Xenobiotica
the fate of foreign compounds in biological systems

ISSN: 0049-8254 (Print) 1366-5928 (Online) Journal homepage: www.tandfonline.com/journals/ixen20

The metabolism and excretion of the dipeptidyl peptidase 4 inhibitor [^{14}C] cetagliptin in healthy volunteers

Jinmiao Lu, Yicong Bian, Hua Zhang, Dong Tang, Xusheng Tian, Xinyi Zhou, Zengyan Xu, Yating Xiong, Zheming Gu, Zhenwen Yu, Tong Wang, Juping Ding, Qiang Yu & Jinsong Ding

To cite this article: Jinmiao Lu, Yicong Bian, Hua Zhang, Dong Tang, Xusheng Tian, Xinyi Zhou, Zengyan Xu, Yating Xiong, Zheming Gu, Zhenwen Yu, Tong Wang, Juping Ding, Qiang Yu & Jinsong Ding (2022) The metabolism and excretion of the dipeptidyl peptidase 4 inhibitor [^{14}C] cetagliptin in healthy volunteers, Xenobiotica, 52:1, 38-45, DOI: 10.1080/00498254.2021.2002973

To link to this article: https://doi.org/10.1080/00498254.2021.2002973

XENOBIOTICA
2022, VOL. 52, NO. 1, 38–45
https://doi.org/10.1080/00498254.2021.2002973

Taylor & Francis
Taylor & Francis Group

RESEARCH ARTICLE

The metabolism and excretion of the dipeptidyl peptidase 4 inhibitor [^{14}C] cetagliptin in healthy volunteers

Jinmiao Lu[a,b], Yicong Bian[c], Hua Zhang[c], Dong Tang[b], Xusheng Tian[b], Xinyi Zhou[b], Zengyan Xu[b], Yating Xiong[d], Zheming Gu[d], Zhenwen Yu[d], Tong Wang[b], Juping Ding[b], Qiang Yu[b] and Jinsong Ding[a]

[a]Xiangya School of Pharmaceutical Sciences, Central South University, Hunan, China; [b]CGeneTech (Suzhou, China) Co., Ltd, Jiangsu, China; [c]Department of Clinical Pharmacology, the First Affiliated Hospital of Soochow University, Suzhou, China; [d]Value Pharmaceutical Services Co., Ltd, Jiangsu, China

ABSTRACT

1. The metabolism and excretion of cetagliptin were investigated in healthy male subjects after a single oral dose of 100mg/50μCi [^{14}C] cetagliptin.
2. The mean concentration-time profile of cetagliptin was similar to that of total radioactivity in plasma after oral administration of [^{14}C] cetagliptin in healthy male subjects. Cetagliptin was rapidly absorbed after oral administration. Unchanged cetagliptin was the most abundant radioactive component in all matrices investigated. Approximately 53.13% of plasma AUC of total radioactivity was accounted for by cetagliptin. Each metabolite plasma AUC was not higher than 2.93% of plasma AUC of total radioactivity. By 336 h after administration, 91.68% of the administered radioactivity was excreted, and the cumulative excretion in the urine and faeces was 72.88% and 18.81%, respectively. The primary route of excretion of radioactivity was via the kidneys.
3. Four metabolites were detected at trace levels, and it involved hydroxylated (M436-1 and M436-3), N- sulphate (M500), and N-carbamoyl glucuronic acid conjugates (M640B) of cetagliptin. These metabolites were detected also in plasma, urine, and faeces at low levels, except that metabolite M640B was not detected in faeces. All metabolites were observed with <10% of parent compound systemic exposure after oral administration.

ARTICLE HISTORY
Received 17 September 2021
Revised 2 November 2021
Accepted 2 November 2021

KEYWORDS
Cetagliptin; metabolism; excretion; metabolite; healthy subjects

Introduction

Type-2 diabetes mellitus (T2DM) is a complex and chronic disease caused by insulin resistance in the peripheral tissues. The high frequency of complications associated with the disease leads to a significant reduction in life expectancy (DeFronzo 1988). Dipeptidyl peptidase-4 (DPP-4) inhibitors represent a therapeutic option for the treatment of type-2 diabetes (Deacon 2004; Ahren and Schmitz 2004). The incretin hormones glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide stimulates meal-induced insulin secretion and contributes to glucose homoeostasis (Kieffer and Habener 1999). They can be rapidly degraded and inactivated by DPP-4 (Gorrell 2005). DPP-4 inhibitors augments endogenous GLP-1 activity, which leads to a clinically significant lowering of diabetic glycaemia (Drucker 2002). They exhibit several properties, including glucose-dependent stimulation of insulin secretion, suppression of glucagon secretion and slowing of gastric emptying, which result in improvements in glycaemic control in patients with type-2 diabetes (Rajput 2009). Several pharmaceutical companies have been developed DPP-4 inhibitors (such as sitagliptin, vildagliptin, linagliptin, teneligliptin) with different chemical structures for the treatment of type-2 diabetes. Although DPP-4 inhibitors share a common mode of action, they differ in terms of their chemical structure, pharmacokinetic, pharmacodynamic and safety profiles, and clinical characteristics (Deacon 2011). The metabolism and excretion of these DPP-4 inhibitors have been investigated in humans, and the biotransformation pathways were clear (Vincent et al. 2007; He et al. 2009; Blech et al. 2010; Nakamaru et al. 2014).

Cetagliptin a novel, orally active, highly specific, and potent inhibitor of DPP-4 that is currently in clinical development for the treatment of patients with type-2 diabetes. It was developed by CGeneTech (Suzhou, China) Co., Ltd. Both rapid absorption and prolonged duration of cetagliptin demonstrate the potential value of cetagliptin for diabetes treatment (Bai et al. 2020). Based on in vitro study results, cetagliptin did not inhibit CYP1A2, CYP2C8, CYP2B6, CYP2C9, CYP2C19, and CYP3A4, only has a moderate inhibitory effection CYP2D6, and did not induce CYP1A2, CYP2B6, and CYP3A4. Plasma protein binding of cetagliptin did not have species differences or concentration dependence. (Lu et al. 2021).

When drugs are administered to living organisms, they undergo a series of events such as absorption, distribution,

CONTACT Jinsong Ding dingjs0221@163.com Xiangya School of Pharmaceutical Sciences, Central South University, Changsha 410013, China; Qiang Yu yuq@cgenetech.com.cn CGeneTech (Suzhou, China) Co., Ltd, Suzhou 215123, Jiangsu, China

metabolism and excretion (ADME) and these events modulate the efficacy and toxicity of drugs. Understanding the clearance mechanism, distribution and the metabolic fate of a drug candidate in humans is a key factor in new drug development, registration and ultimate use. in addition to good pharmacological activity, ADME properties are crucial determinants of the ultimate clinical success of a drug candidate. Labelling compounds with carbon-14 or H-3 (tritium) is by far the most widely used technique and the choice is usually between these two isotopes when planning a human mass balance study (Penner et al. 2009). However, the pros of the tritium use are outweighed by the cons, notably, the lack of biological stability and isotopic effect (switching biotransformation pathway due to reaction kinetics) (Shaffer et al. 2006). In a drug development setting, ^{14}C-labelling is used in the majority of ADME studies (Penner et al. 2009).

However, we lack of the biotransformation pathways of cetagliptin in humans. In order to find out the metabolic pathway for cetagliptin in humans, we report here this study was to investigate the metabolism and excretion of cetagliptin in healthy male volunteers after oral administration of 100 mg/50μCi [^{14}C] cetagliptin ((8 R)-7-[(3R)-3-Amino-1-oxo-4-(2,4,5-trifluorophenyl)butyl]-5,6,7,8-tetrahydro-8-methyl-3-(trifluoromethyl)-imidazo[1,5-a]pyrazine phosphate monohydrate). This study was performed to further establish the pharmacokinetics and metabolism of cetagliptin in humans after oral administration.

Materials and methods

Study drug and dose preparation

[^{14}C] cetagliptin was synthesised as the phosphate salt (specific activity 47.0 mCi/mmol, radiochemical purity 100%) by WuXi AppTec (Shanghai) Co., Ltd. The chemical structure of cetagliptin and the position of the radiolabel are shown in Figure 1. For oral administration, it was blended with nonlabeled cetagliptin for human use (specific radioactivity 0.500μCi/mg, radiochemical purity ≥95%) by Value Pharmacetical Services Co., Ltd. Nonradioactive cetagliptin and reference compound were synthesised and analysed at by CGeneTech (Suzhou, China) Co., Ltd. The dose was prepared as solid powder contained in a vial with 100 mg/50μCi [^{14}C] cetagliptin.

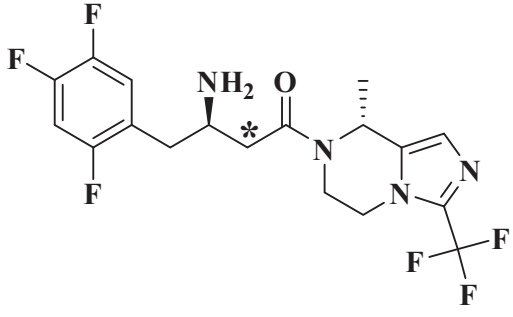

Figure 1. Chemical structure of [^{14}C] cetagliptin, ★ indicates position of C-14 label.

Clinical study design

This was a single centre, open label, and non-randomized study (Bian et al. 2021). The study was conducted at the First Affiliated Hospital of Soochow University (Suzhou, China), the study protocol and its amendments were approved by the ethics committee (chinadrugtrials.org registry number: CTR20200163). The written informed consent was obtained from all the subjects before enrolment. Six healthy, male volunteers, age 18 to 45 years, with weights ranging from 60 to 77 kg, participated in the study. All subjects fulfilled inclusion criteria and did not meet exclusion criteria. Subjects were admitted to the clinical research unit the evening the day before dosing and remained in the unit until the completion of all laboratory collections for the duration of the study. Subjects abstained from food at least 10 h, and abstained from drink at least 1 h before dosing. Subjects were given a single p.o. 100 mg/50μCi dose of [^{14}C] cetagliptin as a 240 ml drinking solution. After administration, the subjects continued to abstain from food for 4 h and water for 1 h. A standardised diet was given to each subject.

Sample collection, storage, and shipping conditions

Blood was collected at 0 (1 h before dosing), 0.5, 1, 2, 3, 4, 5, 6, 8, 12, 24, 48, 72, 96, 120, 144, 192, 240, 288, and 336 h post-dose by either direct venipuncture or an indwelling cannula inserted in a forearm vein using K_2EDTA as anticoagulant. Seven millilitres of venous blood were collected at each time point. Plasma was separated from whole blood by centrifugation for pharmacokinetic analysis. Blood sampling time points for metabolic study after oral dosing was as follows: 1, 4, 8, 24, and 48 h. Eight millilitres of venous blood was collected at each time point. Blood was collected at 0, 1, 4, 8, 24, and 48 h post-dose for the study of total radioactivity distribution ratio of whole blood and plasma. Two millilitres of venous blood were collected at each time point. Urine samples were collected at −2 4 ∼ 0 h pre-dose, and at 0 to 4, 4 to 8, 8 to 12, 12 to 24, 24 to 48, 48 to 72, 72 to 96, 96 to 120, 120 to 144, 144 to 168, 168 to 192, 192 to 216, 216 to 240, 240 to 264, 264 to 288, 288 to 312, and 312 to 336 h post-dose. Faeces were collected at −2 4 ∼ 0 h pre-dose, and at 0 to 24, 24 to 48, 48 to 72, 72 to 96, 96 to 120, 120 to 144, 144 to 168, 168 to 192, 192 to 216, 216 to 240, 240 to 264, 264 to 288, 288 to 312, and 312 to 336 h post-dose. The criteria for termination of sample collection as follows: The total radioactivity of urine and faeces collected from each volunteer exceeded 80% of the administered radioactivity, and radioactivity collected for two consecutive days was less than 1% of the administered radioactivity; radioactivity concentration in blood or plasma was three times less than background at two consecutive time points.

Plasma was stored at −60 °C to −90 °C, whole blood, urine samples and faeces were stored at −10 °C to −30 °C until analysis. All of the samples were shipped on dry ice.

Radioactivity analysis of blood, plasma, urine, and faeces samples

Radioactivity in plasma and urine was analysed by liquid scintillation counting (LSC) on a liquid scintillation analyser (Tri-Carb 4910TR, PerkinElmer Life and Analytical Sciences, Downers Grove, IL). Plasma and urine were mixed with scintillant and counted directly. Radioactivity in whole blood and faeces was also assessed by LSC. Whole blood samples and faecal homogenates were combusted in a biological oxidiser (HTC-501, Hualida Laboratory Equipment Co., Ltd.). The [^{14}C]-labelled carbon dioxide released was trapped in scintillation fluid. Radioactivity was determined by liquid scintillation counting as specified above. Lower limits of quantification (LOQ) of total radioactivity were expressed as nanogram equivalent [^{14}C] cetagliptin per gram of blank matrix. They accounted for 306 ng Eq./g (faeces), 91.8 ng Eq./g (urine), 184 ng Eq./g (whole blood) and 46.8 ng Eq./g (plasma).

Analysis of unchanged cetagliptin

Amounts of unchanged cetagliptin in plasma were measured quantitatively using a validated high-performance liquid chromatography/tandem mass spectrometry (HPLC/MS/MS) assay. Plasma was pre-treated by protein precipitation. Samples were analysed on a HPLC system (HPLC 20-AD, Shimadzu, Kyoto, Japan) with series quadrupole mass spectrometer (5500 QTRAP, Applied Biosystems, Darmstadt, Germany). Cetagliptin and Internal standard (IS) were separated on a column (ZORBAX Eclipse XDB-C18, 2.1 × 50mm, 3.5 μm). The mobile phase of A/B was used, where A was aqueous solution with 0.1% methanol and 1 mmol/l ammonium acetate, and B was Acetonitrile solution. The flow rate was maintained at 0.50 ml/min with an injection volume of 7.0 μl.

Data were acquired using Analyst™ 1.6.2 software (SCIEX, USA). The calibration ranges for cetagliptin were linear from 2.50 to 5000 ng/ml. Assay accuracy ranges were −5.50 to 5.00 and precision (coefficient of variation (%)) was 3.32 of quality control samples.

Sample preparation for metabolite investigation

Plasma samples were treated using the following procedure: (1) extracted with acetonitrile (3x, v/v), vortexed 5 min, and ultrasonic treatment 5 min; (2) incubated 30 min at 4 °C; (3) vortexed 2 min, centrifuged 10 min (10000 g, 4 °C); (4) the residues were reconstituted in 0.5 ml pure water; (5) two washes with methanol (2x, v/v); (6) extraction was combined, and then evaporated to near dryness under a stream of nitrogen; (7) reconstituted in methanol and pure water (1:1, v/v), centrifuged 10 min (10000 g, 4 °C). Supernatants were used for metabolite profiling.

For urine analysis, a pool of equal percent volume from the 0 to 168 h fractions was prepared for each subject (1001, 1002, 1003, 1004, 1005 and 1006). The resulting pools were centrifuged 10 min (10000 g, 4 °C). Supernatants were used for metabolite profiling.

Faeces homogenates were pooled from 0 to 168 h at equal percent weight for each subject, and extracted with acetonitrile (3x, v/v) by vortexing (5 min) and centrifugation (10 min, 10000 *g*, 4 °C). The residues were reconstituted in pure water (1x, v/v), extracted twice with methanol (2x, v/v). Extraction was combined, then evaporated to near dryness under a stream of nitrogen, and reconstituted in methanol/20 mM ammonium acetate, pH 5.50 (1:1, v/v). Reconstitution was centrifuged 10 min (10000 *g*, 4 °C). Supernatants were used for metabolite profiling.

Metabolite pattern analysis

All samples were analysed by reverse-phase HPLC coupled to online radioactivity detection. HPLC/online radioactivity detection systems consisted HPLC system (Shimadzu LC-20AD, Shimadzu, Kyoto, Japan), chromatographic column (ACE Excel 3 C18, 150 × 4.6 mm id), maintained at 40 °C, guard column (ACE, Guard Cartridge, 4.0 × 4.6 mm), radioactivity detector (vARC Radio-Assay System, Model 3). The mobile phase A was aqueous solution with 0.4% methanol, pH 3.2. The mobile phase A was acetonitrile. A gradient method was used. Samples were separated by HPLC column. The HPLC effluent was fractionated into a 96-deepwell Lumaplate™ (PerkinElmer Life and Analytical Sciences) using a fraction collector. Effluent radioactivity was detected by the radioactivity detector. The results were converted using ARC®Convert (ARC Data System 3, Version: 3.0.2.379) and Evaluation software to obtain off-line radioactivity metabolite profiles. Radiochromatograms of each sample were integrated to obtain each radioactive metabolite peak areas, and the percentage of each radioactivity peak in the radiochromatogram (%HPLC). Percentage of major metabolites in total plasma radiation exposure (%AUC) (or in dosage (%Dose)) was calculated from the percentage of major metabolites in the total radioactivity of the sample.

Structural characterization of major metabolites

Major metabolite characterisation was conducted with HPLC/radioactivity detection system and mass spectrometer (Thermo Fisher Scientific, Waltham, MA) equipped with an electrospray ionisation (Turbo Ion Spray). The electrospray interface was operated at 3800 V. and the mass spectrometer was operated in the positive-ion mode. The capillary temperature was set to 320 °C and the collision energy was set to 35 eV. Scan model was Q1 MS and MS/MS. The systems were operated by Xcalibur™ Software (version 4.3).

Pharmacokinetic analysis

The percentage of radioactivity urine and faces in dosage, mean value and standard deviation were calculated using Microsoft Office Excel (version 2010). Radioactivity concentrations of plasma and whole blood were converted into nanogram (ng) cetagliptin in gram (g) plasma and gram (g) whole blood (ng Eq./g). Plasma concentrations of cetagliptin and radioactivity were calculated using WinNonlin software

(version 8.3; Pharsight Corporation, Mountain View, CA). The following pharmacokinetic parameters were determined by fitting the concentration-time profiles to a noncompartmental model with an iterative nonlinear regression program (WinNonlin software version 8.3; Pharsight, Mountain View, CA): time to highest observed drug concentration (T_{max}); highest observed blood or plasma drug concentration (C_{max}); area under the blood or plasma drug concentration-time curve between time 0 and time t (AUC_{0-t}); AUC until time infinity ($AUC_{0-\infty}$); elimination half-life ($t_{1/2}$); apparent clearance (CL/F); apparent volume of distribution of parent drug (Vz/F).

Results

Pharmacokinetics of cetagliptin and total radioactivity

The mean plasma radioactivity concentration versus time profiles and plasma pharmacokinetic parameters of total radioactivity and unchanged cetagliptin in healthy male volunteers after a single oral dose of [^{14}C] cetagliptin are shown in Figure 2 and Table 1. The highest concentrations in plasma (C_{max}) were achieved at 0.75 h postdose with the mean value 415 ng Eq./ml (total radioactivity), and at 0.50 h with the mean value 245 ng/ml (cetagliptin). The terminal elimination half-life ($t_{1/2}$) of radioactivity (28.0 h) and cetagliptin (23.4 h) were similar. Plasma AUC_{0-t} in six healthy volunteers subjects with a mean value of 5960 h·ng Eq./ml for total radioactivity and 2890 h·ng/ml for cetagliptin. The mean C_{max} and AUC_{0-t} of cetagliptin were 59.04% and 48.49% of the C_{max} and AUC_{0-t} of total radioactivity, respectively. Cetagliptin was the major radioactive component in plasma. All blood radioactivity at 24 h was below the limit of quantification. In addition, mean blood-to-plasma ratios of radioactivity calculated at specific time points (between 1 and 8 h) was 0.689 to1.50, indicating that cetagliptin generally distributed almost equally between blood cells and plasma as the main circulating component was the parent drug at the early time points and there was no significant binding between cetagliptin and blood cells.

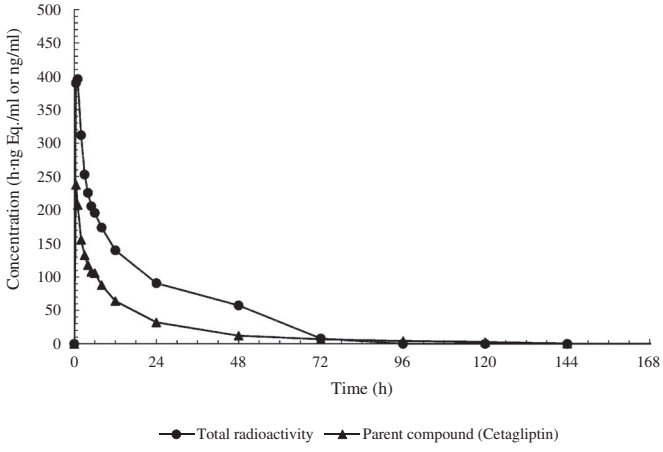

Figure 2. Mean concentration-time profiles of cetagliptin and total radioactivity in plasma after oral administration of [^{14}C] cetagliptin in healthy male volunteers.

Table 1. Plasma pharmacokinetic parameters (mean ± S.D.) of cetagliptin and radioactivity after administration of a single oral 100 mg/50μCi [^{14}C] cetagliptin to healthy volunteers.

Pharmacokinetic Parameters[a]	Total Radioactivity	Cetagliptin
T_{max} (h)	0.750 (0.500, 1.00)[b]	0.500 (0.500, 1.00)[b]
C_{max} (ng Eq./ml[c])	415 ± 84.4	245 ± 74.7
AUC_{0-t} (h·ng Eq./ml[d])	5960 ± 1110	2890 ± 204
$AUC_{0-\infty}$ (h·ng Eq./ml[d])	8480 ± 2660	3000 ± 209
MRT_{0-t} (h)	17.6 ± 3.59	22.4 ± 2.71
$t_{1/2}$ (h)	28.0 ± 10.9	23.4 ± 2.99
Vz/F (L)	465 ± 72.2	1130 ± 163
CL/F (L/h)	13.0 ± 5.01	33.5 ± 2.29

[a]The abbreviation definitions for pharmacokinetic parameters, e.g., C_{max}, T_{max}, AUC, $t_{1/2}$, CL/F, and Vz/F are denoted in the Pharmacokinetic Analysis.
[b]Median and range.
[c]Unit for cetagliptin = ng/ml.
[d]Unit for cetagliptin = h·ng/ml.

Excretion and mass balance in urine and faeces

The cumulative excretion of total radioactivity in urine and faeces after a single dose of 100 mg/50μCi [^{14}C] cetagliptin to healthy male volunteers are shown in Figure 3 and Table 2. Total recovery of the administered radioactivity in urine and faeces was 91.68% by 336 h post-dose with 72.88% and 18.81% in urine and faeces, respectively. 89.97% of the administered radioactivity was excreted in the urine and faeces by 192 h after administration.

Metabolite profiles in plasma, urine and faeces

Metabolite profiles of a pooled sample of in plasma, urine and faeces are shown in Table 3. Figure 4 shows representative HPLC radiochromatograms of plasma, urine and faeces.

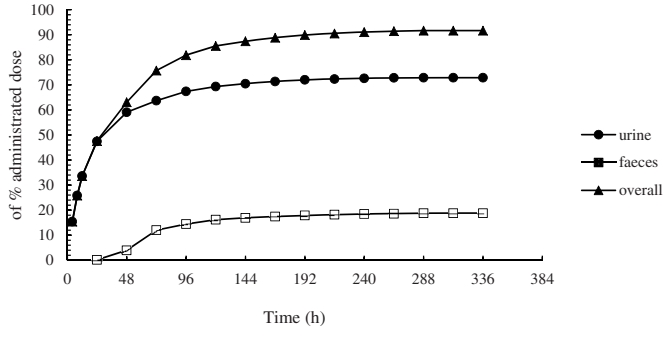

Figure 3. Cumulative excretion of total radioactivity in urine, faeces and overall after a single oral administration of 100 mg/50μCi [^{14}C] cetagliptin.

Table 2. Cumulative excretion of ^{14}C radioactivity in urine and faeces after a single dose of 100 mg/50μCi [^{14}C] cetagliptin.

Subject	Urine (% of dose)	Faeces (% of dose)	Total
1001[a]	74.21	16.72	90.93
1002[a]	71.12	22.80	93.92
1003[b]	74.25	18.78	93.03
1004[b]	71.10	18.90	90.00
1005[b]	76.09	13.45	89.54
1006[b]	70.49	22.19	92.68
mean	72.88	18.81	91.68
S.D.	2.28	3.48	1.78

[a]1001 and 1002 urine and faeces samples were collected from 0 to 336 h.
[b]1003, 1004, 1005, 1006 urine and faeces samples were collected from 0 to 288 h.

Table 3. Metabolite pattern in plasma, urine, and faeces after a single oral dose of 100 mg/50μCi [^{14}C] cetagliptin.

		Plasma		Urine		Faeces	
		%AUC		%Dose (72.88%)		%Dose (18.81%)	
Compound	Metabolite type	Mean	S.D.	Mean	S.D.	Mean	S.D.
Cetagliptin	Parent drug	53.13	10.23	37.53	5.14	3.21	0.896
M436-1	Monooxide of parent drug	1.42	2.45	4.77	1.28	1.27	0.336
M436-3	Monooxide of parent drug	1.71	1.76	2.15	0.918	0.64	0.140
M500	Sulphate conjugate of parent drug	2.46	1.53	0.53	0.821	ND[a]	
M640B	Carbamoyl glucuronic acid conjugate of parent drug						
Solid residue after extraction		NA[b]		NA[b]		6.65	1.25

[a]Not detected.
[b]Not applicable.

Four known metabolites (M436-1, M436-3, M500, M640B) were detected by HPLC-MS/MS. The metabolism of cetagliptin is presented in Figure 5. In plasma, major circulating component was cetagliptin, accounting for 53.13% of the total plasma exposure. No major metabolite was found in plasma, and each metabolite accounting for <2.93%. Cetagliptin was the major radioactive component, comprising 37.53% of the urinary radioactivity. M436-1 represented the major metabolite (4.77% of the administered dose) in urine, and all the other metabolites were minor. In the faeces, total recovery of the administered radioactivity of solid residue after extraction was 6.65% of the administered dose. Cetagliptin was the most abundant component in faeces (3.21% of the administered dose), followed by M436-1. M640B was not detected in faeces.

Structure identification of metabolites

Metabolite structures were characterised by HPLC-MS/MS. The structure of major metabolites, where possible, was supported by comparisons of their retention times on HPLC and mass spectra with reference compounds ([^{14}C] cetagliptin).

HPLC retention time from 34.8 min to 34.9 min was reference compound ([^{14}C] cetagliptin). Cetagliptin displayed a protonated molecular ion $[M+H]^+$ at *m/z* 421.1445. The product ion spectrum of *m/z* 421.1445 showed major fragment ions at *m/z* 174 and 248 by degradation of the C-C bond. Fragment ion *m/z* 206 were formed by direct loss of C_2H_2O from fragment ion *m/z* 248. The subsequent degradation of the C-C bond resulted in *m/z* 86. The product ion mass spectrum of *m/z* 421.1445 produced piperazine ring degradation to give fragment ion *m/z* 259. Full-scan analysis of cetagliptin gave $[M+H]^+$ at *m/z* 421.1438. HPLC retention time from 35.4 to 35.9 min. All fragments ions and retention time were consistent with the structure of reference compound.

Metabolite M436-1 was found in plasma, urine and faeces. HPLC retention time from 30.1 min to 30.6 min. M436-1 displayed an $[M+H]^+$ of *m/z* 437.1388. The fragment ion *m/z* 248, 206 and 86 showed product ions identical to those of reference compound. Due to piperazine ring degradation and dehydrogenation, the fragment ion *m/z* 488 gave *m/z* 257, followed by C-C bond degradation (*m/z* 190). the fragment ion (*m/z* 172) was formed from *m/z* 190 dehydration. All fragments ions were consistent with the structure of M436-1, and it was identified as the hydroxylated of cetagliptin.

Metabolite M436-3 was found in plasma, urine and faeces. HPLC retention time from 32.6 min to 33.6 min. M436-3 displayed an $[M+H]^+$ of *m/z* 437.1389. The fragmentation pathway of M436-3 was the same as that of M436-1. All fragments ions were consistent with the structure of M436-3.

Metabolite M500 was found in plasma and urine. HPLC retention time from 39.1 min to 39.6 min. Full-scan analysis of M500 gave $[M+H]^+$ at *m/z* 501.1004. The fragment ion was formed by the loss of fragment 80 of M500. Subsequent fragmentation pathway was the same as that of parent drug. All fragments ions were consistent with the structure of M500, and it was identified as the N- sulphate of cetagliptin.

Metabolite M640B was found in plasma and urine. HPLC retention time from 39.1 to 39.6 min. M640B displayed an $[M+H]^+$ of *m/z* 641.1647. The product ion mass spectrum of *m/z* 641.1647 produced loss of acyl - glucuronic acid (220) to give *m/z* 421. Subsequent fragmentation pathway was the same as that of parent drug. All fragments ions were consistent with the structure of M640B, and it was identified as the N-carbamoyl glucuronic acid conjugates of cetagliptin.

Safety evaluation

There were no safety concerns related to the single oral administration of 100 mg/50μCi [^{14}C] cetagliptin in six healthy male subjects. One subject reported a total of two clinical AEs in this study. All AEs were mild, transient, and resolved at end of the study. There were no apparent treatment-related clinically relevant changes in vital signs and clinical laboratory tests. No SAE happened, and no subject dropped out because of AE.

Discussion

The determination of metabolic pathways of a drug candidate through the identification of circulating and excreted metabolites is vitally important to understanding its physical and biological effects (Penner et al. 2009). Human metabolites that can raise a safety concern are those present at greater than 10 percent of total drug-related exposure at steady state (US Food and Drug Administration 2020). The ADME studies with radiolabelled drug are considered to be able to provide more detailed metabolism data (Ministry of

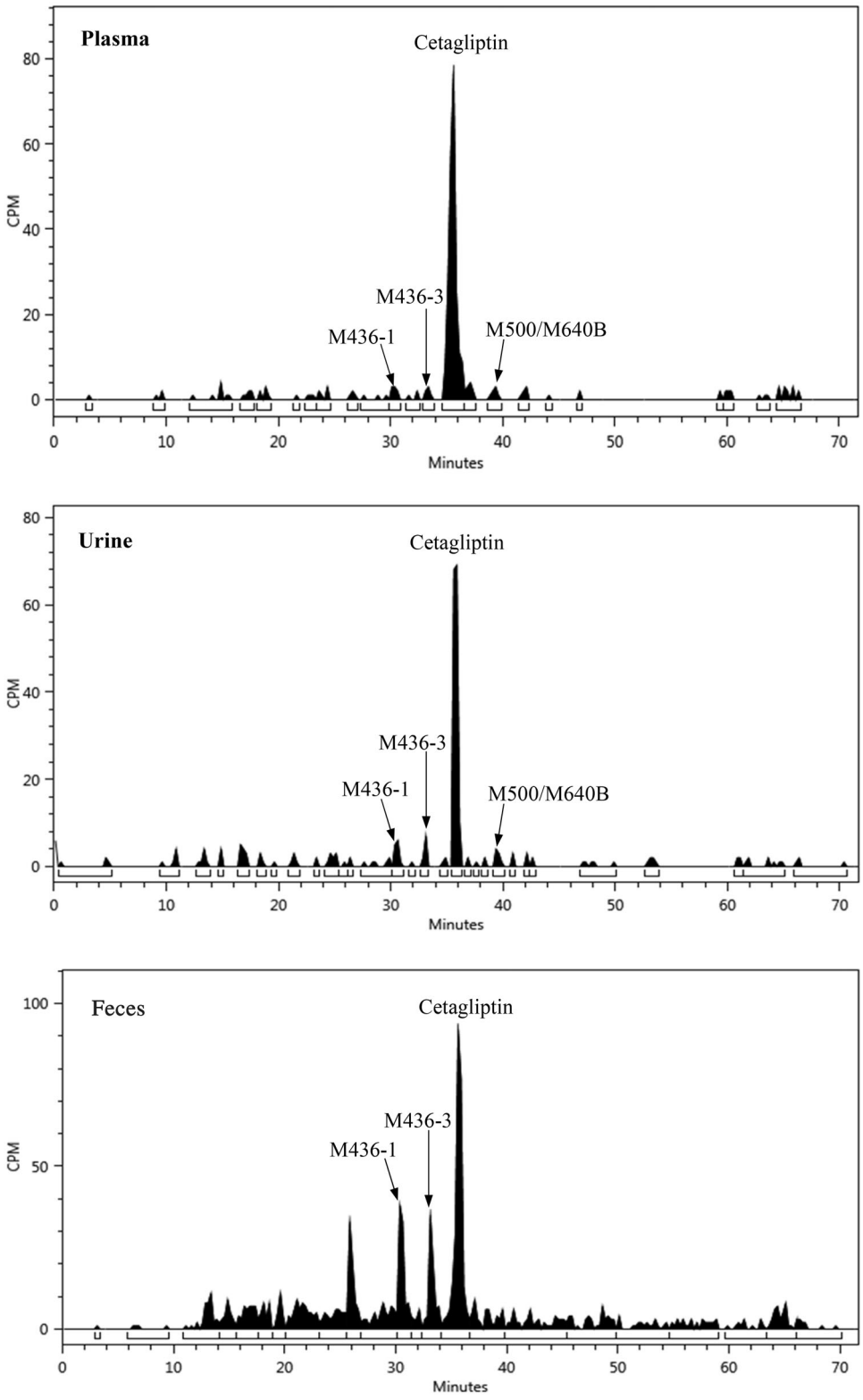

Figure 4. Representative HPLC-LSC radiochromatograms of plasma, urine (0-168 h) and faeces (48-96 h) after oral administration of [^{14}C] cetagliptin. Plasma, urine and faecal homogenate extracts pooled across subjects.

Health labour and Welfare 2001; US Food and Drug Administration 2018).

In this study, we investigated the absorption, metabolism and excretion of cetagliptin after oral administration of 100 mg/50μCi [^{14}C] cetagliptin to healthy volunteers. The radioactivity pharmacokinetic data indicate that total plasma radioactivity concentrations reached C_{max} rapidly (T_{max}: 0.75 h) postdose. Cetagliptin makes up the majority of the radioactivity in plasma (as determined by the ratio of cetagliptin AUC and total radioactivity AUC), and the remaining radioactivity accounted for by the metabolites. Due to their low levels in plasma, these metabolites would not be expected to contribute to the pharmacological activity of cetagliptin. According to the ratio (0.689-1.50) of total

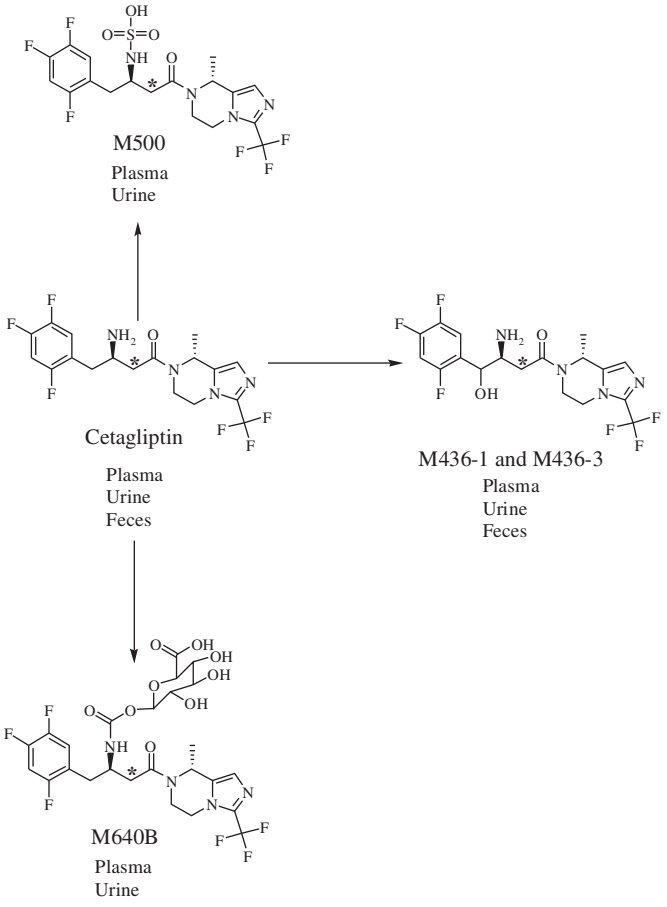

Figure 5. Metabolism pathways of cetagliptin in humans, the position of the ^{14}C radiolabel is indicated by an asterisk.

radioactivity of whole blood to plasma, there was no significant binding between cetagliptin and blood cells.

Total recovery of radioactivity accounted for 91.68% of the dose. The primary recovered dose was eliminated via the urine with 72.88% of the dose. The results indicated that cetagliptin was well absorbed after oral administration and most of the radioactive dose was excreted via the kidney. The primary route of elimination of cetagliptin in healthy subjects is via renal excretion of cetagliptin. Unchanged cetagliptin was the majority of the recovered dose in urine and faeces, accounted for 37.53% and 3.21% of the dose, respectively. The unchanged cetagliptin found in faeces may represent unabsorbed material. Metabolism of cetagliptin was minimal. Four major metabolites were identified, and it involved hydroxylated (M436-1 and M436-3), N-sulphate (M500), and N-carbamoyl glucuronic acid conjugates (M640B) of cetagliptin. Results from the metabolites profiling studies of plasma, urinary and faecal indicate that no metabolite was accounting for >10% of the $AUC_{0-\infty}$ of the total radioactivity found in plasma, and metabolites were minor in urine and faeces. As described above, cetagliptin is significantly cleared through multiple pathways including renal excretion and metabolism from the human body.

Conclusions

In healthy male subjects, cetagliptin was rapidly absorbed after oral administration. Cetagliptin was the major radioactive component in plasma, urine, and faeces. The administered cetagliptin was eliminated primarily by renal excretion. Four major metabolites were detected, each representing 1.42 ~ 2.46% of the radioactivity in plasma. These metabolites were the hydroxylated, N-sulphate and N-carbamoyl glucuronic acid conjugates of cetagliptin, and were detected in urine and faeces at low levels.

Acknowledgments

The authors acknowledge the First Affiliated Hospital of Soochow University (Suzhou, China) for conducting this study. The authors acknowledge Value Pharmaceutical Services Co., Ltd for the radiometric analysis of the human plasma, faeces and urine.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This study was sponsored by CGeneTech (Suzhou, China) Co., Ltd. The work is supported by China national science and technology major project for significant new drug development funding project [2018ZX09301007-001].

ORCID

Jinmiao Lu http://orcid.org/0000-0002-3782-6645

References

Ahren B, Schmitz O. 2004. GLP-1 receptor agonists and DPP-4 inhibitors in the treatment of type 2 diabetes. Horm Metab Res. 36(11/12): 867–876.

Bai H, Lu J, Cheng X, Liu L, Zhang W, Wei Y, Wang Y, Liu J, Ding J, Yu Q, et al. 2020. Development and validation of an ultrasensitive LC-MS/MS method for the quantification of cetagliptin in human plasma and its application in a microdose clinical trial. Biomed Chromatogr. 35(3): e4994.

Bian Y, Zhang H, Ma S, Jiao Y, Yan P, Liu X, Ma S, Xiong Y, Gu Z, Yu Z, et al. 2021. Mass balance, pharmacokinetics and pharmacodynamics of intravenous HSK3486, a novel anaesthetic, administered to healthy subjects. Br J Clin Pharmacol. 87(1):93–105.

Blech S, Ludwig-Schwellinger E, Gräfe-Mody EU, Withopf B, Wagner K. 2010. The metabolism and disposition of the oral dipeptidyl peptidase-4 inhibitor, linagliptin, in humans. Drug Metab Dispos. 38(4): 667–678.

Deacon CF. 2004. Therapeutic strategies based on glucagon-like peptide 1. Diabetes. 53(9):2181–2189.

Deacon CF. 2011. Dipeptidyl peptidase-4 inhibitors in the treatment of type 2 diabetes: a comparative review. Diabetes Obes Metab. 13(1): 7–18.

DeFronzo RA. 1988. Lilly lecture 1987. The triumvirate: beta-cell, muscle, liver. A collusion responsible for NIDDM. Diabetes. 37(6): 667–687.

Drucker DJ. 2002. Biological actions and therapeutic potential of the glucagon-like peptides. Gastroenterology. 122(2):531–544.

Gorrell MD. 2005. Dipeptidyl peptidase IV and related enzymes in cell biology and liver disorders. Clin Sci (Lond)). 108(4):277–292.

He H, Tran P, Yin H, Smith H, Batard Y, Wang L, Einolf H, Gu H, Mangold JB, Fischer V, et al. 2009. Absorption, metabolism, and excretion of

[14C]vildagliptin, a novel dipeptidyl peptidase 4 inhibitor, in humans. Drug Metab Dispos. 37(3):536–544.

Kieffer TJ, Habener JF. 1999. The glucagon-like peptides. Endocr Rev. 20(6):876–913.

Lu J, Tian X, Tang D, Zhou X, Xu Z, Ding Wang JT, Yu Q, Ding J. 2021. In vitro study of the drug-drug interaction potential of cetagliptin and clinical study of pharmacokinetic interaction of cetagliptin and metformin in healthy volunteers. Xenobiotica. 51(10):1122–1131.

Ministry of Health labour and Welfare. 2001. Guidance for Industry. Clinical Pharmacokinetic Studies of Pharmaceuticals. Available from: http://www.nihs.go.jp/phar/pdf/ClPkEng011122.pdf.

Nakamaru Y, Hayashi Y, Ikegawa R, Kinoshita S, Perez Madera B, Gunput D, Kawaguchi A, Davies M, Mair S, Yamazaki H, et al. 2014. Metabolism and disposition of the dipeptidyl peptidase IV inhibitor teneligliptin in humans. Xenobiotica. 44(3):242–253.

Penner N, Klunk LJ, Prakash C. 2009. Human radio labeled mass balance studies: objectives, utilities and limitations. Biopharm Drug Dispos. 30(4):185–203.

Rajput R. 2009. Dipeptidyl peptidase-IV inhibitors: a new drug in the therapeutic armamentarium for treatment of type 2 diabetes mellitus. JIACM. 10(3):128–133.

Shaffer CL, Gunduz M, Thornburgh BA, Fate GD. 2006. Using a tritiated compound to elucidate its preclinical metabolic and excretory pathways in vivo: exploring tritium exchange risk. Drug Metab Dispos. 34(9):1615–1623.

US Food and Drug Administration 2018. Guidance for industry. Liposome Drug Products Chemistry, Manufacturing, and Controls; Human Pharmacokinetics and Bioavailability; and Labeling Documentation. Available from: https://www.fda.gov/media/70837/download.

US Food and Drug Administration. 2020. Guidance for industry. Safety testing of drug metabolites guidance for industry. Available from: https://www.fda.gov/media/72279/download.

Vincent SH, Reed JR, Bergman AJ, Elmore CS, Zhu B, Xu S, Ebel D, Larson P, Zeng W, Chen L, et al. 2007. Metabolism and excretion of the dipeptidyl peptidase4 Inhibitor [^{14}C] sitagliptin in humans. Drug Metab Dispos. 35(4):533–538.