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Absorption, Metabolism, and Excretion of [^{14}C]Vildagliptin, a Novel Dipeptidyl Peptidase 4 Inhibitor, in Humans

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ABSTRACT:

The absorption, metabolism, and excretion of (1-[[3-hydroxy-1-adamantyl) amino] acetyl]-2-cyano-(*S*)-pyrrolidine (vildagliptin), an orally active and highly selective dipeptidyl peptidase 4 inhibitor developed for the treatment of type 2 diabetes, were evaluated in four healthy male subjects after a single p.o. 100-mg dose of [^{14}C]vildagliptin. Serial blood and complete urine and feces were collected for 168 h postdose. Vildagliptin was rapidly absorbed, and peak plasma concentrations were attained at 1.1 h postdose. The fraction of drug absorbed was calculated to be at least 85.4%. Unchanged drug and a carboxylic acid metabolite (M20.7) were the major circulating components in plasma, accounting for 25.7% (vildagliptin) and 55% (M20.7) of total plasma radioactivity area under the curve. The terminal half-life of vildagliptin was 2.8 h. Complete recovery of the dose was achieved within 7 days, with 85.4% recovered in urine (22.6% unchanged drug) and the remainder in feces (4.54% unchanged drug). Vildagliptin was extensively metabolized via at least four pathways before excretion, with the major metabolite M20.7 resulting from cyano group hydrolysis, which is not mediated by cytochrome P450 (P450) enzymes. Minor metabolites resulted from amide bond hydrolysis (M15.3), glucuronidation (M20.2), or oxidation on the pyrrolidine moiety of vildagliptin (M20.9 and M21.6). The diverse metabolic pathways combined with a lack of significant P450 metabolism (1.6% of the dose) make vildagliptin less susceptible to potential pharmacokinetic interactions with comedications of P450 inhibitors/inducers. Furthermore, as vildagliptin is not a P450 inhibitor, it is unlikely that vildagliptin would affect the metabolic clearance of comedications metabolized by P450 enzymes.

Dipeptidyl peptidase 4 (DPP-4, DPP-IV) is a highly specialized aminopeptidase that is present in plasma, the kidney, and the intestinal brush-border membranes, as well as on the surface of capillary endothelial cells, hepatocytes, and a subset of T lymphocytes (Deacon et al., 1995; Mentlein, 1999). DPP-4 is responsible for the rapid inactivation of the incretin glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic peptide. GLP-1, which is released postprandially, stimulates meal-induced insulin secretion and contributes to glucose homeostasis (Gutniak et al., 1997; Kieffer and Habener, 1999). Circulating GLP-1 is rapidly degraded and inactivated by DPP-4 (Deacon et al., 1995; Mentlein, 1999). With the inhibition of the DPP-4 enzyme activity, GLP-1 activity increases markedly, improving glycemic control in experimental and human studies (Balkan et al., 1999; Ahrén et al., 2002, 2004; Reimer et al., 2002). Therefore, administration of a DPP-4 inhibitor to diabetic patients augments endogenous GLP-1 activity, which in turn produces a clinically significant lowering of diabetic glycemia comparable with that observed when GLP-1 is administered by direct infusion (Gutniak et al., 1992; Drucker, 2003; Mest and Mentlein, 2005).

Vildagliptin (Galvus, Novartis, East Hanover, NJ; (1-[[3-hydroxy-1-adamantyl) amino] acetyl]-2-cyano-(*S*)-pyrrolidine) is a potent, orally active, highly selective inhibitor of DPP-4 (Villhauer et al., 2003) and is marketed as an antidiabetic drug in this novel class of action mechanisms (He et al., 2007b). Based on an in vitro recombinant DPP-4 assay, the IC_{50} for vildagliptin is 2 nM. In humans, the efficacy of vildagliptin against the DPP-4 enzyme also shows a low in vivo inhibitory constant (IC_{50} 4.5 nM), a value that suggests higher potency than that reported for another DPP-4 inhibitor, sitagliptin (IC_{50} 26 nM) (Herman et al., 2005; He et al., 2007b). Vildagliptin has shown the ability to inhibit DPP-4, increase plasma concentrations of intact GLP-1 and glucose-dependent insulinotropic peptide, decrease fasting and postprandial glucose, and suppress plasma glucagons in clinical trial in patients with type 2 diabetes. The pharmacokinetics and pharmacodynamics of vildagliptin after various dosing regimens in healthy volunteers and patients with type 2 diabetes have been previously reported (He et al., 2007a,b, 2008; Sunkara et al., 2007).

The purpose of this study was to investigate the disposition and

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ABBREVIATIONS: DPP-4, dipeptidyl peptidase 4; GLP-1, glucagon-like peptide 1; vildagliptin, (1-[[3-hydroxy-1-adamantyl) amino] acetyl]-2-cyano-(*S*)-pyrrolidine; [^{14}C]vildagliptin, (1-[3-hydroxy-adamant-1-yl-amino)-acetyl]-pyrrolidine-2(*S*)-carbonitrile; LSC, liquid scintillation counting; LC/MS/MS, liquid chromatography/tandem mass spectrometry; IS, internal standard; ESI, electrospray ionization; HPLC, high-performance liquid chromatography; LC/MS, liquid chromatography/mass spectrometry; DMSO, dimethyl sulfoxide; CID, collision-induced dissociation; P450, cytochrome P450; UGT, UDP-glucuronosyltransferase; AUC, area under the curve; amu, atomic mass unit.

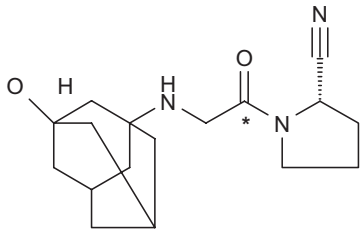

FIG. 1. Chemical structure of [^{14}C]vildagliptin.

biotransformation of vildagliptin in healthy male volunteers after a single 100-mg (47 μCi) p.o. dose of [^{14}C]vildagliptin [(1-[3-hydroxy-adamant-1-yl-amino)-acetyl]-pyrrolidine-2(*S*)-carbonitrile]. A daily dose of 100 mg is the recommended human efficacious dosing regimen for vildagliptin, and no pharmacokinetic gender difference has been observed (He et al., 2007b, 2008). [^{14}C]Vildagliptin has been shown to be highly absorbed in both rats and dogs (He et al., 2009). Vildagliptin was mainly metabolized before excretion in rats and dogs. One major metabolite in excreta involved hydrolysis at the cyano moiety to yield a carboxylic acid metabolite (M20.7) in rats and dogs. Another predominant metabolic pathway included the hydrolysis of the amide bond (M15.3) in the dog.

Materials and Methods

Study Drug. [^{14}C]Vildagliptin (specific activity 0.47 μCi/mg, radiochemical purity >99%) was synthesized by the Isotope Laboratory of Novartis Pharmaceuticals Corporation (East Hanover, NJ). The chemical structure of vildagliptin and the position of the radiolabel are shown in Fig. 1.

Metabolites. Synthetic standards of metabolites M20.2, M20.7, and M15.3 were also obtained from Novartis Pharmaceuticals Corporation.

Human Studies. The study protocol and the informed consent document were approved by an independent institutional review board. The written informed consent was obtained from all the subjects before enrollment.

Four healthy, nonsmoking, male white subjects, age 18 to 45 years, with weights ranging from 77 to 93 kg, participated in the study. Subjects were confined to the study center for at least 20 h before administration of the study drug until 168 h (7 days) postdose. After an overnight fast, the subjects were given a single p.o. 100-mg dose of [^{14}C]vildagliptin as a 250-ml drinking solution. The radioactive dose given per subject was 47 μCi (1.85 MBq). After administration, the subjects continued to abstain from food for an additional 4 h.

Blood was collected at 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 16, 24, 36, 48, 72, 96, 120, 144, and 168 h postdose by either direct venipuncture or an indwelling cannula inserted in a forearm vein. Eighteen milliliters of venous blood was collected at each time point in heparinized tubes. Plasma was separated from whole blood by centrifugation, transferred to a screw-top polypropylene tube, and immediately frozen.

Urine samples were collected at predose and at 0 to 4, 4 to 8, 8 to 12, 12 to 16, 16 to 24, 24 to 36, 36 to 48, 48 to 72, 72 to 96, 96 to 120, 120 to 144, and 144 to 168 h postdose. Feces were collected as passed from time of dosing until at least 168 h postdose. All of the samples were stored at −20°C or less until analysis.

Radioactivity Analysis of Blood, Plasma, Urine, and Feces Samples. Radioactivity was measured in plasma and blood by liquid scintillation counting (LSC) on a liquid scintillation analyzer (Tri-CARB 2500; Canberra Industries, Meriden, CT). Plasma was mixed with scintillant and counted directly; whole blood samples were digested with tissue solubilizer (Soluene 350; PerkinElmer Life and Analytical Sciences, Waltham, MA), decolorized with hydrogen peroxide, stored in the dark to reduce luminescence, and then counted. Radioactivity in urine and feces was also assessed by LSC. Urine was mixed with liquid scintillant and counted directly. Feces was homogenized in combusted with a biological oxidizer (Packard Oxidizer 306; PerkinElmer Life and Analytical Sciences) before LSC.

The total radioactivity given with the dose was set to 100%. The radioactivity at each sampling time for urine and feces was defined as the percentage of dose excreted in the respective matrices. The radioactivity measured in plasma was converted to nanogram-equivalents of vildagliptin based on the specific activity of the dose.

Analysis of Unchanged Vildagliptin. Amounts of unchanged vildagliptin in plasma and urine were measured quantitatively using a validated liquid chromatography/tandem mass spectrometry (LC/MS/MS) assay. Aliquots of plasma (200 μl) or human urine (100 μl diluted with 100 μl of water) and 200 μl of internal standard (IS) solution ($^{13}C_5{}^{15}N$-vildagliptin) were transferred to individual wells in a 1-ml, 96-well polypropylene plate. Extraction of the samples was performed using a Quadra-96 model 320 workstation (TomTec, Hamden, CT). Before extraction of samples, a 10-mg Oasis HLB 96-well solid-phase extraction plate (Waters, Milford, MA) was conditioned with 300 μl of methanol, followed by 300 μl of water. The samples were applied to the preconditioned extraction plate. The plate was washed with 300 μl of 5% methanol (containing 2% ammonium hydroxide), 300 μl of 20% methanol (containing 2% ammonium hydroxide), and 300 μl of water. After vacuum-drying each well, the samples were eluted with 2 × 75 μl of 80% methanol (containing 0.1% trifluoroacetic acid) and evaporated under nitrogen (35°C) to a volume of ~50 μl using an Evaporex solvent evaporator (Apricot Designs, Monrovia, CA). The samples were diluted with 50 μl of 15% methanol (containing 0.5% ammonium hydroxide) and mixed before injection.

Samples were analyzed on a Micromass Quattro LC (Waters) operated in multiple reaction monitoring mode with electrospray ionization (ESI^+) as an interface. Vildagliptin and IS were separated on a Polaris 5-μm C18-A 50 × 2.0-mm column (45°C) (Metachem Technologies, Torrance, CA) with isocratic elution. The mobile phase of A/B (1:3, v/v) was used, where A was methanol/10 mM ammonium acetate, pH 8.0 (5:95, v/v), and B was acetonitrile/methanol (10:90, v/v). The flow rate was maintained at 0.2 ml/min with an injection volume of 10 μl. Multiple reaction monitoring transitions for the drug and IS were m/z 304.2 → m/z 154.1 and m/z 310.3 → m/z 160.0, respectively. The dynamic range of the assay was from 1.93 to 2020 ng/ml for plasma and 5.13 to 5010 ng/ml for urine.

Sample Preparation of Plasma, Urine, and Feces for Metabolite Investigation. Semiquantitative determination of main and trace metabolites was obtained for plasma, urine, and feces (based on peak areas) using high-performance liquid chromatography (HPLC)-radiodetection with off-line microplate solid scintillation counting and structural characterization by liquid chromatography/mass spectrometry (LC/MS). Plasma samples (3.5–4.5 ml) from each subject at 0.5, 1, 2, 3, 6, 12, 16, and 24 h postdose were protein-precipitated with acetonitrile/ethanol (90:10 v/v) containing 0.1% acetic acid and removed by centrifugation. Recoveries of radioactivity after plasma sample preparation averaged 95%. The supernatant was evaporated to near dryness under a stream of nitrogen using the Zymark Turbo-Vap LV (Zymark Corp., Hopkinton, MA), and the residues were reconstituted in acetonitrile/5 mM ammonium acetate containing 0.1% trifluoroacetic acid (10:90 v/v). Aliquots (80–85 μl) of concentrated plasma extracts were injected onto the HPLC column. For urine analysis, a pool of equal percent volume from the 0- to 48-h fractions (10% of urine volume from each time point, e.g., 0–24 and 24–48 h) was prepared for each subject. An aliquot was centrifuged, and 100 μl was injected onto the HPLC column without further purification. Recoveries of radioactivity after centrifugation of urine samples were 100%. Feces homogenates were pooled from 0 to 96 h at equal percent weight for each subject (10% of feces homogenates from each time point, e.g., 0–24, 24–48, and 48–72 h) and extracted twice with methanol by vortexing and centrifugation. The average recovery of sample radioactivity in the methanolic extracts was 87%. Aliquots of combined supernatant (5 ml) were evaporated to dryness under a stream of nitrogen using the Zymark Turbo-Vap LV, and the residues were reconstituted in 0.2 ml of acetonitrile/5 mM ammonium acetate containing 0.1% trifluoroacetic acid (10:90 v/v). Aliquots (60–80 μl) of concentrated fecal extracts were injected onto the HPLC column.

HPLC Instrumentation for Metabolite Pattern Analysis. Vildagliptin and its metabolites in urine, plasma, and feces were analyzed by HPLC with off-line radioactivity detection using a Waters Alliance 2690 HPLC system

(4.6 × 150 mm, 4 μm, maintained at 30°C) and a guard column of the same type. The mobile phase consisted of 5 mM ammonium acetate containing 0.1% trifluoroacetic acid, pH 2.3 (solvent A), and acetonitrile (solvent B), and a gradient method was used. The mobile phase was initially composed of solvent A (100%) and held for 4 min. The mobile phase composition was then linearly programmed to solvent A/solvent B (87:13) over 26 min and held for 2 min and to solvent A/solvent B (40:60) in 0.5 min and held for 4 min. A short gradient was programmed to solvent A/solvent B (5:95) over 0.5 min, and these conditions were held for 4 min. The mobile phase condition was returned to the starting solvent mixture over 0.5 min. The system was allowed to equilibrate for 10 min before the next injection. A flow rate of 1.0 ml/min was used for all the analyses. The HPLC effluent was fractionated into a 96-deep-well Lumaplate (PerkinElmer Life and Analytical Sciences) using a fraction collector (FC 204; Gilson Inc., Middleton, WI) with a collection time of 8.4 s/well. Samples were dried under a stream of nitrogen, sealed, and counted for 1 to 15 min/well on a TopCount microplate scintillation counter (PerkinElmer Life and Analytical Sciences).

The amounts of metabolites of parent drug in plasma or excreta were derived from the radiochromatograms (metabolite patterns) by dividing the radioactivity in original sample in proportion to the relative peak areas. Parent drug or metabolites were expressed as concentrations (in nanogram-equivalent per milliliter) in plasma or as percentage of dose in excreta. These values are to be considered as semiquantitative only, unlike those determined by the validated quantitative LC/MS/MS assay.

In Vitro Human Blood Distribution and Protein Binding. A single 10-μl aliquot of the stock solution in ethanol containing [^{14}C]vildagliptin (35×10^6 dpm) and known amounts of unlabeled vildagliptin was spiked to 1 ml of human fresh blood or plasma ($n = 3$) to achieve final concentrations of 10 to 10,000 ng/ml.

For the blood distribution study, triplicate determinations of the hematocrit in blood were made, and single blood samples (1 ml) were prepared from each of the three male human volunteers. After gentle mixing, a single 200-μl aliquot of blood containing [^{14}C]vildagliptin was pipetted for radioactivity analysis. Then the blood samples were incubated at 37°C for 30 min and centrifuged at ~3000*g* for ~15 min at 37°C. The resultant plasma was analyzed for radioactivity using a single 200-μl aliquot.

For the protein binding study, single plasma samples (1 ml) were prepared from each of the three male human volunteers. The pH was adjusted to 7.4 by adding 10 μl of 0.1 N HCl/ml plasma and gently vortexing the sample. After a single aliquot of plasma containing [^{14}C]vildagliptin (200 μl) was pipetted for radioactivity analysis, each sample (~0.8-ml aliquot) was transferred to the sample reservoir of individual micropartition centrifuge tube (Centrifree Micropartition Centrifuge Tube; Millipore Corporation, Billerica, MA). The membrane had a molecular mass cutoff of 30,000 Da. Samples were centrifuged for 20 min at ~1000*g* at 37°C. The ultrafiltrate contained the free fraction, and 200-μl aliquots were analyzed for radioactivity. Nonspecific binding studies were conducted in 0.2 M sodium phosphate buffer, pH 7.4, under the same conditions described above.

For radioactivity analysis of blood or plasma samples in the blood distribution study, 200 μl of blood was pipetted onto individual Combusto-Pads (PerkinElmer Life and Analytical Sciences), air-dried, and combusted in a Packard 308 oxidizer before counting in a liquid scintillant (PerkinElmer Life and Analytical Sciences). For radioactivity analysis in the protein binding study, aliquots (200 μl) of plasma samples and filtrates were mixed with 2 ml of a liquid scintillant (NEN Formula 989; PerkinElmer Life and Analytical Sciences) in a vial for direct counting. The radioactivity in all the samples was determined by LSC in a Packard spectrometer (Hewlett Packard, Palo Alto, CA). M20.7 human protein binding was also determined over the concentration range from 10 to 1000 ng/ml using the same ultrafiltration method as described above.

In Vitro Metabolism in Human Liver Slices. [^{14}C]Vildagliptin was incubated with liver slice preparations from one human subject. The human tissue was obtained through the Association of Human Tissue Users (Tucson, AZ). Each of the human organs had been perfused with Belzer's University of Wisconsin solution but was rejected for transplantation. The incubations were carried out at 5 and 20 μM substrate concentrations for 1, 2, 4, 10, 18, and 24 h. The incubates were analyzed by HPLC with online radioactivity detection. Human liver slices with a diameter of approximately 200 ± 25 μm were prepared from 8-mm diameter tissue cores using a Vitron (Tucson, AZ) sterile tissue slicer. The individual organs were cored transversely and sliced using a Vitron sterile tissue slicer in ice-cold oxygenated (95% O_2/5% CO_2) V7 preservation media. The viability of the human liver slices was assessed by determining the intracellular K^+ content and measurement of ATP content in 0.1% dimethyl sulfoxide (DMSO) and vildagliptin-exposed slice incubates. The slices were placed onto roller culture inserts and maintained at 37°C in Dulbecco's modified Eagle's/F-12 media without phenol red (Invitrogen, Carlsbad, CA) and supplemented with 10 ml/l Antibiotic AntiMycotic solution (Invitrogen), 10% Nu Serum, and Mito/Serum Extender, 1 ml/l (BD Biosciences, Franklin Lakes, NJ). After a preincubation period of 90 min, fresh media containing [^{14}C]vildagliptin in 0.1% DMSO were added. At the various time points, the slice and media were transferred to separate vials, and the roller culture vial and insert were bathed in methanol (3.0 ml), which was then collected. Before HPLC analysis, the human liver slices were disrupted by homogenization with MeOH/H_2O (50:50) followed by brief sonication. The incubation media were extracted with methanol, and the methanol wash was evaporated to dryness. All the fractions were pooled, and the protein was pelleted at approximately 40,000*g* for 10 min at 20°C. The pellet was re-extracted with methanol, and the resultant supernatant was evaporated to dryness and combined with the pooled sample.

Structural Characterization of Metabolites by LC/MS/MS. Metabolite characterization was conducted with a Finnigan LCQ ion-trap mass spectrometer (Thermo Fisher Scientific, Waltham, MA) equipped with an ESI source. The effluent from the HPLC column was split, and approximately 500 μl/min was introduced into the atmospheric ionization source after diverting to waste during the first 4 min of each run to protect the mass spectrometer from nonvolatile salts. The electrospray interface was operated at 5000 V, and the mass spectrometer was operated in the positive ion mode. Collision-induced dissociation (CID) studies were performed using helium gas at the collision energy of 35% (arbitrary unit).

Metabolism of Vildagliptin in Human Liver Microsomes and by Recombinant Cytochromes P450. The metabolism of [^{14}C]vildagliptin (specific activity of 154.5 μCi/mg) was examined in pooled human liver microsomes ($n = 46$ donors, mixed gender) and in microsomal preparations from baculovirus-infected insect cells expressing recombinant human cytochrome P450 (P450) enzymes (BD Gentest, Woburn, MA). The recombinant P450 enzymes examined in this study were CYP1A1, CYP1A2, CYP1B1, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C18, CYP2C19, CYP2D6, CYP2E1, CYP2J2, CYP3A4, CYP3A5, and CYP4A11. Human liver microsomes (1 mg of microsomal protein/ml) or recombinant P450 enzymes (100 pmol of P450/ml) were preincubated with [^{14}C]vildagliptin (47 μM, 0.5% final DMSO concentration, v/v) in 100 mM potassium phosphate buffer, pH 7.4, and 5 mM $MgCl_2$, final concentrations, at 37°C for 3 min. The reactions were initiated by the addition of NADPH (1 mM, final concentration) and incubated for 30 min; reactions were then quenched by the addition of half the reaction volume of cold acetonitrile. The precipitated protein was removed by centrifugation, and an aliquot of each sample was analyzed by HPLC with in-line radioactivity detection as described above.

P450 Inhibition Assessment by Vildagliptin and M20.7. The ability of vildagliptin and its metabolite M20.7 to inhibit P450 enzyme activity was assessed using pooled human liver microsomes ($n = 50$ donors, mixed gender; XenoTech, LLC, Lenexa, KS). To determine individual P450 activities, several probe substrate reactions were used that are known to be P450 enzyme-selective. The reactions used and corresponding probe substrate concentrations included phenacetin *O*-deethylation (5 μM, CYP1A2), bupropion hydroxylation (25 μM, CYP2B6), paclitaxel 6α-hydroxylation (10 μM, CYP2C8), diclofenac 4′-hydroxylation (5 μM, CYP2C9), *S*-mephenytoin 4′-hydroxylation (15 μM, CYP2C19), bufuralol 1′-hydroxylation (5 μM, CYP2D6), chlorzoxazone 6-hydroxylation (10 μM, CYP2E1), and midazolam 1′-hydroxylation (5 μM, CYP3A4/5). The probe concentrations used were less than or approximately equal to their reported K_m values. The conditions for each probe reaction were previously established to ensure linearity with time and protein concentration and to limit probe substrate turnover to ~<10% (results not shown). Increasing concentrations of the vildagliptin or authentic synthetic M20.7 (up to 100 μM) were incubated at 37°C individually with human liver

100 mM potassium phosphate buffer, pH 7.4, 1 mM NADPH, 5 mM $MgCl_2$, 1 mM EDTA, and 0.2% DMSO. After incubation, the reactions were quenched by addition of an equal volume of cold acetonitrile. Probe substrate turnover was determined by LC/MS/MS analysis (PE Sciex API300 mass spectrometer; Applied Biosystems, Foster City, CA; Shimadzu LC, Shimadzu, Kyoto, Japan) of metabolite formation. Reference standards for probe metabolites were obtained from commercial sources as follows: acetaminophen (Sigma-Aldrich, St. Louis, MO); 1′-hydroxybufuralol, hydroxybupropion, 6-hydroxychlorzoxazone, 4′-hydroxy-*S*-mephenytoin, and 1′-hydroxymidazolam (Ultrafine Chemicals, Manchester, UK); and 6-hydroxypaclitaxel and 4′-hydroxydiclofenac (BD Biosciences, San Jose, CA). Chromatographic separation was achieved on a Supelco (Bellefonte, PA) Discovery DP-Amide C16 column (50 × 2.1 mm, 4 μm, 0.3 ml/min flow rate, 25°C). The chromatographic solvents were as follows: A = 0.1% formic acid in 10 mM ammonium acetate, pH ~4.7, B = acetonitrile; the gradient elution program (%B) was 0→1 min (5%), 1→4 min (from 5% to 95%), 4→6 min (95%), 6→6.5 min (from 95% to 5%). Probe metabolites derived from phenacetin, bupropion, midazolam, bufuralol, and paclitaxel were analyzed using ESI in positive ion mode, whereas the metabolites of the remaining probes (diclofenac, chlorzoxazone, *S*-mephenytoin) were analyzed in negative ion mode.

UDP Glucuronosyltransferase Enzyme Involvement in the Glucuronidation of Vildagliptin. The enzymes involved in the glucuronidation of vildagliptin to form M20.2 were determined using a panel of recombinant human UDP glucuronosyltransferase (UGT) enzymes, including UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A7, UGT1A8, UGT1A9, UGT1A10, UGT2B4, UGT2B7, UGT2B15, and UGT2B17 (BD Gentest). In the initial assessment, incubations (100 μl, 37°C) consisted of (final concentrations): vildagliptin (20 μM), UDP-glucuronic acid (5 mM), alamethicin (0.25 mg/mg protein), enzyme protein (1 mg/ml), $MgCl_2$ Tris-HCl buffer (pH 7.6, 50 mM), and acetonitrile (<0.2%). The enzyme protein had been preincubated with the alamethicin for 15 min on ice immediately before the experiments. The reactions were initiated by the addition of UDP-glucuronic acid after a 3-min preincubation and terminated after 60 min by the addition of acetonitrile (200 μl). The incubation samples were evaporated, reconstituted in 100 μl of 10:90 (v/v) acetonitrile/water, and centrifuged at ~4000*g* (10 min). A 25-μl aliquot was analyzed by LC/MS (Finnigan hybrid LTQ ion trap, ESI, positive mode). Chromatographic separation was achieved using an Ace3 C18 (50 × 3 mm, 3.5 μm, 0.25 ml/min flow rate, 35°C). The chromatographic solvents were as follows: A = 0.1% formic acid in 10 mM ammonium acetate, pH ~4.7, B = acetonitrile; the gradient elution program (%B) was 0→1 min (10%), 1→5 min (from 10 to 30%), 5→6 min (from 30 to 98%), 6→7 min (98%), and 7→8 min (from 98 to 10%).

DPP-4-Catalyzed Formation of M20.7. Vildagliptin (0.1 μM) was incubated with human recombinant DPP-IV (expressed in Sf9 cells; Sigma-Aldrich) in Tris-HCl buffer (50 mM, pH 8.0) at 37°C. After 60 min, the reaction was terminated by the addition of acetonitrile (200 μl). The incubation samples were evaporated, reconstituted in 100 μl of 10:90 (v/v) acetonitrile/water, and centrifuged at ~4000*g* (10 min). A 25-μl aliquot was analyzed by LC/MS. The LC/MS method was as described above (see UGT involvement).

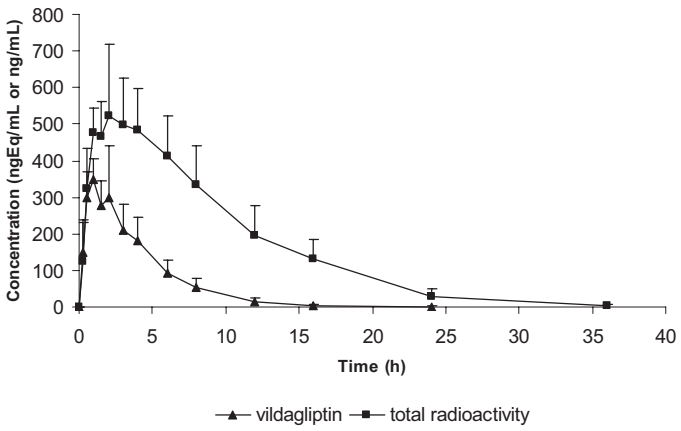

FIG. 2. Plasma concentrations of radioactivity (squares) and vildagliptin parent

TABLE 1

Pharmacokinetic parameters (mean ± S.D.) after a single 100-mg p.o. dose of [^{14}C]vildagliptin

Pharmacokinetic Parameters[a]	Plasma Vildagliptin[b]	Plasma Radioactivity[c]	Blood Radioactivity[c]
C_{max} (ng/ml) or (ngEq/ml)	397 ± 92	594 ± 153	470 ± 87
t_{max} (h)	1.1 ± 0.6	2.1 ± 1.3	2.0 ± 1.4
AUC_{0-t} (ng · h/ml) or (ngEq · h/ml)[d]	1620 ± 460		
$AUC_{0-\infty}$ (ng · h/ml) or (ngEq · h/ml)	1610 ± 460	6000 ± 1610	3850 ± 1580
$t_{1/2}$ (h)	2.8 ± 1.0	4.6 ± 0.3	5.1 ± 2.5
CL/F (l/h)	65.2 ± 15.5		
Vz/F (l)	269 ± 125		

[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* under *Materials and Methods*.
[b] Vildagliptin was determined by validated LC/MS/MS.
[c] Total radioactivity was determined by LSC.
[d] *t* was 24 or 48 h.

Pharmacokinetic Analysis. The following pharmacokinetic variables were determined by fitting the concentration-time profiles to a noncompartmental model with an iterative nonlinear regression program (WinNonlin software version 4.0; Pharsight, Mountain View, CA): area under the blood or plasma drug concentration-time curve between time 0 and time t (AUC_{0-t}); AUC until time infinity ($AUC_{0-\infty}$); highest observed blood or plasma drug concentration (C_{max}); time to highest observed drug concentration (t_{max}); apparent terminal half-life ($t_{1/2}$); apparent volume of distribution of parent drug (V_z/F) calculated as dose/(AUC · λ_z), where F is bioavailability and λ_z is the terminal rate constant; and apparent clearance (CL/F), calculated as dose/$AUC_{0-\infty}$.

Results

In Vitro Human Blood Distribution and Protein Binding. The mean human blood/plasma ratio (C_b/C_p) and fraction of [^{14}C]vildagliptin bound to red blood cells (f_{BC}) were 1.0 and 0.44, respectively, indicating approximately equal distribution between plasma and blood cells. The blood distribution was independent of concentration between 10 and 10,000 ng/ml.

The mean plasma protein binding of vildagliptin in humans was low (9.3%) and also independent of concentration. The nonspecific binding of the compound to centrifuge tubes and/or membranes was low (<12%), suggesting that ultrafiltration is a suitable method. In addition, M20.7 showed no plasma protein binding in humans over the concentration range of 10 to 1000 ng/ml and was independent of concentration.

Absorption. The absorption of vildagliptin was rapid after oral administration, with the peak plasma concentration of vildagliptin observed at an average of 1.1 h (range, 0.5–2 h). The percentage of drug absorbed was estimated to be at least 85.4%, because this amount of the radioactivity was recovered in urine.

Pharmacokinetics of Vildagliptin and Total Radioactivity. The mean plasma concentration-time profiles and pharmacokinetic parameters of total radioactivity and unchanged vildagliptin in healthy male volunteers after a single oral dose of [^{14}C]vildagliptin are shown in Fig. 2 and Table 1, respectively. The highest concentrations in plasma (C_{max}) were achieved at 2.1 h postdose with the mean value 594 ng-Eq/ml (total radioactivity) and at 1 h postdose with the mean value of 397 ng/ml (vildagliptin) in all four subjects. Radioactivity and parent levels at 48 h were below the limit of quantification. The terminal elimination half-life ($t_{1/2}$) of radioactivity and vildagliptin averaged 4.6 and 2.8 h, respectively. Based on $AUC_{0-\infty}$ values, approximately 25.7 and 55% of the circulating radioactivity were attrib-

TABLE 2

Cumulative excretion of ^{14}C radioactivity in urine and feces after a single p.o. 100-mg dose of [^{14}C]vildagliptin to humans, mean ± S.D.

Time Period (h)	Urine	Feces	Total
h	*% dose*	*% dose*	*% dose*
0–24	72.7 ± 4.8	1.37 ± 2.0	
0–48	81.6 ± 4.2	9.93 ± 8.0	
0–72	83.8 ± 4.4	13.2 ± 4.8	
0–96	84.7 ± 4.4	14.3 ± 3.7	
0–168	85.4 ± 4.4	14.8 ± 3.5	100 ± 1

respectively. In addition, mean blood-to-plasma ratios of radioactivity calculated at specific time points (between 0.25 and 1 h) averaged near 1.1, indicating that vildagliptin distributed almost equally between blood cells and plasma as the main circulating component was the parent drug at the early time points, consistent with the in vitro finding (C_b/C_p ratio of vildagliptin ~1). However, the total radioactivity AUC_b/AUC_p ratio was 0.64, suggesting that metabolite(s) should be distributed more to plasma than blood cells. The CL/F and V_z/F values of vildagliptin were 65.2 l/h and 269 liters, respectively. With a human bioavailability of 84%, the estimated CL was 55 l/h, which is slightly higher than that after an intravenous infusion dose (41 l/h) (He et al., 2007a). A measured V_z value has not been reported in the literature, but V_z can be estimated to be 229 liters based on data from this study.

Excretion and Mass Balance in Urine and Feces. After a single p.o. 100-mg dose of [^{14}C]vildagliptin, radioactivity was excreted predominantly in the urine (Table 2). At 168 h after dosing, the excretion in urine and feces averaged 85.4 ± 4.4 and 14.8 ± 3.5% of the administered dosed, respectively. In total, the cumulative excretion of radioactivity was complete in all four subjects, averaging 100 ± 1.10%. Elimination of radioactivity was rapid. More than 90% of the entire radioactivity recovered in urine and feces was excreted in the first 48 h after dose administration.

Metabolite Profiling. Tables 3 and 4 summarize the pharmacokinetics of vildagliptin and its metabolites in plasma and percentage of vildagliptin and its metabolites in excreta, respectively, after a single p.o. 100-mg dose of [^{14}C]vildagliptin to humans. A representative HPLC radiochromatogram of circulating metabolites is shown in Fig. 3A. In plasma, major circulating components were unchanged drug and M20.7 (a carboxylic metabolite), accounting for 25.7% (vildagliptin) and 55% (M20.7) of the total plasma exposure. At early time points (up to 1 h) postdose, the predominant radiolabeled plasma component was vildagliptin, accounting for 70.2 to 88.3% of the total radioactivity. At later time points (12–24 h), the predominant component was M20.7, accounting for 78.4 to 89.9% of the total radioactivity. Other metabolites detected in plasma included M15.3 (carboxylic metabolite formed from hydrolysis of the amide bond) and M20.2 (glucuronic acid conjugate of vildagliptin), accounting for 8.1 and 9.3%, respectively, of the total plasma exposure.

Representative HPLC radiochromatograms for metabolites in urine and feces are shown in Fig. 3, B and C, respectively. Approximately 27.1% of the administered dose was excreted as unchanged drug in urine and feces. In addition to the unchanged drug, five metabolites were radiochemically quantifiable in the urine or feces. In urine, M20.7 represented the major metabolite (49.6% of the administered dose), and all the other metabolites were minor (each <4.5% of the dose). Unchanged vildagliptin in urine averaged at 22.6% of the dose. In the feces, M20.7 was also the major metabolite, accounting for 6.89% of the dose; unchanged vildagliptin accounted for 4.54% of the dose. In summary, the urinary and fecal metabolites totally accounted for >90% of the total radioactivity recovered.

In Vitro Metabolism in Human Liver Slices. Incubation of [^{14}C]vildagliptin with human liver slices formed three metabolites: M15.3, M20.7, and M20.2 (formed at late time points in the incubations at 20 μM substrate concentration).

Metabolite Characterization by Mass Spectrometry. Metabolite structures were characterized by LC/MS/MS using a combination of full and product ion scanning techniques. The structure of major metabolites, where possible, was supported by comparisons of their retention times on HPLC and mass spectra with those of synthetic standards (vildagliptin, M20.2, M20.7, and M15.3).

Vildagliptin displayed a protonated molecular ion (MH^+) at *m/z* 304. The product ion spectrum of *m/z* 304 showed major fragment ions at *m/z* 97 and 154 (Fig. 4). The ion at *m/z* 154 corresponded to the amino acetyl pyrrolidine carbonitrile moiety, and *m/z* 97 corresponded to the pyrrolidine carbonitrile moiety.

Metabolite M15.3. M15.3 was found in urine, feces, and plasma. Mass spectral analysis showed a protonated molecular ion at *m/z* 226 [78 atomic mass units (amu) lower than the parent drug]. The product ion mass spectrum of *m/z* 226 showed fragment ions at *m/z* 76 and 151. The ion at *m/z* 151 corresponded to the hydroxy adamantyl moiety, suggesting modification had occurred at the other half of vildagliptin. Based on comparison of the HPLC retention time and CID spectrum with the synthetic standard, M15.3 was identified as the carboxylic acid metabolite resulting from hydrolysis at the amide bond.

Metabolite M20.2. M20.2 was found in urine and plasma. Its full-scan mass spectrum displayed a protonated molecular ion at *m/z* 480, 176 amu higher than that of the parent drug, suggesting that it was a glucuronide conjugate. Based on comparison of the HPLC retention time and CID spectrum with the synthetic standard, M20.2 was identified as the *O*-glucuronide of vildagliptin.

Metabolite M20.7. M20.7 was found in urine, feces, and plasma. Its full-scan MS displayed a protonated molecular ion at *m/z* 323, 19 amu higher than that of the parent drug. The product ion mass spectrum of *m/z* 323 showed fragment ions at *m/z* 305, 173, and 116. The ion at *m/z* 305 occurred from the loss of water in the molecule. The ions at *m/z* 173 and 116 were 19 amu higher than those observed in the parent drug (*m/z* 154 and 97, respectively), suggesting modification had occurred at the pyrrolidine carbonitrile moiety. Based on comparison of the HPLC retention time and CID spectrum with the synthetic

TABLE 3

Pharmacokinetic parameters (mean ± S.D.) of vildagliptin and its metabolites in plasma after a single p.o. 100-mg dose of [^{14}C] vildagliptin

Pharmacokinetic Parameters[a]	M15.3[b]	M20.2[b]	M20.7[b]	Vildagliptin[b]
C_{max} (ngEq/ml)	54.9 ± 20.4	90.9 ± 23.1	230 ± 50	399 ± 100
t_{max} (h)	3.8 ± 1.5	1.8 ± 0.5	6.0 ± 0.0	1.3 ± 0.5
$AUC_{0-168\ h}$ (ngEq · h/ml)	489 ± 166	559 ± 120	3310 ± 1190	1550 ± 520
$AUC_{0-\infty}$ (ngEq · h/ml)	490 ± 164	572 ± 124	3350 ± 970	1550 ± 510
AUC (%)	8.1	9.3	55	25.7

[a] The abbreviation definitions for pharmacokinetic parameters, e.g., C_{max}, t_{max}, and AUC are denoted in the *Pharmacokinetic Analysis* under *Materials and Methods.*

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