

Metabolism And Excretion of the Dipeptidyl Peptidase 4 Inhibitor [¹⁴C]Sitagliptin in Humans

Stella H. Vincent, James R. Reed, Arthur J. Bergman, Charles S. Elmore, Bing Zhu, Shiyao Xu, David Ebel, Patrick Larson, Wei Zeng, Li Chen, Stacy Dilzer, Kenneth Lasseter, Keith Gottesdiener, John A. Wagner, and Gary A. Herman

Departments of Drug Metabolism (S.H.V., J.R.R., C.S.E., B.Z., S.X.) and Clinical Pharmacology (D.E., P.L., K.G., J.A.W., G.A.H.), Merck Research Laboratories, Rahway, New Jersey; Department of Drug Metabolism, Merck Research Laboratories, West Point, Pennsylvania (A.J.B., W.Z., L.C.); and Clinical Pharmacology Associates, Miami, Florida (S.D., K.L.)

Received September 27, 2006; accepted January 3, 2007

ABSTRACT:

The metabolism and excretion of [¹⁴C]sitagliptin, an orally active, potent and selective dipeptidyl peptidase 4 inhibitor, were investigated in humans after a single oral dose of 83 mg/193 μ Ci. Urine, feces, and plasma were collected at regular intervals for up to 7 days. The primary route of excretion of radioactivity was via the kidneys, with a mean value of 87% of the administered dose recovered in urine. Mean fecal excretion was 13% of the administered dose. Parent drug was the major radioactive component in plasma, urine, and feces, with only 16% of the dose excreted as metabolites (13% in urine and 3% in feces), indicating that sitagliptin was eliminated primarily by renal excretion. Approximately 74% of plasma AUC of total radioactivity was accounted for by parent

drug. Six metabolites were detected at trace levels, each representing <1 to 7% of the radioactivity in plasma. These metabolites were the *N*-sulfate and *N*-carbamoyl glucuronic acid conjugates of parent drug, a mixture of hydroxylated derivatives, an ether glucuronide of a hydroxylated metabolite, and two metabolites formed by oxidative desaturation of the piperazine ring followed by cyclization. These metabolites were detected also in urine, at low levels. Metabolite profiles in feces were similar to those in urine and plasma, except that the glucuronides were not detected in feces. CYP3A4 was the major cytochrome P450 isozyme responsible for the limited oxidative metabolism of sitagliptin, with some minor contribution from CYP2C8.

Dipeptidyl peptidase 4 (DPP-4) is a ubiquitous proline-specific serine protease responsible for the rapid inactivation of incretins, including glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic peptide (Gorrell, 2005). GLP-1, which is released upon nutrient ingestion, stimulates meal-induced insulin secretion and contributes to glucose homeostasis (Kieffer and Habener, 1999). Stabilization of GLP-1 via DPP-4 inhibition is a new therapeutic approach for type 2 diabetes (Drucker, 2003; Holst, 2004; Mest and Mentlein, 2005; Nielsen, 2005).

Sitagliptin (Januvia), also known as MK-0431 (Fig. 1), is an orally active, potent and selective DPP-4 inhibitor with an IC₅₀ value of 18 nM (Kim et al., 2005). Sitagliptin has been shown to inhibit plasma DPP-4 activity in a dose-dependent manner and to enhance active GLP-1 levels in normal volunteers (Bergman et al., 2005, 2006; Herman et al., 2005b) and patients with type 2 diabetes (Herman et al., 2004). Furthermore, in patients with type 2 diabetes, single doses of sitagliptin enhanced insulin and C-peptide release, decreased glucagon secretion, and reduced plasma glucose levels after an oral glucose

tolerance test (Herman et al., 2004), whereas 12-week treatment with sitagliptin significantly reduced HbA_{1c} and fasting plasma glucose (Herman et al., 2005a; Scott et al., 2005).

The metabolism and excretion of [¹⁴C]sitagliptin were studied in male human volunteers after oral administration of 83 mg/193 μ Ci. In preclinical species, [¹⁴C]sitagliptin was shown to be eliminated by biliary and/or renal excretion of parent drug (Beconi et al., 2007). Metabolism was minimal, and it involved *N*-sulfation (M1), *N*-carbamoyl glucuronidation (M4), hydroxylation (M6) followed by ether glucuronidation (M3), and oxidative desaturation followed by cyclization (M2 and M5) (Fig. 1). Synthetic standards of metabolites M1, M2, and M5 were tested for DPP-4 inhibition and shown to be ~300-, 1000-, and 1000-fold less active, respectively, than parent drug.

Materials and Methods

Chemicals and Dose Preparation. [¹⁴C]Sitagliptin was synthesized as the phosphate salt with a specific activity of 2.36 μ Ci/mg free base (1.9 μ Ci/mg salt) by the Labeled Compound Synthesis Group [Merck Research Laboratories (MRL), Rahway, NJ]. The chemical purity was 99.7%, as determined by HPLC. The dose was prepared as a capsule formulation containing 20 mg/48.3 μ Ci [¹⁴C]sitagliptin (25 mg of phosphate salt). The 2,5-difluoro analog of sitagliptin used to saturate nonspecific binding sites on the solid phase extraction cartridges was provided by Process Research (MRL, Rahway, NJ). The

Article, publication date, and citation information can be found at <http://dmd.aspetjournals.org>.

doi:10.1124/dmd.106.013136.

ABBREVIATIONS: DPP-4, dipeptidyl peptidase 4; GLP-1, glucagon-like peptide-1; LC-MS/MS, liquid chromatography-tandem mass spectrometry; LC-MS, LC-mass spectrometry; MRL, Merck Research Laboratories; sitagliptin, (2*R*)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro-[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine; HPLC, high-performance liquid chromatography; MRM, multiple reaction monitoring; AUC, area under the curve; ADME, absorption, distribution, metabolism, and excretion.

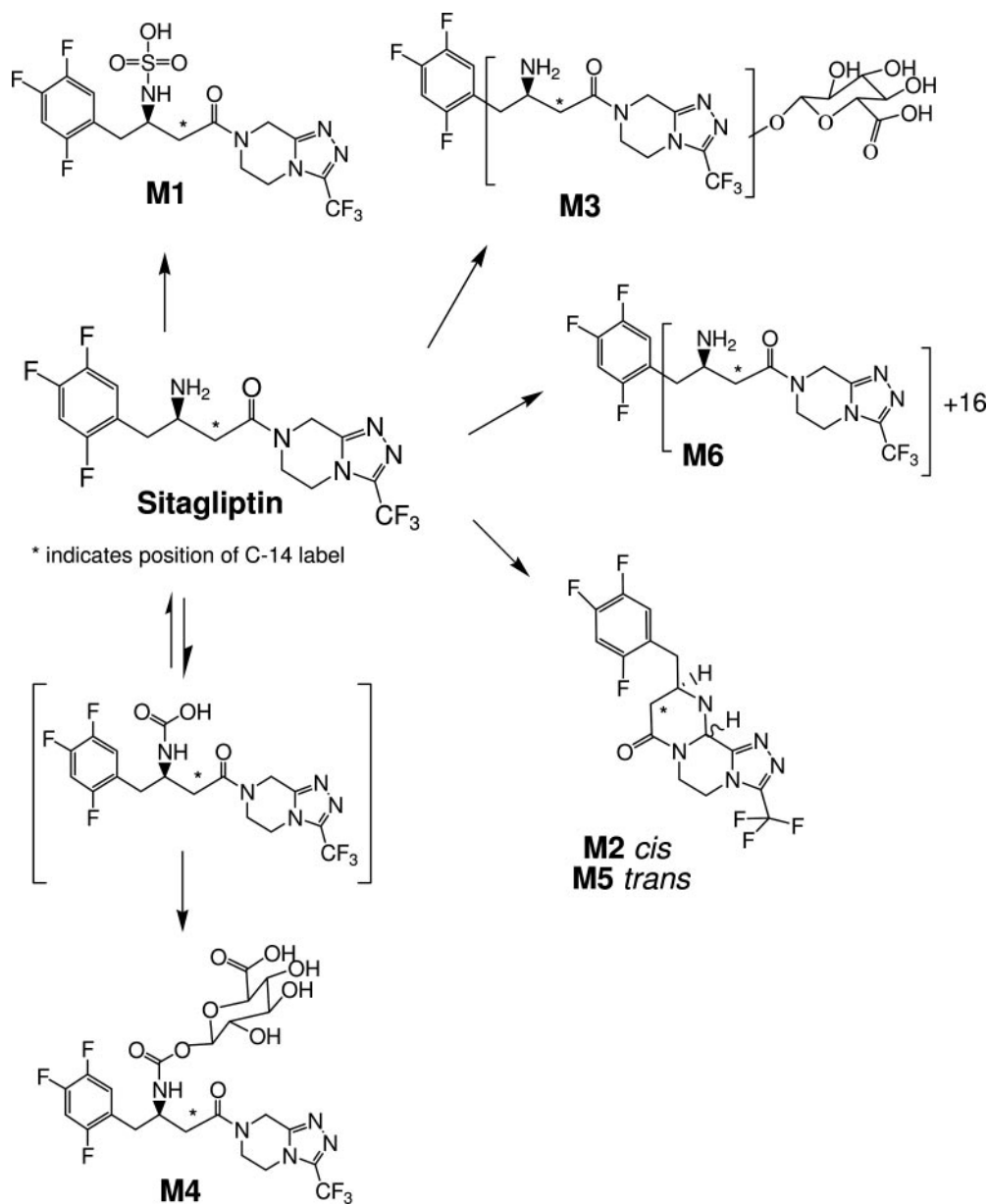


Fig. 1. Main biotransformation pathways for [^{14}C]sitagliptin in humans

synthetic standard of metabolite M1 was synthesized by the Labeled Compound Synthesis Group (MRL, Rahway, NJ), and M2 and M5 were synthesized by Basic Chemistry (MRL, Rahway, NJ). The solid phase extraction cartridges, Varian C_{18} Bond Elut columns, were purchased from Varian Inc. (Harbor City, CA).

Subjects and Dose Administration. The study was conducted at Clinical Pharmacology Associates in Miami, FL, in six healthy male volunteers, 27 to 43 years old, weighing 60 to 95 kg. Subjects were admitted to the clinical research unit the evening before dosing and remained in the unit until the completion of all laboratory collections for the duration of the study (approximately 7 days). Subjects abstained from food and drink except water from midnight the evening before dosing, and consumed approximately 240 ml of water approximately 2 h before drug administration. Water was restricted 1 h before and 1 h after drug administration. Each subject ingested four capsules containing a total of 83 mg/ $193 \mu\text{Ci}$ [^{14}C]sitagliptin with approximately 240 ml of water. A standardized lunch was given at approximately 4 h postdose, a standardized dinner was given at approximately 10 h postdose, and a snack was allowed in the evening. Blood was collected in EDTA-coated tubes at selected time points up to and including 7 days postdose, and spun in a centrifuge to obtain plasma. Urine and feces were collected daily for 7 days. Plasma was

stored at -70°C , and urine and feces at -20°C . Safety and tolerability were assessed by clinical and laboratory evaluations prestudy, predose, postdose, and post-study. Vital signs and ECGs were also evaluated at selected intervals.

Radioactivity excretion data from one subject (AN 803) showed that there was substantially lower overall recovery compared with the other five subjects, suggesting incomplete collection of radioactivity. This subject was subsequently re-dosed with a nonlabeled 100-mg dose of sitagliptin, followed by collection of blood and urine samples for 72 h postdose.

Determination of Radioactivity and Sample Processing for Metabolite Profiling. The concentration of radioactivity in aliquots (0.5 ml) of plasma taken at 0, 0.5, 1.5, 2, 3, 5, 6, 10, 15, 24, 36, 48, 60, 72, 96, 120, 144, and 168 h after dosing was determined by liquid scintillation counting. For metabolite profiling, approximately 4 ml of plasma taken at 1, 4, 8, 12, and 18 h from the six subjects were pooled for each time point. The resulting pools were mixed with 4 ml of 8 M urea and applied to 20-g Varian C_{18} Bond Elut columns, using the following procedure: 1) two washes with 15 ml of methanol; 2) two washes with 15 ml of water; 3) equilibration with 30 ml of $50 \mu\text{M}$ 2,5-difluoro analog of sitagliptin (to saturate sites of nonspecific adsorption); 4) washes with methanol (two times, 15 ml) and water (two times, 15 ml); 5) loading of the plasma; 6) three washes with 15 ml of water; and 7) elution with 12 ml of

TABLE 1

Excretion of radioactivity in human urine and feces after an oral dose of [¹⁴C]sitagliptin

Six healthy volunteers received 83 mg/193 μ Ci of [¹⁴C]sitagliptin in four dry-filled capsules. Radioactivity in fecal homogenates and urine was determined and expressed as percentage of administered dose. Subject 0803* was considered an outlier and data from this subject were not used for the calculation of the mean and standard deviation values.

	Percentage of Dose by Subject						Mean	S.D.
	0800	0801	0802	0803*	0804	0805		
Urine							<i>n</i> = 5	
0–6 h	43	38	44	16	46	47	44	3.7
6–12 h	17	17	26	4.2	18	21	20	3.9
12–24 h	13	16	14	3.4	12	14	14	1.5
24–168 h	9.4	11	9.4	1.4	8.3	7.7	9.2	1.4
0–168 h	82	82	93	25	85	90	87	5.2
Feces								
0–24 h	2.1	0.1	0.3	4.4	0.3	0.3	0.6	0.9
24–48 h	15	18.2	3.1	0.5	5.4	6.9	9.8	6.6
48–168 h	0.4	1.4	6.9	<0.1	3.0	1.6	2.7	2.6
0–168 h ^a	18	20	10	4.9	8.9	8.7	13	5.3
Total	100	102	103	30	94	99	100	4.1

^a Includes radioactivity on fecal wipes.

methanol containing 10% formic acid. Previous studies had shown that sitagliptin and its metabolites were stable under acidic conditions (5% HClO₄). The column eluates were evaporated under N₂ and the samples were reconstituted in 0.3 ml of water/methanol/acetic acid (90:10:0.1, by volume) and analyzed by LC-MS/MS and radiometric detection.

Concentrations of radioactivity and percentage of radioactive dose excreted into the urine and feces were determined at Charles River Laboratories (Wilmington, MA). Feces were homogenized with water (4 ml/g feces) and weighed aliquots were analyzed by combustion followed by liquid scintillation counting of the trapped ¹⁴CO₂. Radioactivity in weighed aliquots of urine was determined directly by liquid scintillation counting.

For metabolite profiling, 0- to 168-h pools of urine and 0- to 96-h pools of fecal homogenates were prepared for each subject based on the volume recovered at each time point. Aliquots from each urine or feces pool were treated with an equal volume of acetonitrile. The mixtures were placed on melting ice for 10 min and centrifuged at 3000 rpm for 10 min. The resulting supernatants were dried under N₂ at 30°C overnight, reconstituted in 350 μ l (urine) or 600 to 750 μ l (feces) of water/methanol/acetic acid (90:10:0.1, by volume), and analyzed by LC-MS/MS coupled with radiometric detection. Also, representative metabolite profiles were generated by analyzing samples consisting of equal volumes of urine or feces extracts from five subjects (excluding subject 803) or all six subjects. For confirmation of metabolites by MRM transition monitoring and MS/MS fragmentation, fecal samples were subjected to solid phase extraction, using a procedure similar to the method described for plasma.

Qualitative LC-MS/MS Analysis. LC-MS analysis was conducted on a PE Sciex API 3000 mass spectrometer (PerkinElmerSciex Instruments, Boston, MA), which was interfaced with a PerkinElmer HPLC system (PerkinElmer Life and Analytical Sciences, Boston, MA) equipped with two Series 200 micro pumps and a PerkinElmer Series 200 autosampler. A Thermo Hypersil Fluophase PFP column (3.0 \times 150 mm, 5 μ m; Thermo Electron Corporation, Waltham, MA) was used for chromatographic separation. The column was eluted with a mixture of 5 mM ammonium acetate in water plus 0.05% acetic acid (mobile phase A) and 5 mM ammonium acetate in methanol plus 0.05% acetic acid (mobile phase B). The gradient was begun with 18% B for 2 min, and increased linearly to 80% B over 33 min and then to 95% B in 5 min, followed by a hold at 95% B for 5 min. The effluent from the HPLC, pumped at a rate of 0.6 ml/min, was diverted at a 5:1 ratio into the radiometric flow detector and into the mass spectrometer, respectively. Scintillation cocktail (Packard Ultima Flo-M, Downers Grove, IL) was pumped at a rate of 1.2 ml/min in the radiometric detector. The contributions of parent drug and metabolites were calculated from the amount of radioactivity eluting in each peak relative to the total radioactivity in the HPLC chromatogram. LC-MS and LC-MS/MS experiments were carried out using the TurboIonSpray interface operated in the positive ion mode. The source voltage was 3500 V and the probe temperature, 300°C. Metabolites were identified by selective ion monitoring of the following precursor \rightarrow product transitions (MRM): M1, *m/z* 488.2 \rightarrow 408.2 and *m/z* 488.2 \rightarrow 193.0; M2, M3, and M5, *m/z* 406.2 \rightarrow 174 and *m/z* 406 \rightarrow 191; M4, *m/z* 628.2 \rightarrow 408 and *m/z* 628.2 \rightarrow 452; and M6, *m/z*

424.2 \rightarrow 406.1 and *m/z* 424.2 \rightarrow 191. The presence of parent drug and metabolites was confirmed by a signal of the MRM transition for each metabolite at least 2- to 3-fold above background at the correct retention time. Also, product ion scan experiments were carried out to compare the MS/MS fragmentation of M1 with that of the synthetic standard.

Identification of P450 Isozymes Involved in the Metabolism of Sitagliptin. Sitagliptin (25 μ M) was incubated with cell membranes containing singly expressed P450 isoforms (CYP2A6, 2B6, C8, C9, C19, 2D6, and 3A4), cytochrome *b*₅, and an NADPH-regenerating system in 0.05 M potassium phosphate buffer. The final concentration of the P450 isoforms and cytochrome *b*₅ in the incubation mixture was 0.5 μ M. The relative contribution of individual P450s to the metabolism of sitagliptin was determined by preincubating human liver microsomes (2 mg protein/ml) with monoclonal antibodies against human CYP3A4 and 2C8 for 1 h at room temperature, followed by the addition of 10 μ M sitagliptin. Control incubations were carried out using a control antibody. The mixtures were incubated at 37°C for 5 min, NADPH was added, and incubations continued for another 30 min. The reactions from both sets of incubations were quenched by the addition of acetonitrile containing 2% formic acid, and supernatants were analyzed by LC-MS/MS, for the formation of metabolites M2, M5, and M6 using MRM.

Quantitative LC-MS/MS Analysis. Concentrations of sitagliptin in plasma were determined by direct on-line LC-MS/MS analysis using a Cohesive Technologies (Franklin, MA) high turbulence liquid chromatography system, as described in more detail elsewhere (Bergman et al., 2006). Analyte and internal standard were detected using selected reaction monitoring with TurboIonSpray interface in the positive ion mode. The lower limit of quantification for the plasma assay was 0.5 ng/ml (1.23 nM) and the linear calibration range was 0.5 to 1000 ng/ml (1.23–2455 nM).

Pharmacokinetic Calculations. Plasma concentrations of sitagliptin and radioactivity were converted into molar units (nM or nM Eq) using the molecular weight of 407.321 before pharmacokinetic analysis. Area under the plasma concentration-time curve to the last time point where radioactivity was above the lower limit of quantitation (AUC_{0–last}) was calculated for both sitagliptin concentrations and radioactivity using the linear trapezoidal method for ascending concentrations and the log trapezoidal method for descending concentrations. Sitagliptin and radioactivity plasma C_{max} and T_{max} were obtained by inspection of the plasma concentration data.

Results

Excretion of Radioactivity. The excretion of radioactivity in human urine and feces after a single 83 mg/193 μ Ci oral dose of [¹⁴C]sitagliptin is summarized in Table 1. The results indicated that most of the radioactive dose was excreted via the kidneys, with a mean value of \sim 87% of the administered dose recovered within 7 days in urine (range: \sim 83–94%) in five of six subjects who participated in this study. Fecal excretion averaged \sim 13% (*n* = 5) of the

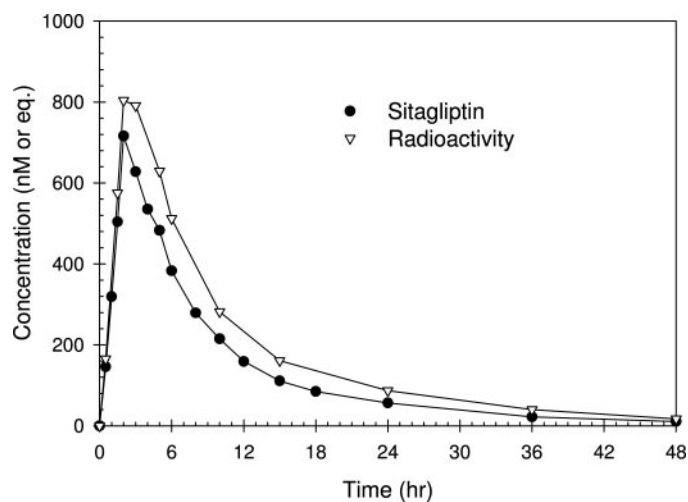


FIG. 2. Mean concentration-time profiles of sitagliptin and total radioactivity in plasma after oral administration of [^{14}C]sitagliptin in healthy young men. Healthy volunteers received 83 mg/193 μCi [^{14}C]sitagliptin in four dry-filled capsules. Radioactivity in plasma was determined by liquid scintillation counting; mean \pm standard deviation values ($n = 5$) are shown.

administered dose (range: ~ 9 –20%), with a mean total recovery of radioactivity in urine and feces of $\sim 100\%$ (range: ~ 94 –105%).

The recovery of radioactivity in one of the subjects (subject 803) was much lower than the average recovery in the other five subjects (30 versus 100% of the dose). The data from this subject were not used in the calculation of the mean and standard deviation values reported in Table 1 and Fig. 2. In a subsequent study with nonradio-labeled sitagliptin (100 mg), it was determined that the pharmacokinetics and renal excretion of sitagliptin in this subject were similar to historical data. Pharmacokinetic analysis of the plasma and urine samples after this dosing revealed that approximately 66% of the sitagliptin dose was excreted unchanged in urine over the 72-h collection period (data not shown). Also, the renal clearance of MK-0431 was generally similar to that observed in other subjects (439 ml/min). These results suggested that the results obtained for this subject after the 83.04-mg [^{14}C]sitagliptin were very likely spurious, and therefore, this subject was excluded from the primary analysis.

Radioactivity and Sitagliptin Levels in Plasma. Concentrations of radioactivity (expressed as [^{14}C]sitagliptin nmol-Eq) and sitagliptin (nM) in human plasma after oral dosing of [^{14}C]sitagliptin are de-

icted in Fig. 2, and pharmacokinetic parameters are summarized in Table 2. The highest concentrations in plasma (C_{max}) were achieved at 2 to 4 h postdose and ranged from 706 to 993 nmol-Eq (total radioactivity) and 523 to 930 nM (sitagliptin) in five of the six subjects (excluding subject AN 803). Radioactivity levels at 60 to 168 h postdose were below the limit of quantification, ~ 25 nM Eq. Plasma $\text{AUC}_{0\text{-last}}$ values in the five subjects ranged from 7.19 to 9.21 $\mu\text{mol-Eq} \cdot \text{h}$, with a mean value of 8.20 $\mu\text{M Eq} \cdot \text{h}$ for total radioactivity, and from 5.12 to 6.83 (mean 6.04) $\mu\text{M} \cdot \text{h}$ for sitagliptin. The mean $\text{AUC}_{0\text{-last}}$ of radioactivity was 74% of the $\text{AUC}_{0\text{-last}}$ of sitagliptin. Data from subject AN803 were not used in the calculation of mean and standard deviation values, because of the low recovery of the radioactive dose in this subject.

Metabolite Profiles in Plasma. Metabolite profiles in plasma pooled across subjects at 1 and 8 h are illustrated in Fig. 3. Similar profiles were observed at 4, 12, and 18 h. Due to the low levels of radioactivity and the limited volume of plasma, it was not possible to obtain individual metabolite profiles. Radioactivity in plasma was composed primarily of parent drug at all time points examined, with approximately 90% of circulating radioactivity detected at 1 h, approximately 80% detected at 4, 8, and 18 h, and approximately 78% detected at 12 h. Six known metabolites were detected, each accounting for <1 to 8% of the circulating radioactivity between 1 and 18 h postdose (Table 3). The most abundant metabolites in plasma were M5 (4–7% of radioactivity) and M2 (1–6%), both of which are formed by oxidative desaturation of the piperazine ring followed by cyclization (Fig. 1). Other metabolites include M6 (a group of hydroxylated derivatives; 1–4%), M1 (*N*-sulfate conjugate; 2–4%), M4 (*N*-carbamoyl glucuronide conjugate; 1%), and M3 (ether glucuronide conjugate of a hydroxylated derivative; $<1\%$).

Metabolite Profiles in Urine and Feces. The metabolite profile of a pooled sample of urine collected at 0 to 168 h postdose is shown in Fig. 4. Similar profiles were obtained for urine collected from individual subjects, including subject AN 803. Parent drug was the major radioactive component, comprising ~ 84 to 88% of the urinary radioactivity. All six metabolites detected in plasma were excreted in small amounts into urine (<1 –5% of the urinary radioactivity, <1 to $\sim 4\%$ of the dose, each).

The metabolite profile of a pooled human fecal sample collected at 0 to 96 h is illustrated in Fig. 4. Feces collected between 4 and 7 days postdose contained negligible amounts of radioactivity and were not analyzed. Parent drug was the major radioactive component in feces,

TABLE 2

Individual plasma pharmacokinetic parameters of sitagliptin and radioactivity after administration of a single oral 83-mg dose of [^{14}C]sitagliptin to healthy young men

Six healthy volunteers received 83 mg/193 μCi [^{14}C]sitagliptin in four dry-filled capsules. Concentrations of radioactivity and sitagliptin in plasma were determined by liquid scintillation counting and LC-MS/MS, respectively.

Subject	Sitagliptin			Radioactivity			Sitagliptin/Radioactivity	
	$\text{AUC}_{0\text{-last}}^a$	C_{max}	T_{max}	$\text{AUC}_{0\text{-last}}$	C_{max}	T_{max}	$\text{AUC}_{0\text{-last}}$ Ratio	C_{max} Ratio
	$\mu\text{M} \cdot \text{h}$	nM	h	$\mu\text{M Eq} \cdot \text{h}$	nM Eq	h		
800	5.27	869	2	7.30	955	2	0.72	0.91
801	6.83	771	2	8.55	833	2	0.80	0.93
802	6.67	702	5	9.21	888	5	0.72	0.79
803 ^b	3.45	447	1.5	4.64	572	1.5	0.74	0.78
804	6.31	930	1.5	8.73	993	2	0.72	0.94
805	5.12	523	4	7.19	706	3	0.71	0.74
AM ^c	6.04	759	2 ^d	8.20	875	2 ^d	N.C.	N.C.
S.D. ^c	0.80	158	N.C.	0.90	113	N.C.	N.C.	N.C.
GM ^c	6.00	745	N.C.	8.16	869	N.C.	0.74	0.86

N.C., not calculated.

^a last = last time point where plasma radioactivity is above the lower limit of quantitation.

^{b,c} Subject 0803 was considered an outlier and data from this subject were not used for the calculation of the arithmetic mean (AM), geometric mean (GM), or S.D. values.

^d Median.

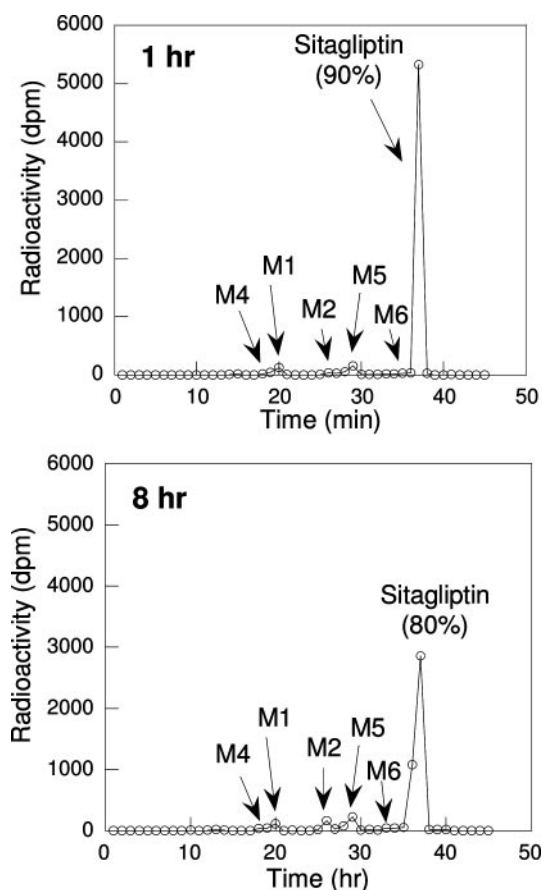


FIG. 3. HPLC radiochromatograms of human plasma after oral administration of [¹⁴C]sitagliptin. Healthy volunteers received 83 mg/193 μ Ci [¹⁴C]sitagliptin. Plasma pooled across subjects was subjected to solid phase extraction followed by LC-MS/MS analysis coupled with radiometric detection.

TABLE 3

Relative contribution of [¹⁴C]sitagliptin and metabolites to the radioactivity in human plasma following oral administration

Compound	Contribution (Percentage of Radioactivity)				
	1 h	4 h	8 h	12 h	18 h
Sitagliptin	90	81	80	78	81
M1	3	4	3	3	2
M2	1	3	4	6	6
M3	<1	<1	<1	<1	<1
M4	<1	1	1	<1	<1
M5	4	6	7	7	6
M6	1	4	3	2	2

comprising ~51 to 86% of the radioactivity. The metabolite profiles in feces were similar in all subjects (not shown) and were also similar to the profiles in urine and plasma, with the exception that the conjugates M3 and M4 were not detected in feces, presumably because they had been hydrolyzed to their corresponding aglycones, M6 and sitagliptin, respectively. Metabolites M1, M2, M5, and M6 were detected in small amounts (~1–12% of the fecal radioactivity, <1 to ~1.6% of the dose, each).

Safety Evaluation. There were no clinical or laboratory adverse experiences reported in this study. In addition, there were no apparent treatment-related clinically relevant changes in vital signs, ECG, or laboratory safety parameters.

Identification of Cytochrome P450 Involved in Sitagliptin Metabolism. After 1-h incubations of 10 μ M [¹⁴C]sitagliptin with

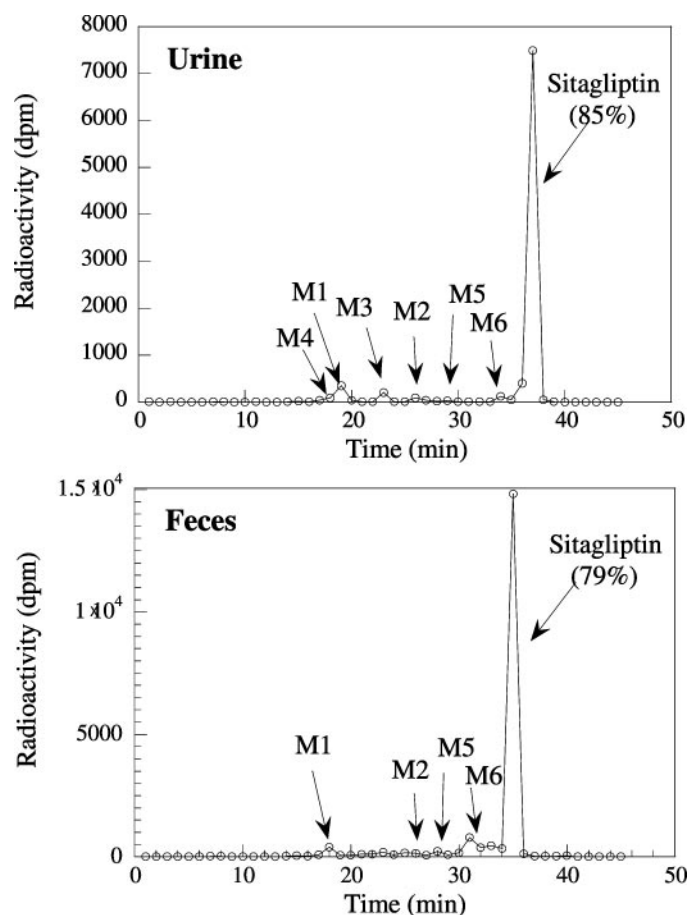


FIG. 4. HPLC radiochromatogram of pooled human urine (0–168 h) and feces (0–96 h) after oral administration of [¹⁴C]sitagliptin. Healthy volunteers received 83 mg/193 μ Ci [¹⁴C]sitagliptin. Urine and fecal homogenate extracts pooled across subjects were analyzed by LC-MS/MS coupled with radiometric detection.

NADPH-enriched human liver microsomes, ~2% turnover was observed. The only metabolites detected by LC-MS/MS were a hydroxylated derivative (M6), and the cyclized products M2 and M5 (Beconi et al., 2007). Incubations with recombinant P450s indicated that CYP3A4 and, to a much smaller extent, CYP2C8 were capable of catalyzing the formation of M2, M5, and M6. Due to the low turnover, the relative contribution of these P450s could not be determined accurately. However, based on LC-MS/MS analysis, it could be discerned that the formation of M2 and M5 in human liver microsomes could be inhibited to a much larger extent by anti-CYP3A4 than anti-CYP2C8 antibody. Also, the formation of M6 could be inhibited by anti-CYP3A4 only.

Discussion

After oral administration of [¹⁴C]sitagliptin to healthy volunteers, the total recovery of radioactivity was approximately 100%. The results of this study demonstrate that the primary route of elimination of sitagliptin in healthy subjects is via renal excretion of intact drug. Approximately 16% of the oral radioactive dose was excreted as metabolites (13% in urine, 3% in feces), and ~10% of the radioactivity dose was excreted unchanged in feces. The unchanged sitagliptin found in feces may represent unabsorbed material, drug cleared by biliary excretion, or back-converted M1 (*N*-sulfate) and/or M4 (*N*-carbamoyl glucuronide). Nonetheless, these results indicated that sitagliptin was well absorbed after oral administration, as ~87% of the radioactivity was recovered in urine. These data are corroborated by

the high bioavailability (Bergman et al., 2005) and high recovery of parent drug in urine after the administration of unlabeled sitagliptin to healthy subjects (Herman et al., 2005a; Bergman et al., 2006).

Examination of the sitagliptin and radioactivity pharmacokinetic data indicate that sitagliptin makes up the majority (74%) of the radioactivity in plasma after an oral dose (as determined by the ratio of sitagliptin AUC and radioactivity AUC), with the remaining radioactivity accounted for by the metabolites shown in Fig. 1. Due to their low affinity for the DPP-4 enzyme (M1, M2, and M5) and their low levels in plasma, these metabolites would not be expected to contribute to the pharmacological activity of sitagliptin.

Similar observations were made in rats and dogs (Beconi et al., 2007), where, as in humans, sitagliptin was eliminated primarily unchanged into urine (dog) or urine and bile (rat). Also, all the metabolites observed in human plasma, urine, and feces were observed also in rat and/or dog plasma, urine, bile, and feces, as well as in incubations in vitro with rat, dog, and human liver preparations (Beconi et al., 2007). Results from in vitro experiments with recombinant P450s and monoclonal anti-P450 antibodies indicated that the oxidative metabolism of sitagliptin in human liver microsomes is catalyzed primarily by CYP3A4 with some minor contribution from CYP2C8. Because sitagliptin is eliminated primarily unchanged into urine, it is not expected to be a victim of metabolism-based drug interactions.

Acknowledgments. We thank the following individuals, all of MRL: Dr. David Liu and Christopher Kochansky for help in the qualitative LC-MS/MS analysis, Drs. Dennis Dean and Allen N. Jones for overseeing the synthesis and analysis of [¹⁴C]sitagliptin, and Drs. Ronald Franklin, Greg Winchell, David Evans, and Tom Baillie for helpful discussions and support. Also, we acknowledge the contribution of Paul Zavorskas and other personnel of Charles River Laboratories (Worcester, MA) in the radiometric analysis of the human feces and urine.

References

- Beconi MG, Reed JR, Teffera Y, Xia Y-Q, Kochansky CJ, Liu, DQ, Xu S, Elmore CS, Ciccotto S, Hora D, Stearns RA, and Vincent SH (2007) Disposition of the dipeptidyl peptidase 4 inhibitor sitagliptin in rats and dogs. *Drug Metab Dispos* **35**:525–532.
- Bergman A, Krishna R, Ebel D, Liu F, Stone J, Wang A, Zeng W, Chen L, Dilzer S, Lasseter K, et al. (2005) Lack of a pharmacokinetic food effect and evaluation of the definitive bioavailability of sitagliptin (MK-0431), an oral dipeptidyl peptidase-IV inhibitor. *J Clin Pharm* **45**:1089.
- Bergman AJ, Stevens C, Zhou YY, Yi B, Laethem M, De Smet M, Snyder K, Hilliard D, Tanaka W, Zeng W, et al. (2006) Pharmacokinetic and pharmacodynamic properties of multiple oral doses of sitagliptin, a dipeptidyl peptidase-IV inhibitor: a double-blind, randomized, placebo-controlled study in healthy male volunteers. *Clin Ther* **28**:55–72.
- Drucker DJ (2003) Therapeutic potential of dipeptidyl peptidase IV inhibitors for the treatment of type 2 diabetes. *Expert Opin Investig Drugs* **12**:87–100.
- Gorrell MD (2005) Dipeptidyl peptidase IV and related enzymes in cell biology and liver disorders. *Clin Sci (Lond)* **108**:277–292.
- Herman G, Hanefeld M, Wu M, Chen X, Zhao P, and Stein P (2005a) Effect of MK-0431, a dipeptidyl peptidase IV (DPP-IV) inhibitor, on glycemic control after 12 weeks in patients with type 2 diabetes. *Diabetes* **54** (Suppl 1):A134.
- Herman GA, Stevens C, Van Dyck K, Bergman A, Yi B, De Smet M, Snyder K, Hilliard D, Tanen M, Tanaka W, et al. (2005b) Pharmacokinetics and pharmacodynamics of single doses of sitagliptin, an inhibitor of dipeptidyl peptidase-IV, in healthy subjects. *Clin Pharm Ther* **78**:675–688.
- Herman GA, Zhao PL, Dietrich B, Golor G, Schroeder A, Keymeulen B, Lasseter KC, Kipnes MS, Hilliard D, Tanen M, et al. (2004) The DP-IV inhibitor MK-0431 enhances active GLP-1 and reduces glucose following an OGTT in type 2 diabetics. *Diabetes* **53** (Suppl 2):A82.
- Holst JJ (2004) Treatment of type 2 diabetes mellitus with agonists of the GLP-1 receptor or DPP-IV inhibitors. *Expert Opin Emerg Drugs* **9**:155–166.
- Kieffer TJ and Habener JF (1999) The glucagon-like peptides. *Endocr Rev* **20**:876–913.
- Kim D, Wang L, Beconi M, Eiermann GJ, Fisher MH, He H, Hickey G-J, Kowalchick JE, Leiting B, Lyons K, et al. (2005) (2R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine: a potent, orally active dipeptidyl peptidase IV inhibitor for the treatment of type 2 diabetes. *J Med Chem* **48**:141–151.
- Liu, DQ, Arison BA, Stearns RA, Kim D, and Vincent SH (2007) Characterization of two cyclic metabolites of sitagliptin. *Drug Metab Dispos* **35**:521–524.
- Mest HJ and Mentlein R (2005) Dipeptidyl peptidase inhibitors as new drugs for the treatment of type 2 diabetes. *Diabetologia* **48**:616–620.
- Nielsen LL (2005) Incretin mimetics and DPP-IV inhibitors for the treatment of type 2 diabetes. *Drug Discov Today* **10**:703–710.
- Scott R, Herman G, Zhao P, Chen X, Wu M, and Stein P (2005) Twelve-week efficacy and tolerability of MK-0431, a dipeptidyl peptidase IV (DPP-IV) inhibitor, in the treatment of type 2 diabetes (T2D). *Diabetes* **54** (Suppl 1):A10.

Address correspondence to: Dr. Stella Vincent, Merck Research Laboratories, RY 80-141, P.O. Box 2000, Rahway, NJ 07065. E-mail: Stella_Vincent@Merck.com
