemic goals remains a question.

The incretins such as glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP) secreted from enteroendocrine K and L cells could stimulate pancreatic beta cells to secrete insulin,[6,7] both incretins are rapidly degraded by the enzyme dipeptidyl peptidase-4 (DPP4). Early studies have shown DPP4 revolved in the pathogenesis of IR,[8] hyperglycemia,[9] dyslipidemia,[10,11] obesity,[12,13] oxidative stress,[14] inflammation,[15–17] and malignant tumors.[18] Inhibition of DPP4 enzymes and prolonged endogenous GLP-1 and GIP concentrations in the blood has become a new target for managing DM2.[19] A clinical study[20] showed that linagliptin was involved in significantly fewer cardiovascular events. Like a placebo, linagliptin did not affect the risk for the secondary kidney outcome in participants with DM2 and CKD and/or CV disease.[21] Up until now, over ten DPP4 inhibitory drugs classified as gliptins have been approved by the FDA:[22] sitagliptin, linagliptin, vildagliptin, teneligliptin, saxagliptin, omarigliptin, gemigliptin, alogliptin, anagliptin, trelagliptin, evogliptin, and gosogliptin.[23] Unlike other DPP4 inhibitors, linagliptin is almost wholly bound (99%) to plasma proteins (mostly the DPP-4 enzyme) in the early animal study.[24] Human studies have indicated that hepatobiliary clearance is the predominant mechanism of clearance of linagliptin, which is perhaps connected with its extensive binding characteristics.[24,25] Such characteristics suggested that kidney excretion is a non-primary way of eliminating linagliptin. Linagliptin administration does not require dosage adjustment in clinic patients with declining renal function.[26] Pharmacokinetics (PK) and pharmacodynamics of linagliptin are unique in that they are described by target-mediated non-linear PK and a comprehensive safety window.[27,28] Another, linagliptin significantly improved glycemic control in type 2 diabetes inadequately controlled on basal insulin.[29] At present, a generic linagliptin tablet is being developed by Chengdu Brilliant Pharmaceutical Co., Ltd. According to China National Medical Products Administration, a bioequivalence study was required to support registration.

Oral drug absorption is an intricate process and can be influenced by numerous factors. Food plays a primary role in the bioavailability of drugs orally administered. By affecting the solubility and intestinal permeability,[30] food may alter the absorption, metabolism, excretion, and other processes of the drug in the gastrointestinal tract via numerous food-drug interactions.[31] Ahmad Y Abuhelwa's study[32] has demonstrated that drug dissolution or solubility, drug stability, drug release, and intestinal permeability all was significantly affected by food intake. Following a meal, gastric emptying rate, dissolution, GI luminal metabolism, pH, and bile flow were reconfigured, contributing to delayed drug absorption.[33] Christina S Won's study[34] about mechanisms underlying food-drug interactions also manifested that fruit juices, teas, and other commonly consumed could inhibit the activity of intestinal cytochrome P450 or phase II conjugation enzymes, decrease the expression of uptake and efflux transport proteins, which caused the changed in bioavailability of drugs.

This study aimed to assess the bioequivalence between generic linagliptin tablets and branded preparation under fasting and fed conditions. In addition, the food effect on PK of linagliptin tablet was also evaluated in healthy Chinese volunteers.

Materials and methods

Subjects

Healthy Chinese volunteers aged over 18 years with a body mass index of 19–26 kg/m^2 were qualified for inclusion. All volunteers were evaluated during screening visits, including physical examinations, vital signs, electrocardiogram (12-lead), laboratory tests (coagulation function, hematology, blood chemistry, urinalysis), serologic tests (HBV surface antigen, antibodies including HCV, HIV, and syphilis) and a history of medication. Participants agreed to use effective contraception from two weeks before screening through 3 months after the end of the trial. Primary exclusion criteria included (a) clinically meaningful abnormality in vital signs, electrocardiogram, physical examinations, or laboratory results according to the physician's judgment; (b) the presence or history of endocrine, cardiovascular, metabolic, psychiatric, and neurological diseases; (c) allergy to any drugs or food; (d) Over 400 mL of blood lost; (e) surgery operated within four weeks before entry into the study or scheduled during this study; (f) pregnancy or lactation ; (g) consume plenty of tea, coffee, or caffeinated beverages; (h) heavy smokers ≥5 cigarettes per day or alcoholics weekly alcohol ≥14 units; (i) Substance abuse and positive substance abuse screening tests including morphine, 3,4-methylenedioxyamphetamine, ketamine, methamphetamine, tetrahydrocannabinol, and cocaine.

Study drugs and reagents

The generic (or test) preparation, a linagliptin tablet, 5 mg (batch no: 200401, Exp: April 2023), was provided by Chengdu Brilliant Pharmaceutical Co., Ltd., and the branded (or reference) preparation, linagliptin tablet 5 mg (batch no: AA6924A, Exp: January 2022) was obtained from Boehringer Ingelheim International GmbH, Germany.

Study design

This randomized, open-label, single-center, single-dose, and two-sequence crossover study to evaluate the bioequivalence of test and reference linagliptin under fed and fasting conditions was conducted in healthy volunteers (Fig. 1). This clinical trial strictly adheres to the ethical guidelines of The Declaration of Helsinki on human medical research (as revised in 2013). The protocol and informed consent of the clinical trial were approved by the Ethics Committee of the Third Hospital of Changsha (Approval No. 2020EC-007). Written informed consent was obtained from the patient. This study was registered at www.chinadrugtrials.org.cn (registration number: CTR20201729).

Sixty-four volunteers who satisfied all the criteria for inclusion were included in this study, and 32 participants were assigned to the fasting and fed groups, respectively. Subjects were randomly divided into two sequence groups (T-R, R-T). Statistical Analysis System (SAS) 9.4 software randomly assigned participants into two groups. The washout period was 36 days between treatment periods. After an overnight fast of at least 10 hours, subjects in the fasting cohort received 240 mL of water after oral administration of tablets of the test or reference linagliptin. A restriction was placed on water consumption one hour before and after administration. The subjects in the fed cohort ingested a standard high-fat meal (800–1,000 kcal) 30 minutes prior to the administration. All subjects were checked in real-time to confirm the drug was entirely swallowed.

Blood Sample

Fasting blood samples (4 mL) were obtained predose and at 15, 30, 45, 60, 75, 90, and 105 min and 2, 2.25, 2.5, 3, 3.5, 4, 5, 6, 8, 12, 24, 48, and 72 h after dosing. Fed blood samples (4 mL) were

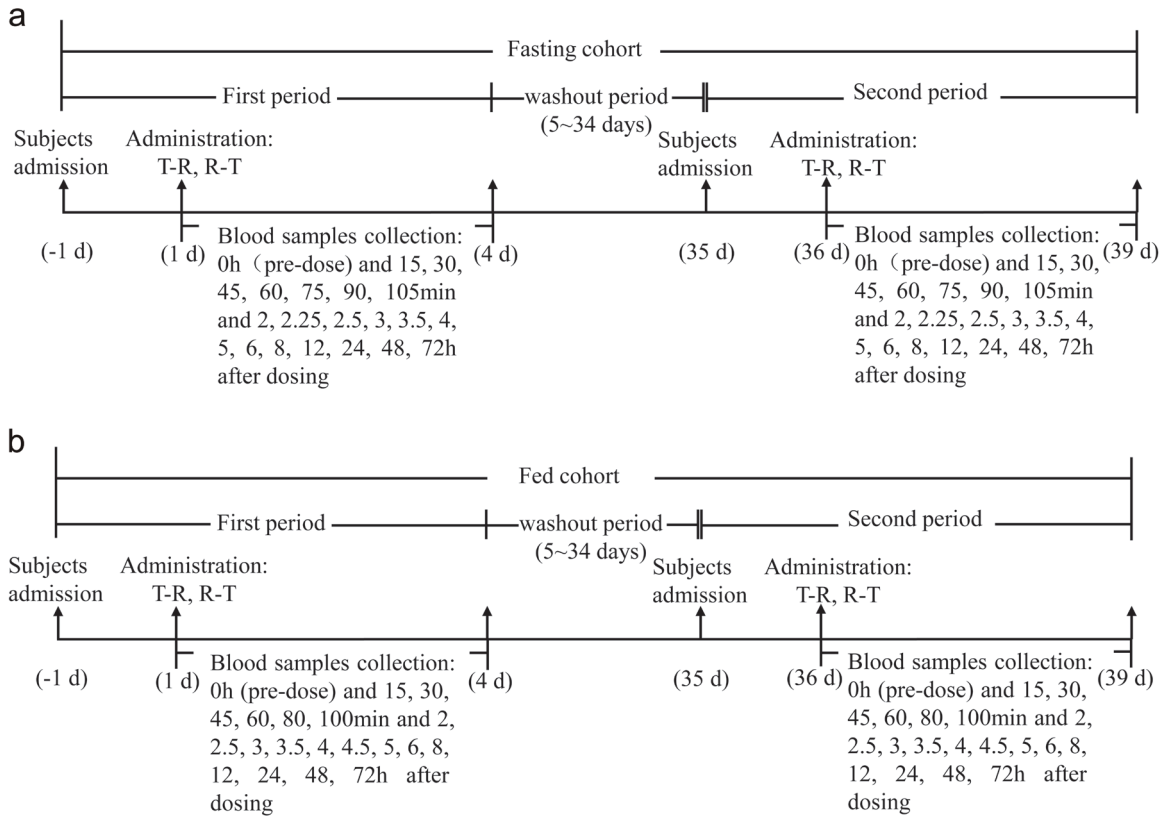

Fig. 1. The flowchart of the clinical trial design in fasting cohort (a) and fed cohort (b).

obtained predose and at 15, 30, 45, 60, 80, and 100 min and 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 8, 12, 24, 48, and 72 h after dosing. Immediately after collecting blood samples, plasma was centrifuged at 1,700 g (2–8°C, 10 minutes) and stored at −60°C until further analysis.

Analytical method and method validation

A liquid-liquid extraction method was applied to extract linagliptin from plasma. A validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was used to analyze plasma levels of linagliptin. Briefly, linagliptin from the plasma was extracted using a liquid-liquid extraction technique and separated on a 2.0×50 mm, 5 µm CAPCELL PAK C18 column (Shiseido Co Ltd). Water containing salt solution: formic acid LC: ultrapure water (5:1:1,000, V/V/V) (solvent A) and acetonitrile (solvent B) made up the mobile phase. With a flow rate of 0.400 mL/min, elution was performed as follows: 0.01–0.5 min, linearly increase B from 20% to 90%; 0.5–1.5 min, isocratic A: B = 10:90; 1.51–2.5 min, the system was switched back to the initial proportion (20% B), the column was equilibrated for 3 min with a column heater at 40 °C. An electrospray mass spectrometer operated in multiple reaction monitoring modes with positive ionization. The optimal parameters for the tandem mass spectrometer were as follows: curtain gas, ion spray voltage, temperature, ion source gas 1, and ion source gas 2 were instrumented in the following settings: 30 psi, 5,500 V, 550°C, 50 psi, and 60 psi, respectively. Potentials for declustering, entrance, and collision cell exits were set to 90 V, 12 V, and 11V for linagliptin, 110 V, 8 V, and 8V for linagliptin-13C-d3, respectively. The collision energy was set at 35 eV. In using electrospray ionization to monitor multiple reactions, the transition m/z was 473.4 to 420.4 for linagliptin and 477.4 to 420.5 for linagliptin-13C-d3. Software Analyst 1.6.3 was used to analyze the mass spectrum data (AB SCIEX, Foster City, California). The linear range, intra-day precision, inter-day precision and accuracy were 0.20 to 12.0 ng/mL, 2.9% to 8.1%, 2.5% to 6.9%, and within 97.6% to 101.0%, respectively. Seven calibration plasma samples for linagliptin were prepared by spiking standard solutions (at the concentrations of 4.0, 8.0, 20.0, 60.0, 120.0, 200.0, and 240.0 ng/mL, respectively) into drug-free blank plasma with appropriate volume ratio. The concentrations of quality control (QC) samples were 4.0, 12.0, 80.0, 160.0 ng/mL. The method was validated in light of the guideline on Bioanalytical Method Validation from the EMA,[15] including specificity, linearity, the lower limit of quantification, accuracy, precision, matrix effect, extraction recovery, stability, and the QC during the subject sample analysis.

Pharmacokinetic Parameters and Statistical Analyses

Major PK parameters, including maximum plasma drug concentration (C_{max}), time to reach maximum plasma concentration (T_{max}), and area under the plasma concentration-time curve (AUC) from zero to time 72 h (AUC_{0-72}), were calculated based on plasma concentration-time data. PK parameters were analyzed with WinNonlin 8.0 (Pharsight Corporation, Sunnyvale, California) using noncompartmental models. A linear mixed ANOVA model was used to analyze the log-transformed C_{max} and AUC_{0-72}, with subjects within the series as a random effect and period, series, and formula as fixed effects. The Mann-Whitney U test was used to

Table 1. Demographic characteristics of subjects

Demographics	Fasting (n = 32)	Fed (n = 32)
Male/female, n	24/8	24/8
Age, y	26.0 ± 5.8	24.9 ± 6.1
Height, cm	168 ± 8.0	167 ± 7.0
Weight, kg	62.4 ± 7.8	62.9 ± 7.6
BMI, kg/m^2	21.8 ± 1.6	22.3 ± 1.8

BMI, body mass index. Age, height, weight, and BMI data are expressed as mean ± SD.

compare the PK profile between the fasting and fed groups. SPSS (version 22.0) was used to conduct statistical analysis. The ratios of geometric least square mean and the corresponding 90% confidence intervals (CI) of the C_{max} and AUC_{0-72} were computed, and bioequivalence was demonstrated between the test and reference formulations if the 90% CI of C_{max} and AUC_{0-72} fell within the predetermined range 80.00–125.00%. A nonparametric test was applied to analyze T_{max}. Statistical analyses were carried out using SAS version 9.4 (SAS Institute, Inc., Cary, North Carolina), and p-values of <0.05 were considered statistically significant.

Safety Assessment

A safety assessment was conducted by vital sign measurements, physical examinations, electrocardiograms, and laboratory tests at baseline, at the indicated time points following drug administration, and the next day after study completion. Every adverse event was recorded.

Result

Demographic Characteristics

In total, 64 healthy subjects participated in the study, with 32 individuals in the fasting cohort and 32 in the fed cohort, respectively. Of the 64 subjects who completed the study, one subject withdrew from the fed cohort. Detailed demographic information about all patients is presented in Table 1.

BE Studies and Effect of Food on PK

The mean serum concentration-time curves of two formulations of linagliptin products, each administered as a single 5 mg oral dose

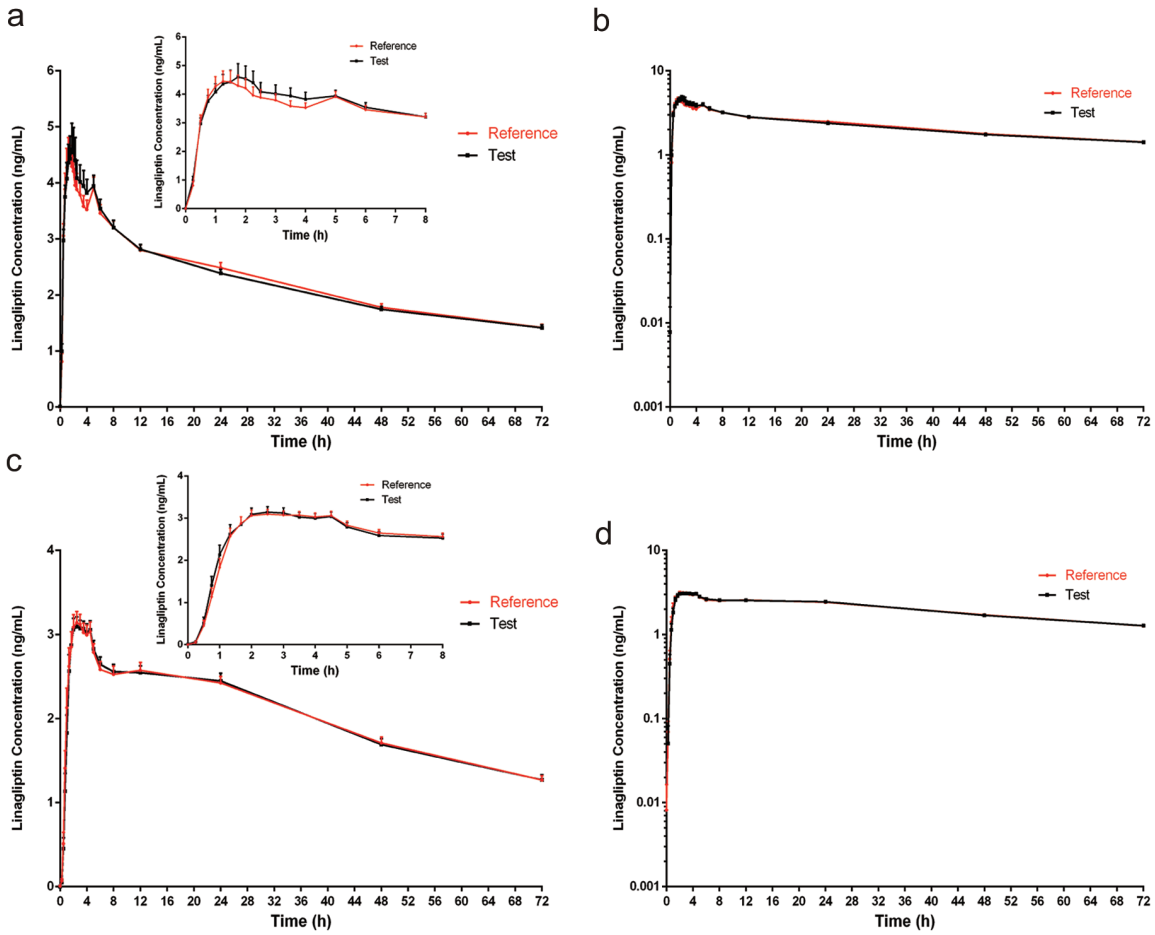

Fig. 2. Mean (±SEM) plasma concentration-time curves (a) and semilogarithmic curves (b) of the test and the reference products of Linagliptin in healthy subjects under fasting conditions. Mean (±SEM) plasma concentration-time curves (c) and semilogarithmic curves (d) of the test and the reference products of Linagliptin in healthy subjects under fed conditions.

Table 2. Bioavailability parameters of the test and reference formulations under fasting and fed conditions

Parameter	Fasting		Fed	
	Test (n = 32)	Reference (n = 32)	Test (n = 32)	Reference (n = 31)[a]
T_{max} (h)	1.9 (0.5, 8.0)	1.5 (0.5, 12.0)	2.0 (0.8, 24.0)	3.0 (0.8, 4.5)
C_{max} (ng/mL)	5.5 ± 2. 6	5.2 ± 2.1	3.5 ± 0.7	3.5 ± 0.6
AUC_{0-72} (h*ng/mL)	159.3 ± 25.2	160.9 ± 27. 7	145.1 ± 28.0	144.9 ± 28.7

T_{max}, time to maximum plasma concentration; C_{max}, maximum plasma concentration; AUC_{0-72}, area under the concentration curve from time zero to 72h. All data are expressed as mean ± SD, except for t_{max}, which is expressed as median (range). [a]One subject experienced carried "Skin expert antibacterial ointment" during the second period of check-up and withdrew from the second period without taking a reference drug.

to 64 healthy Chinese volunteers, are shown in Figure 2. Table 2 shows the values of the primary PK parameters for linagliptin following administration under fasting or fed conditions. There were no significant differences between the test drug and the reference drug, whether in the fasting or the fed group. As to the effect of food on the PK, we found the concurrent intake of food increased the time to reach maximum plasma concentration by approximately 1 hour and decreased C_{max} by about 30%, the AUC_{0-72} in the fed condition was also decreased compared to the fasting condition. The results from the Mann-Whitney U test (Table 3) indicated that Changes among C_{max} ($p < 0.05$) and AUC_{0-72} ($p < 0.05$) were statistically significant in test drug and reference drug when comparing fed condition with fasting condition, T_{max} was increased in the fed state, but not statistically significant. As shown in Table 4, the 90% CIs for the ratio (test/reference) of log-transformed C_{max} and AUC_{0-72} were 95.9–110.9% and 96.8–101.9% in the fasting group, and 98.2–103.4% and 97.7–103.5% in the fed group, respectively. These were within the acceptance range of 80–125%, indicating that the test preparation was equivalent to the reference preparation in healthy Chinese subjects under both the fasting and fed conditions.

Safety Assessment

From Table 5, there were six AEs (Adverse Events) reported in four (6.3%) of 64 subjects in the fasting group. These AEs were mild (Grade I) and thought to be associated with the administration of the drug. Especially five AEs occurred in the test drug period, whereas one AE was reported in the reference drug period. Finally, one AE was improved when five AEs had completely recovered at the last scheduled visit.

Also, six AEs (Adverse Events) were reported in five (7.9%) of 63 subjects in the fed group. These AEs were mild (Grade I) and thought to be associated with the administration of the drug. Among these, three AEs occurred in the test drug period, whereas in the reference drug period, two AEs were reported. Finally, three AEs completely recovered at the last scheduled visit. No serious adverse events occurred in the fasting or the fed cohorts, nor were any AEs leading to withdrawal. There was no statistically significant difference between the test and reference products in the incidence of adverse events.

No severe AEs or AEs were leading to withdrawal in the fasting and fed cohorts. The difference in the incidence of AEs between the test and reference products was not statistically significant.

Table 3. Effects of food on the PK parameters of linagliptin

Parameter	Mann-Whitney U test	
	Test	Reference
T_{max} (h)	0.831	0.069
C_{max} (ng/mL)	<0.01*	<0.01*
AUC_{0-72} (h*ng/mL)	0.003*	0.01*

*$p < 0.05$. AUC_{0-72}, area under the concentration curve from time zero to 72h; C_{max}, maximum plasma concentration; PK, pharmacokinetics; T_{max}, time to maximum plasma concentration.

Discussion

The bioequivalence of generic linagliptin to the branded tablet in healthy Chinese subjects was established on fasting and fed Chinese subjects through the two-period, crossover, phase I study. Of course, the 5 mg dose was safe and well tolerated, consistent with previous clinical studies.[35,36] No adverse events or clinically significant changes in the study were reported.

Compared with some of the standard therapies for DM2, DPP4 inhibitors showed fewer limitations.[37] Compared with other DPP4 inhibitors, linagliptin showed more obvious advantages. Firstly, linagliptin is considered safe in renal failure.[38] Linagliptin undergoes enterohepatic cycling and is excreted primarily (85%) in the bile, while the elimination of other DPP-4 inhibitors is performed mainly through renal excretion, with 60~85% of each dose eliminated as an unchanged parent compound in the urine.[39] Secondly, as a weak competitive inhibitor of CYP3A4, linagliptin just resulted in a little decrease in the clearance of other drugs metabolized by CYP3A4. As a substrate for CYP3A4, linagliptin has a similar exposure regardless of whether CYP3A4 inhibition or induction is present. Thus, linagliptin is considered to have a low potential for clinically relevant interactions. Moreover, a study[40] suggested that compared with other DPP-4 inhibitors, linagliptin possessed the best overall balance between potency and the clinical pharmacokinetic characteristics of distribution, metabolism, and elimination.

With a high-fat meal, we observed that the T_{max} of linagliptin was delayed about 1 h, and C_{max} was decreased by about 30% in the study. Meanwhile, linagliptin concentrations in plasma were slightly higher in a fed condition beyond 12 hours after dosing. Food-induced increase in AUC_{0-72h} was consistent with the observation in Graefe-Mody U's study.[41] Food delayed the absorption rate of linagliptin but did not affect the extent of absorption. Food was involved in drug absorption and distribution via various mechanisms, including a direct drug-food interaction or a change in physiological conditions. Considering linagliptin is a substrate for CYP3A4, food may affect the absorption rate by regulating the activity of CYP3A4. Also, the solubility of the drug *in vivo* was different in fasting and fed conditions,[42] food increased viscosity of the stomach contents and slowed down gastric emptying rate, which may be the reason for the decreased C_{max} and prolonged T_{max} of linagliptin in the fed group.

Considering the presentation of DM2 was different between patients of Asian and Caucasian origin,[43–46] whether these differences based on ethnicity affect the PK characteristics of linaglip-

Table 4. Bioequivalence between the Test (T) and Reference (R) linagliptin tablets in healthy Chinese subjects under fasting and fed conditions

Parameter	Fasting				Fed			
	(T/R) GMR	90% CI (%)	Intraindividual Variability (%)	power	(T/R) GMR	90% CI (%)	Intraindividual Variability (%)	power
C_{max} (ng/mL)	103.1	95.9–110.9	17.3	99.5	100.7	98.2–103.4	5.8	100.0
AUC_{0-72} (h*ng/mL)	99.3	96.8–101.9	6.1	100.0	100.5	97.7–103.5	6.5	100.0

C_{max}, the maximum plasma concentration; AUC_{0-72}, area under the curve to time 72h; GMR, geometric mean ratio; CI, confidential interval.

tin? From a review published in 2017,[37] we acknowledged that small changes in PK parameters were observed when linagliptin was given to Caucasian, Japanese, and Chinese patients, but this is not considered clinically relevant.

Further directions

Of course, the study also existed a shortcoming. Blood DPP-4 levels could be measured to assess trends in DPP-4 levels over time before and after drug administration. Therefore, we could evaluate the influence of drugs on DPP-4 levels *in vivo* by determining the DPP-4 level in the blood samples of each subject. Meanwhile, we could evaluate linagliptin's action mode and characteristics *in vivo* more comprehensively by combining them with the PK results.

Conclusions

In the study, two formulations were well-tolerated in healthy Chinese volunteers. All AEs were mild. Linagliptin showed a clean safety profile in this study with an AE rate similar to that of the placebo of the reference drug. Our data recommended that the generic linagliptin capsule is safe and may be a cost-effective alternative to the branded linagliptin for DM2 patients in China, especially those with kidney insufficiency.

Acknowledgments

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Funding

None.

Conflict of interest

XL has been an editorial board member of the *Journal of Exploratory Research in Pharmacology* since January 2020. KY is the project manager of Brilliant Pharmaceuticals Co., Ltd. The authors have no other conflicts of interest to declare.

Author contributions

All the authors contributed substantially to the manuscript. XL participated in the study concept and design; XL, BX and YL contributed to assay performance and data analysis; XL, KY, PZ and SQT performed the administration; XL and FY designed the study; FY and GYX contributed to manuscript writing; XL and FY contributed to the critical revision of the manuscript.

Ethical statement

This clinical trial strictly adheres to the ethical guidelines of The Declaration of Helsinki on human medical research (as revised

Table 5. Incidence of adverse events of subjects in the fasting (n = 32) and fed group (n = 32)

AEs, n (%)	Fasting		Fed		All (N = 64)
	Test (n = 32)	Reference (n = 32)	Test (n = 32)	Reference (n = 31)[a]	
All AEs	3 (9.4%)	1 (3.1%)	3 (9.4%)	2 (6.5%)	9 (14.1%)
Leukocytosis	1 (3.1%)	0	0	1 (3.1%)	2 (3.1%)
Elevated platelet	1 (3.1%)	0	0	0	1 (1.6%)
Prolonged thrombin time	0	0	1 (3.1%)	0	1 (1.6%)
Increased triglycerides	0	1 (3.1%)	3 (9.4%)	0	4 (6.3%)
Elevated Serum creatine phosphokinase	0	0	0	1 (3.1%)	1 (1.6%)
Anemia	1 (3.1%)	0	0	0	1 (1.6%)
Dizzy	1 (3.1%)	0	0	0	1 (1.6%)
Weak	1 (3.1%)	0	0	0	1 (1.6%)

AEs, adverse events. [a]One subject experienced carried "Skin expert antibacterial ointment" during the second period of check-up and withdrew from the second period without taking a reference drug.

in 2013). The protocol and informed consent of the clinical trial were approved by the Ethics Committee of the Third Hospital of Changsha (Approval No. 2020EC-007). Written informed consent was obtained from the patient. This study was registered at www.chinadrugtrials.org.cn (registration number: CTR20201729).

Data sharing statement

No additional data are available.

References

[1] Stumvoll M, Goldstein BJ, van Haeften TW. Type 2 diabetes: principles of pathogenesis and therapy. Lancet 2005;365(9467):1333–1346. doi:10.1016/S0140-6736(05)61032-X, PMID:15823385.

[2] Multhaup ML, Seldin MM, Jaffe AE, Lei X, Kirchner H, Mondal P, *et al*. Mouse-human experimental epigenetic analysis unmasks dietary targets and genetic liability for diabetic phenotypes. Cell Metab 2015;21(1):138–149. doi:10.1016/j.cmet.2014.12.014, PMID:25565211.

[3] Farmer A, Fox R. Diagnosis, classification, and treatment of diabetes. BMJ 2011;342:d3319. doi:10.1136/bmj.d3319, PMID:21659368.

[4] Gudmundsdottir V, Pedersen HK, Mazzoni G, Allin KH, Artati A, Beulens JW, *et al*. Whole blood co-expression modules associate with metabolic traits and type 2 diabetes: an IMI-DIRECT study. Genome Med 2020;12(1):109. doi:10.1186/s13073-020-00806-6, PMID:33261667.

[5] Saeedi P, Petersohn I, Salpea P, Malanda B, Karuranga S, Unwin N, *et al*. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9th edition. Diabetes Res Clin Pract 2019;157:107843. doi:10.1016/j.diabres.2019.107843, PMID:31518657.

[6] Mentlein R. Mechanisms underlying the rapid degradation and elimination of the incretin hormones GLP-1 and GIP. Best Pract Res Clin Endocrinol Metab 2009;23(4):443–452. doi:10.1016/j.beem.2009.03.005, PMID:19748062.

[7] Deacon CF. Circulation and degradation of GIP and GLP-1. Horm Metab Res 2004;36(11-12):761–765. doi:10.1055/s-2004-826160, PMID:15655705.

[8] Sim AY, Barua S, Kim JY, Lee YH, Lee JE. Role of DPP-4 and SGLT2 Inhibitors Connected to Alzheimer Disease in Type 2 Diabetes Mellitus. Front Neurosci 2021;15:708547. doi:10.3389/fnins.2021.708547, PMID:34489627.

[9] Uto A, Miyashita K, Endo S, Sato M, Ryuzaki M, Kinouchi K, *et al*. Transient Dexamethasone Loading Induces Prolonged Hyperglycemia in Male Mice With Histone Acetylation in Dpp-4 Promoter. Endocrinology 2021;162(12):bqab193. doi:10.1210/endocr/bqab193, PMID:34480538.

[10] Czogała W, Czogała M, Kwiecińska K, Bik-Multanowski M, Tomasik P, Hałubiec P, *et al*. The Expression of Genes Related to Lipid Metabolism and Metabolic Disorders in Children before and after Hematopoietic Stem Cell Transplantation-A Prospective Observational Study. Cancers (Basel) 2021;13(14):3614. doi:10.3390/cancers13143614, PMID:34298827.

[11] Lin CP, Huang PH, Chen CY, Wu MY, Chen JS, Chen JW, *et al*. Sitagliptin attenuates arterial calcification by downregulating oxidative stress-induced receptor for advanced glycation end products in LDLR knockout mice. Sci Rep 2021;11(1):17851. doi:10.1038/s41598-021-97361-w, PMID:34497344.

[12] Lamers D, Famulla S, Wronkowitz N, Hartwig S, Lehr S, Ouwens DM, *et al*. Dipeptidyl peptidase 4 is a novel adipokine potentially linking obesity to the metabolic syndrome. Diabetes 2011;60(7):1917–1925. doi:10.2337/db10-1707, PMID:21593202.

[13] Sell H, Blüher M, Klöting N, Schlich R, Willems M, Ruppe F, *et al*. Adipose dipeptidyl peptidase-4 and obesity: correlation with insulin resistance and depot-specific release from adipose tissue in vivo and in vitro. Diabetes Care 2013;36(12):4083–4090. doi:10.2337/dc13-0496, PMID:24130353.

[14] Zheng T, Chen T, Liu Y, Gao Y, Tian H. Increased plasma DPP4 activity predicts new-onset hypertension in Chinese over a 4-year period: possible associations with inflammation and oxidative stress. J Hum Hypertens 2015;29(7):424–429. doi:10.1038/jhh.2014.111, PMID:25411054.

[15] Ghorpade DS, Ozcan L, Zheng Z, Nicoloro SM, Shen Y, Chen E, *et al*. Hepatocyte-secreted DPP4 in obesity promotes adipose inflammation and insulin resistance. Nature 2018;555(7698):673–677. doi:10.1038/nature26138, PMID:29562231.

[16] Zheng TP, Yang F, Gao Y, Baskota A, Chen T, Tian HM, *et al*. Increased plasma DPP4 activities predict new-onset atherosclerosis in association with its proinflammatory effects in Chinese over a four year period: A prospective study. Atherosclerosis 2014;235(2):619–624. doi:10.1016/j.atherosclerosis.2014.05.956, PMID:24968315.

[17] Zheng T, Baskota A, Gao Y, Chen T, Tian H, Yang F. Increased plasma DPP4 activities predict new-onset hyperglycemia in Chinese over a four-year period: possible associations with inflammation. Metabolism 2015;64(4):498–505. doi:10.1016/j.metabol.2014.12.004, PMID:25592717.

[18] Carl-McGrath S, Lendeckel U, Ebert M, Röcken C. Ectopeptidases in tumour biology: a review. Histol Histopathol 2006;21(12):1339–1353. doi:10.14670/HH-21.1339, PMID:16977585.

[19] Deacon CF. Dipeptidyl peptidase 4 inhibitors in the treatment of type 2 diabetes mellitus. Nat Rev Endocrinol 2020;16(11):642–653. doi:10.1038/s41574-020-0399-8, PMID:32929230.

[20] Gallwitz B, Rosenstock J, Rauch T, Bhattacharya S, Patel S, von Eynatten M, *et al*. 2-year efficacy and safety of linagliptin compared with glimepiride in patients with type 2 diabetes inadequately controlled on metformin: a randomised, double-blind, non-inferiority trial. Lancet 2012;380(9840):475–483. doi:10.1016/S0140-6736(12)60691-6, PMID:22748821.

[21] Perkovic V, Toto R, Cooper ME, Mann JFE, Rosenstock J, McGuire DK, *et al*. Effects of Linagliptin on Cardiovascular and Kidney Outcomes in People With Normal and Reduced Kidney Function: Secondary Analysis of the CARMELINA Randomized Trial. Diabetes Care 2020;43(8):1803–1812. doi:10.2337/dc20-0279, PMID:32444457.

[22] Sneha P, Doss CG. Gliptins in managing diabetes - Reviewing computational strategy. Life Sci 2016;166:108–120. doi:10.1016/j.lfs.2016.10.009, PMID:27744054.

[23] Deacon CF, Lebovitz HE. Comparative review of dipeptidyl peptidase-4 inhibitors and sulphonylureas. Diabetes Obes Metab 2016;18(4):333–347. doi:10.1111/dom.12610, PMID:26597596.

[24] 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(1):55–62. doi:10.1211/jpp/61.01.0008, PMID:19126297.

[25] Graefe-Mody U, Retlich S, Friedrich C. Clinical pharmacokinetics and pharmacodynamics of linagliptin. Clin Pharmacokinet 2012;51(7):411–427. doi:10.2165/11630900-000000000-00000, PMID:22568694.

[26] Kalra S, Unnikrishnan AG, Agrawal N, Singh AK. Linagliptin and newer DPP-4 inhibitors: newer uses and newer indications. Recent Pat Endocr Metab Immune Drug Discov 2011;5(3):197–202. doi:10.2174/187221411797265926, PMID:21913883.

[27] Deeks ED. Linagliptin: a review of its use in the management of type 2 diabetes mellitus. Drugs 2012;72(13):1793–1824. doi:10.2165/11209570-000000000-00000, PMID:22913735.

[28] 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(10):1171–1178. doi:10.1177/0091270008323753, PMID:18812608.

[29] Yki-Järvinen H, Rosenstock J, Durán-Garcia S, Pinnetti S, Bhattacharya S, Thiemann S, *et al*. Effects of adding linagliptin to basal insulin regimen for inadequately controlled type 2 diabetes: a ≥52-week randomized, double-blind study. Diabetes Care 2013;36(12):3875–3881. doi:10.2337/dc12-2718, PMID:24062327.

[30] Vaculikova E, Placha D, Pisarcik M, Peikertova P, Dedkova K, Devinsky F, *et al*. Preparation of risedronate nanoparticles by solvent evaporation technique. Molecules 2014;19(11):17848–17861. doi:10.3390/molecules191117848, PMID:25375330.

[31] Shono Y, Jantratid E, Janssen N, Kesisoglou F, Mao Y, Vertzoni M, *et al*. Prediction of food effects on the absorption of celecoxib based on biorelevant dissolution testing coupled with physiologically based pharmacokinetic modeling. Eur J Pharm Biopharm 2009;73(1):107–114. doi:10.1016/j.ejpb.2009.05.009, PMID:19465123.
[32] Abuhelwa AY, Williams DB, Upton RN, Foster DJ. Food, gastrointestinal pH, and models of oral drug absorption. Eur J Pharm Biopharm 2017;112:234–248. doi:10.1016/j.ejpb.2016.11.034, PMID:27914234.
[33] Baxevanis F, Kuiper J, Fotaki N. Fed-state gastric media and drug analysis techniques: Current status and points to consider. Eur J Pharm Biopharm 2016;107:234–248. doi:10.1016/j.ejpb.2016.07.013, PMID:27422208.
[34] Won CS, Oberlies NH, Paine MF. Mechanisms underlying food-drug interactions: inhibition of intestinal metabolism and transport. Pharmacol Ther 2012;136(2):186–201. doi:10.1016/j.pharmthera.2012.08.001, PMID:22884524.
[35] Friedrich C, Shi X, Zeng P, Ring A, Woerle HJ, Patel S. Pharmacokinetics of single and multiple oral doses of 5 mg linagliptin in healthy Chinese volunteers. Int J Clin Pharmacol Ther 2012;50(12):889–895. doi:10.5414/CP201802, PMID:23073142.
[36] Sarashina A, Sesoko S, Nakashima M, Hayashi N, Taniguchi A, Horie Y, *et al*. Linagliptin, a dipeptidyl peptidase-4 inhibitor in development for the treatment of type 2 diabetes mellitus: a Phase I, randomized, double-blind, placebo-controlled trial of single and multiple escalating doses in healthy adult male Japanese subjects. Clin Ther 2010;32(6):1188–1204. doi:10.1016/j.clinthera.2010.06.004, PMID:20637971.
[37] Ceriello A, Inagaki N. Pharmacokinetic and pharmacodynamic evaluation of linagliptin for the treatment of type 2 diabetes mellitus, with consideration of Asian patient populations. J Diabetes Investig 2017;8(1):19–28. doi:10.1111/jdi.12528, PMID:27180612.
[38] Guedes EP, Hohl A, de Melo TG, Lauand F. Linagliptin: farmacology, efficacy and safety in type 2 diabetes treatment. Diabetol Metab Syndr 2013;5(1):25. doi:10.1186/1758-5996-5-25, PMID:23697612.
[39] Russo E, Penno G, Del Prato S. Managing diabetic patients with moderate or severe renal impairment using DPP-4 inhibitors: focus on vildagliptin. Diabetes Metab Syndr Obes 2013;6:161–170. doi:10.2147/DMSO.S28951, PMID:23650450.
[40] Golightly LK, Drayna CC, McDermott MT. Comparative clinical pharmacokinetics of dipeptidyl peptidase-4 inhibitors. Clin Pharmacokinet 2012;51(8):501–514. doi:10.1007/BF03261927, PMID:22686547.
[41] Graefe-Mody U, Giessmann T, Ring A, Iovino M, Woerle HJ. A randomized, open-label, crossover study evaluating the effect of food on the relative bioavailability of linagliptin in healthy subjects. Clin Ther 2011;33(8):1096–1103. doi:10.1016/j.clinthera.2011.07.005, PMID:21803422.
[42] Zhang H, Xia B, Sheng J, Heimbach T, Lin TH, He H, *et al*. Application of physiologically based absorption modeling to formulation development of a low solubility, low permeability weak base: mechanistic investigation of food effect. AAPS PharmSciTech 2014;15(2):400–406. doi:10.1208/s12249-014-0075-1, PMID:24435225.
[43] Chan JC, Malik V, Jia W, Kadowaki T, Yajnik CS, Yoon KH, *et al*. Diabetes in Asia: epidemiology, risk factors, and pathophysiology. JAMA 2009;301(20):2129–2140. doi:10.1001/jama.2009.726, PMID:19470990.
[44] Ramachandran A, Snehalatha C, Vijay V. Low risk threshold for acquired diabetogenic factors in Asian Indians. Diabetes Res Clin Pract 2004;65(3):189–195. doi:10.1016/j.diabres.2004.03.012, PMID:15331198.
[45] Ma RC, Chan JC. Type 2 diabetes in East Asians: similarities and differences with populations in Europe and the United States. Ann N Y Acad Sci 2013;1281:64–91. doi:10.1111/nyas.12098, PMID:23551121.
[46] Takeuchi M, Okamoto K, Takagi T, Ishii H. Ethnic difference in inter-