Disposition of the Dipeptidyl Peptidase 4 Inhibitor Sitagliptin in Rats and Dogs

Maria G. Beconi, James R. Reed, Yohannes Teffera, Yuan-Qing Xia, Christopher J. Kochansky, David Q. Liu, Shiyao Xu, Charles S. Elmore, Suzanne Ciccotto, Donald F. Hora, Ralph A. Stearns, and Stella H. Vincent


Received September 27, 2006; accepted December 15, 2006

ABSTRACT:

The pharmacokinetics, metabolism, and excretion of sitagliptin [MK-0431; (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 dipeptidyl peptidase 4 inhibitor, were evaluated in male Sprague-Dawley rats and beagle dogs. The plasma clearance and volume of distribution of sitagliptin were higher in rats (40–48 ml/min/kg, 7–9 l/kg) than in dogs (9 ml/min/kg, 3 l/kg), and its half-life was shorter in rats, ~2 h compared with ~4 h in dogs. Sitagliptin was absorbed rapidly after oral administration of a solution of the phosphate salt. The absolute oral bioavailability was high, and the pharmacokinetics were fairly dose-proportional. After administration of [14C]sitagliptin, parent drug was the major radioactive component in rat and dog plasma, urine, bile, and feces. Sitagliptin was eliminated primarily by renal excretion of parent drug; biliary excretion was an important pathway in rats, whereas metabolism was minimal in both species in vitro and in vivo. Approximately 10 to 16% of the radiolaabeled dose was recovered in the rat and dog excreta as phase I and II metabolites, which were formed by N-sulfation, N-carbamoyl glucuronidation, hydroxylation of the triazolopiperazine ring, and oxidative desaturation of the piperazine ring followed by cyclization via the primary amine. The renal clearance of unchanged drug in rats, 32 to 39 ml/min/kg, far exceeded the glomerular filtration rate, indicative of active renal elimination of parent drug.

Dipeptidyl peptidase 4 (DPP-4) is a ubiquitous proline-specific serine protease responsible for the rapid inactivation of the incretin glucagon-like peptide 1 (GLP-1) (Mentlein et al., 1993; Gorrell, 2005). DPP-1 and GLP-1 analogs have been shown to decrease fasting and postprandial glucose in diabetic patients when given as a continuous intravenous infusion or by subcutaneous administration (Zander et al., 2002; Gautier et al., 2005). The effectiveness of orally administered DPP-4 inhibitors has been demonstrated in obese Zucker rats (Balkan et al., 1999; Pospisilik et al., 2002), mice (Mu et al., 2006), and humans (Ahren et al., 2004; Herman et al., 2004, 2005a,b; Scott et al., 2005; Cornell, 2006). Thus, stabilization of GLP-1 via DPP-4 inhibition represents a new therapeutic approach for type 2 diabetes (Herman et al., 2005b; Scott et al., 2005). In support of the suitability of the rat and dog as toxicology species, the disposition of sitagliptin was evaluated in these species and compared with that in humans. Results from in vitro (rat, dog, human) and in vivo studies in rats and dogs are discussed herein. The absorption, metabolism, and excretion of sitagliptin in humans are reported in the accompanying article (Vincent et al., 2007).

Materials and Methods

Chemicals and Reagents. Sitagliptin was prepared as the hemifumarate and phosphate salts by the Departments of Medicinal Chemistry and Process Research, respectively, Merck Research Laboratories (MRL; Rahway, NJ). [Trifluoromethyl-3-3H]sitagliptin (specific activity 9.44 mCi/mg, radioactivity purity 98%), [2-amine-3-[14C]butyl]sitagliptin (specific activity 132 mCi/mg, radioactivity purity ~99%), and the N-sulfate of sitagliptin (M1; Fig. 1) were prepared by the Labeled Compound Synthesis Group, MRL (Rahway, NJ). 3H- or 14C-labeled sitagliptin was diluted with unlabeled sitagliptin to achieve the final specific activities used in the studies described below. The 2,5-difluoro analog of sitagliptin, used as an internal standard in the quantitative liquid chromatography-tandem mass spectrometry (LC-MS/MS) assay, was provided by the Department of Medicinal Chemistry, MRL. Synthetic standards of metabolites were prepared by the Labeled Compound Synthesis Group (MRL).

Received September 27, 2006; accepted December 15, 2006

Article, publication date, and citation information can be found at http://dmd.aspetjournals.org. doi:10.1124/dmd.106.013110.

ABBREVIATIONS: DPP-4, dipeptidyl peptidase 4; BDC, bile duct-cannulated; GLP-1, glucagon-like peptide-1; LC-MS/MS, liquid chromatography-tandem mass spectrometry; MK-0431, sitagliptin, (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; MRL, Merck Research Laboratories; HPLC, high-performance liquid chromatography; LC-MS, liquid chromatography-mass spectrometry; amu, atomic mass unit(s).
Metabolism and excretion in rats. Male Sprague-Dawley rats were surgically prepared 2 days before dosing as described by Krieter et al. (1994). In brief, cannulas were inserted into the bile duct and duodenum of rats under deep anesthesia. The cannulas were tunneled under the skin, externalized, and threaded through a Covance (Princeton, NJ) harness and tethering system. The biliary and duodenal cannulas were connected to permit recirculation of bile, and the rats were housed individually in metabolism cages and observed frequently during the recovery period. On the day of dosing, the biliary and duodenal cannulas were disconnected and a solution of 13.4% sodium taurocholate/0.9% saline/0.05% potassium chloride was infused through the duodenal cannula while the bile was collected. Groups of three bile duct-cannulated (BDC) rats (n = 400) received a single dose of [14C]sitagliptin either intravenously (2 mg/kg, via a tail vein) or by oral gavage (5 and 20 mg/kg). The specific activity was ~21.5 μCi/mg for the i.v. and 5 mg/kg for the p.o. dose, and ~5 μCi/mg for the 20 mg/kg p.o. dose. Bile and urine were collected into prefiltered bottles containing a known volume of 0.5 M formate buffer, pH 3.0, at specified time intervals as follows: 0 to 2, 2 to 4, 4 to 6, 6 to 8, 8 to 24, 24 to 48, and 48 to 72 h postdose for bile; and 0 to 8, 8 to 24, 24 to 48, and 48 to 72 h postdose for urine. In a separate study, intact rats were dosed with [14C]sitagliptin (9.7 μCi/mg) at 2 mg/kg i.v. and 5 and 5 mg/kg p.o. (n = 3/route), and urine and feces were collected at 24-h intervals for 5 days.

Plasma for metabolite profiling was collected from intact male Sprague-Dawley rats (350–450 g) dosed orally with [14C]sitagliptin (26 μCi/mg) at 20 mg/kg. Blood was collected at 1, 4, and 8 h postdose from two rats at each time point.

Metabolism and excretion in dogs. The in-life phase of the study was conducted at Charles River (Worcester, MA) in BDC male beagle dogs (11–14 kg) fitted a priori with cannulas in the common bile duct, duodenal lumen, and iliac vein. The surgical procedures were conducted according to a protocol approved by the institutional Animal Care and Use Committee. In brief, the dogs were anesthetized and their gall bladders removed and replaced with a balloon catheter. An outflow catheter was inserted in the proximal portion of the common bile duct so that its tip was immediately proximal to the sphincter of Oddi. The operation of the system was tested several times to ensure proper inflation of the balloon, collection of bile, and bidirectional flushing. Using a trocar, the catheters were brought individually through the skin and externalized. The animals were assessed 14 days after surgery based on several clinical parameters such as bilirubin and alanine aminotransferase levels, catheter patency, and bile flow rate. The BDC dogs were dosed intravenously (0.5 mg/kg) or orally (2 mg/kg) with [14C]sitagliptin (n = 3 per route). Each dog in the i.v. and oral groups received approximately 115 and 450 μCi of [14C]sitagliptin, respectively. Bile and urine were collected in prefiltred bottles containing a known volume of formate buffer, pH 3.0, as described above for the rat study, except that samples were collected for 5 days. Feces were collected at 24-h intervals for 5 days. The cages used to house the dogs were washed with a mixture of ethanol and water, and the washes and debris were analyzed for radioactivity by combustion and/or liquid scintillation counting. Blood was collected from the same dogs at several time points between 0.25 and 72 h postdose.

Plasma Protein Binding. [1H]Sitagliptin (~1 × 10⁶ dpm) was mixed with known amounts of unlabeled sitagliptin to achieve final concentrations of 0.1 to 200 μM and evaporated to dryness. Fresh plasma was added to the dry extracts, and the samples were vortexed and incubated at 37°C for 30 min. After incubation, one aliquot was obtained from each sample for total radioactivity determination, and triplicate 1-ml aliquots were subjected to ultracentrifugation at 100,000 rpm [Beckman Coulter (Fullerton, CA) ultracentrifuge model TL-100, rotor TLA 120.2] at 37°C for 3 h. From each test tube, four 200-ml aliquots were removed sequentially from the top and assayed for radioactivity. A blank plasma sample was processed in the same way and used to determine the protein concentration of these fractions. The unbound fraction was calculated as the ratio of concentration of radioactivity in the fraction with the lowest protein concentration and the concentration of total radioactivity in plasma.

Analytical Methods. LC-MS/MS quantitation assay. Concentrations of sitagliptin in rat and dog plasma were determined by LC-MS/MS after on-line extraction. Plasma samples (30 μl) including study samples, standards, quality

(M1) and the Department of Medicinal Chemistry, MRL (M2 and M5). Human, rat, dog, and monkey liver microsomes were prepared following procedures described in the literature (Raucy and Lasker, 1991). Cryopreserved human, dog, and monkey hepatocytes were obtained from In Vitro Technologies (Baltimore, MD).

In Vitro Incubations. [14C]Sitagliptin (10 μM) was incubated at 37°C with liver microsomes (2 mg of protein) in 0.5 ml of 0.1 M KH2PO4 (pH 7.4) containing 1 mM MgCl2 and an NADPH-regenerating system (10 mM glucose 6-phosphate, 1.4 units/ml glucose-6-phosphate dehydrogenase, and 1 mM NADP). Control incubations (without NADPH) were conducted for each species under the same conditions. Reactions were started by adding NADP (or buffer for controls). In separate experiments designed to measure the formation of the carbamoyl glucuronide, incubations were carried out in 0.5 M NaHCO3 buffer containing liver microsomes (2 mg/ml), 50 μg/ml alamethicin, 5 mM saccharolactone, and 5 mM UDP-glucuronic acid. The incubation mixtures were saturated with CO2 before starting the reactions with substrate. Incubations were carried out for 1 h at 37°C, and the reaction was quenched with 12.5 μl of perchloric acid and 25 μl of acetonitrile. Samples were centrifuged at 14,000 rpm for 10 min and transferred to HPLC vials, and 50-μl aliquots were analyzed by LC-MS and radiometric detection for metabolite profiling.

Incubations of [14C]Sitagliptin (10 μM) with hepatocytes (2-ml suspension, 1 × 10⁶ cells/ml) were conducted for 0 (control), 1, 2, and 4 h. The samples were mixed with 4 ml of acetonitrile, and aliquots of the supernatant (200 μl) were subjected to ultracentrifugation at 100,000 rpm [Beckman Coulter (Fullerton, CA) ultracentrifuge model TL-100, rotor TLA 120.2] at 37°C for 3 h. From each test tube, four 200-ml aliquots were removed sequentially from the top and assayed for radioactivity. A blank plasma sample was processed in the same way and used to determine the protein concentration of these fractions. The unbound fraction was calculated as the ratio of concentration of radioactivity in the fraction with the lowest protein concentration and the concentration of total radioactivity in plasma.

Pharmacokinetic analysis: The in-life phase of the study was conducted at Charles River (Worcester, MA) in BDC male beagle dogs (11–14 kg) fitted a priori with cannulas in the common bile duct, duodenal lumen, and iliac vein. The surgical procedures were conducted according to a protocol approved by the institutional Animal Care and Use Committee. In brief, the dogs were anesthetized and their gall bladders removed and replaced with a balloon catheter. An outflow catheter was inserted in the proximal portion of the common bile duct so that its tip was immediately proximal to the sphincter of Oddi. The operation of the system was tested several times to ensure proper inflation of the balloon, collection of bile, and bidirectional flushing. Using a trocar, the catheters were brought individually through the skin and externalized. The animals were assessed 14 days after surgery based on several clinical parameters such as bilirubin and alanine aminotransferase levels, catheter patency, and bile flow rate. The BDC dogs were dosed intravenously (0.5 mg/kg) or orally (2 mg/kg) with [14C]sitagliptin (n = 3 per route). Each dog in the i.v. and oral groups received approximately 115 and 450 μCi of [14C]sitagliptin, respectively. Bile and urine were collected in prefiltred bottles containing a known volume of formate buffer, pH 3.0, as described above for the rat study, except that samples were collected for 5 days. Feces were collected at 24-h intervals for 5 days. The cages used to house the dogs were washed with a mixture of ethanol and water, and the washes and debris were analyzed for radioactivity by combustion and/or liquid scintillation counting. Blood was collected from the same dogs at several time points between 0.25 and 72 h postdose.

Plasma Protein Binding. [1H]Sitagliptin (~1 × 10⁶ dpm) was mixed with known amounts of unlabeled sitagliptin to achieve final concentrations of 0.1 to 200 μM and evaporated to dryness. Fresh plasma was added to the dry extracts, and the samples were vortexed and incubated at 37°C for 30 min. After incubation, one aliquot was obtained from each sample for total radioactivity determination, and triplicate 1-ml aliquots were subjected to ultracentrifugation at 100,000 rpm [Beckman Coulter (Fullerton, CA) ultracentrifuge model TL-100, rotor TLA 120.2] at 37°C for 3 h. From each test tube, four 200-ml aliquots were removed sequentially from the top and assayed for radioactivity. A blank plasma sample was processed in the same way and used to determine the protein concentration of these fractions. The unbound fraction was calculated as the ratio of concentration of radioactivity in the fraction with the lowest protein concentration and the concentration of total radioactivity in plasma.

Analytical Methods. LC-MS/MS quantitation assay. Concentrations of sitagliptin in rat and dog plasma were determined by LC-MS/MS after on-line extraction. Plasma samples (30 μl) including study samples, standards, quality

FIG. 1. Biotransformation pathways of sitagliptin in the rat and dog.
controls, double blanks, and single blanks were dispensed into 96-well plates. An equal volume of the internal standard solution (2.5-difluoro analog of sitagliptin) at 1 μg/ml in 0.5 M formic acid in water/acetonitrile (80:20 v/v) was added into each well. The samples were vortexed for 5 min, centrifuged at 2000g for 10 min at 10°C, and extracted on-line. An in-house assembled parallel on-line extraction system, described in detail by Xia et al. (2001) was used. The system consisted of a LEAP (Carboro, NC) HTS PAL autosampler coupled with two Shimadzu LC-AD pumps (Shimadzu, Columbia, MD), two PerkinElmer Series 200 micro pumps (PerkinElmer Life and Analytical Sciences, Boston, MA), two 10-port switching valves, a FluoroSep-RP phenyl analytical column (2 × 50 mm, 5 μm; Phenomenex, Torrance, CA) eluted at 0.8 ml/min, two Oasis HLB extraction columns (Waters, Milford, MA) washed at 4 ml/min, and two in-line precolumn filters. The total analysis time per sample, including loading, elution, and equilibration steps, was 2.5 min (0.5 min for on-line extraction and 2.0 min for the analytical separation). Sitagliptin and the internal standard eluted at 1.3 min. Analyte detection was achieved with positive TurboIonSpray tandem mass spectrometry using multiple reaction monitoring of transitions unique to each compound, m/z 408.3→235.2 and m/z 390.2→235.1 for sitagliptin and internal standard, respectively. The PE Sciex Analyst 1.1 software (Applied Biosystems, Foster City, CA) was used for data acquisition and peak integration. The peak area ratios of sitagliptin to the internal standard were plotted as a function of the nominal concentrations of sitagliptin. The standard calibration curves were constructed by fitting the weighted (1/x²) data points to a quadratic equation using least squares. The lower limit of quantification was 1 ng/ml (2.5 nM) for rat and 5 ng/ml (12 nM) for dog using 30 μl of plasma. The performance of the calibration standards was better than 95.5% CV, and accuracy was between −7.5 and 5.3%. The within-run precision of the quality control samples was 11% or better, (excluding the lower limit of quantification) and between-run precision was better than 5%. The accuracy of the quality control samples was between 87 and 111%. At the lower limit of quantification, the precision was better than 14% and accuracy better than 11%. Matrix ion suppression was evaluated using postcolumn infusion of sitagliptin and injection of the blank plasma to the on-line LC-MS/MS system, and was determined to be minimal. Sitagliptin and the internal standard were shown to be stable in rat and dog plasma for 4 h at room temperature and for 24 h at 10°C. Pharmacokinetic calculations. Pharmacokinetic parameters were calculated by established noncompartmental methods using the Watson software (version 6.4.0.04; Thermo Fisher Scientific, Inc., Waltham, MA). The area under the plasma concentration versus time curve from 0 to t (AUC_{0-t}), where t is last time point with concentrations above the lower limit of quantitation, was calculated using linear trapezoidal interpolation in the ascending slope and logarithmic trapezoidal interpolation in the descending slope. The portion of the AUC from the last measurable concentration to infinity (AUC_{last}) was estimated by C_{last}/k, where C_{last} represents the last measurable concentration and k is the elimination rate constant. The latter was determined from the concentration versus time curve at the terminal phase by linear regression of the semilogarithmic plot. The renal clearance of sitagliptin in rats was estimated by multiplying the plasma clearance of unbound drug (total clearance divided by unbound fraction in plasma) with the fraction of dose excreted unchanged into urine. Determination of total radioactivity. Concentrations of total radioactivity in plasma, bile, urine, cage washes, and samples from in vitro experiments were determined by direct counting in a Packard 1900TR (PerkinElmer Life and Analytical Sciences) or a Beckman Coulter LS-6500 liquid scintillation analyzer, after mixing 50 to 100 μl with 6 ml of ScintiSafe Gel scintillation cocktail (Fisher Scientific, Pittsburgh, PA). Total radioactivity levels in feces and cage debris were determined by combusting triplicate aliquots of homogenates (1:3 specimen/water, w/w) in a Packard model 307 oxidizer, followed by determination of radioactivity in a Beckman Coulter LS-6500 liquid scintillation analyzer.

Processing of samples for metabolite profiling. Urine and bile from rats (0–24 h) and dogs (0–72 h) were pooled according to volume and across animals (three per sample), mixed with acetonitrile (−0.3–1 ml/ml), vortexed, and centrifuged at −14,000 rpm. The supernatants were analyzed using LC-MS coupled with radiometric monitoring. Plasma samples were pooled across animals, mixed with an equal volume of acetonitrile, vortexed (10 min), and centrifuged at 4000 rpm for 15 min. The resulting supernatants were analyzed directly or after evaporation under nitrogen and reconstitution in water/acetonitrile/acetic acid (90:10:0.1, by volume).
The i.v. pharmacokinetics were dose-proportional between 0.5 and 5 mg/kg in rats and from 0.5 to 1.5 mg/kg in dogs. The mean plasma clearance and volume of distribution values were higher in rats (40–48 ml/min/kg and ~7–9 l/kg) than in dogs (~9 ml/min/kg and ~3 l/kg), and the half-life was shorter, ~2 h in rats compared with ~4 h in dogs. After p.o. administration, absorption was rapid in both species (T<sub>max</sub> 0.5–2 h in most animals). The increase in plasma AUC<sub>0-∞</sub> was approximately dose-proportional between 2 and 180 mg/kg in rats, and between 0.4 and 30 mg/kg in dogs. The mean bioavailability was ~59% in rats and 89 to 97% in dogs (Table 2).

**Plasma Protein Binding.** The fraction of [3H]sitagliptin (0.1–200 μM) bound to plasma proteins in vitro, as determined by ultracentrifugation, was 28 to 36% in rats, 31 to 37% in dogs, and 34 to 46% in humans.

**Identification of Metabolites.** The structures of the metabolites of sitagliptin shown in Fig. 1 were elucidated by LC-MS (Sciex API 3000 triple quadrupole mass spectrometer) using a combination of full-scan and product ion-scan (MS/MS) analyses of samples from hepatocyte incubations, as well as rat and dog bile. Full-scan analysis of sitagliptin gave [M + H]<sup>+</sup> at m/z 408. The product ion mass spectrum of m/z 408 showed predominant fragment ions at m/z 193, 235, and 174 (Fig. 2). The ions at m/z 193 and 235 were assigned to the triazolopiperazine moiety, whereas the ion at m/z 174 was assigned to the trifluorophenyl-containing portion of the molecule. All product ions from portions of the molecule containing the primary amino group had a characteristic loss of a 17-amu fragment corresponding to the mass of ammonia.

Metabolite M1 displayed an [M + H]<sup>+</sup> of m/z 488 consistent with sulfate conjugation. The product ion mass spectrum of m/z 488 showed product ions identical to those of parent drug after the loss of 80 amu from m/z 488 (spectrum not shown). The structure of the sulfate conjugate was confirmed by comparison with a synthetic standard (HPLC retention time and mass spectrum).

Metabolite M4 displayed an [M + H]<sup>+</sup> of m/z 628 consistent with formation of a carbamoyl glucuronide (+220 amu). The product ion mass spectrum (Fig. 3) of m/z 628 produced sequential loss of gluconic acid (176 amu) and carbon dioxide (44 amu) to give m/z of 452 and 408, respectively. This is a typical fragmentation pattern for a carbamoyl glucuronic acid conjugate (Liu and Pereira, 2002; Beconi et al., 2003).

Metabolites M2 and M5 displayed a [M + H]<sup>+</sup> of m/z 406, 2 amu lower than parent. The product ion mass spectra of m/z 406 were
Dose and 37 and 59% of the oral dose were recovered in urine and

Metabolite M3 displayed an [M + H]+ of m/z 600 corresponding to the addition of one oxygen and glucuronic acid. The product ion mass spectrum of m/z 600 produced ions of m/z 424 and 406 corresponding to a sequential loss of glucuronic acid and water (Fig. 4). The product ions of m/z 174 and 191 indicated an intact trifluorophenyl moiety and loss of water from an oxygenated triazolopiperazine moiety, respectively. This suggested that M3 could be a glucuronic acid conjugate of a hydroxylated metabolite with the hydroxyl group on the triazolopiperazine moiety. The exact site of hydroxylation, however, could not be determined based on the mass spectral data, and identification by NMR was not possible because of the low amounts of M3 in all in vitro and in vivo samples.

Metabolite M6 gave an [M + H]+ at m/z 424, 16 mass units higher than sitagliptin. Upon collision-induced dissociation, it gave a prominent ion at m/z 251 which was also 16 mass units higher than the corresponding ion in the parent compound. Further fragmentation of m/z 251 gave m/z 233 (loss of water). This suggested that M6 could be a hydroxylated metabolite. The exact site of hydroxylation could not be determined based on the mass spectral data.

The glutathione conjugates detected in rat hepatocytes and rat bile gave an [M + H]+ at m/z 711, and a major fragment at m/z 582 formed by loss of 129 amu (pyroglutamate) (Fig. 5), which is characteristic of glutathione conjugates (Baillie and Davis, 1993). The exact structures of the glutathione conjugates could not be determined from the mass spectral data. The conjugates were postulated to be formed by oxidative defluorination of the trifluoro-phenyl ring and addition of reduced glutathione, as described in the literature (Rietjens et al., 1997; Park et al., 2001).

Excretion of Radioactivity. After administration of [14C]sitagliptin to rats at 2 mg/kg i.v. and 5 mg/kg p.o., ~54 and 40% of the i.v. dose and 37 and 59% of the oral dose were recovered in urine and feces, respectively (Table 3). Most of the excretion occurred within 24 h, 89% of the i.v. dose and 90% of the oral dose. In bile duct-cannulated rats, ~55% of the i.v. dose (2 mg/kg) and 53 and 54% of the p.o. doses (5 and 20 mg/kg) were recovered in urine (Table 3). Excretion into bile (21% of the i.v. and 20–29% of the p.o. doses) was lower than expected based on the recovery of radioactivity in feces after i.v. administration to intact rats (40% of the dose). Feces were not collected in the BDC rat study. The high recovery of radioactivity from the oral doses in bile and urine (~74% of the dose), indicated that absorption was high.

In male BDC beagle dogs dosed with [14C]sitagliptin at 0.5 mg/kg i.v. and 2 mg/kg p.o., most of the dose was excreted into urine, ~74 and 77% of the i.v. and p.o. doses, respectively (Table 3). Approximately 8% of the dose was excreted into bile after both routes of administration. As described below, most of the radioactivity in the rat and dog excreta was comprised of sitagliptin, indicating that the drug was eliminated primarily unchanged.

In Vivo Metabolism in Rats and Dogs. In Fig. 6, radiochromatograms of extracts of rat and dog urine and bile after oral administration of [14C]sitagliptin at 5 mg/kg in the rat and 2 mg/kg in the dog are presented, which were acquired using electrospray LC-MS (LCQ Deca XP ion trap mass spectrometer) coupled with on-line radiometric detection. The mass spectra of the metabolites were comparable to those obtained on the triple quadrupole instrument and thus not shown. Similar profiles were obtained after i.v. administration in both species, and at 20 mg/kg p.o. in the rat. Parent drug accounted for the majority of the radioactivity in rat urine and bile (~99% and 78%, respectively) and dog urine (95%). Most of the radioactivity in dog bile was comprised of M4, the carbamoyl glucuronide (53%), and parent drug (38%). Minor metabolites in rat and dog bile and urine were M1 (N-sulfate conjugate of parent drug; rat bile only), M2 and M5 (cis and trans cyclized derivatives, respectively; rat and dog urine; dog bile), M3 (ether-linked glucuronide of a hydroxylated product; rat urine and bile), and M6 (hydroxylated metabolite; rat urine, rat and dog bile). Rat bile also contained low amounts of a glutathione adduct

FIG. 4. Product ion spectrum and proposed fragmentation of the sitagliptin hydroxy glucuronic acid conjugate (M3).
levels in rat plasma, each representing metabolites excreted into rat urine and bile were observed at low rats and dogs. Representative profiles are shown in Fig. 7. All of the comprising 80 to 85% of the circulating radioactivity in orally dosed bile, respectively.

Excreta were collected for 3 days from BDC rats and 5 days from intact rats and BDC dogs; values are mean ± standard deviation (n = 3).

<table>
<thead>
<tr>
<th>Species</th>
<th>Dose</th>
<th>Recovery of Radioactivity (% Dose)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>2 mg/kg i.v.</td>
<td>54 ± 5.8  N.D.  40 ± 3.2  94 ± 9.0</td>
</tr>
<tr>
<td></td>
<td>5 mg/kg p.o.</td>
<td>37 ± 2.6  N.D.  59 ± 2.5  96 ± 5.1</td>
</tr>
<tr>
<td>BDC Rat</td>
<td>2 mg/kg i.v.</td>
<td>55 ± 1.4  21 ± 1.2  N.D.  N.D.</td>
</tr>
<tr>
<td></td>
<td>5 mg/kg p.o.</td>
<td>54 ± 7.3  20 ± 6.7  N.D.  N.D.</td>
</tr>
<tr>
<td></td>
<td>20 mg/kg p.o.</td>
<td>53 ± 7.7  29 ± 5.8  N.D.  N.D.</td>
</tr>
<tr>
<td>BDC Dog</td>
<td>0.5 mg/kg i.v.</td>
<td>75.0 ± 4.5  8.1 ± 4.2  9.3 ± 2.0  95 ± 4.9*</td>
</tr>
<tr>
<td></td>
<td>2 mg/kg p.o.</td>
<td>77.5 ± 1.7  7.8 ± 5.3  4.2 ± 2.1  94 ± 6.0*</td>
</tr>
</tbody>
</table>

N.D., not determined.

* Total recovery in dogs includes radioactivity in cage washes and debris.

(Fig. 1), comprising approximately 4 to 5% of the biliary radioactivity (<1% of the total dose). The metabolite profiles in urine and feces from intact rats (not shown) were similar to the profiles in urine and bile, respectively.

Parent drug was the major radioactive component of rat plasma, comprising 80 to 85% of the circulating radioactivity in orally dosed rats and dogs. Representative profiles are shown in Fig. 7. All of the metabolites excreted into rat urine and bile were observed at low levels in rat plasma, each representing ~2 to 7% of circulating radioactivity; the only exception was M4, which was not detected in rat plasma. The only metabolites detected in dog plasma were M2 and M5. M2 was found at trace levels at the early time points, and contributed up to 8% of the circulating radioactivity between 7 and 24 h, whereas the contribution of M5 to circulating radioactivity increased steadily from 4% at 1 h to 14% at 5 h, 25% at 7 h, and 48% at 24 h. It was estimated that M2 and M5 contributed to ~5 and 19%, respectively, of the AUC of total radioactivity in dog plasma after oral administration of [14C]sitagliptin (data not shown).

**In Vitro Metabolism in Rats, Dogs, Monkeys, and Humans.** The metabolism of [14C]sitagliptin (10 μM) in vitro was minimal in rat, dog, monkey, and human liver microsomes and hepatocytes (2–13% turnover in liver microsomes after 1 h, and 1–15% in hepatocytes after 4 h; data not shown). LC-MS/MS analysis of microsomal and hepatocyte incubation extracts revealed the presence of M2 and M5 in male rat, dog, monkey, and human liver microsomes, and human hepatocytes; M3 in male rat hepatocytes; and M6 in male monkey liver microsomes, human liver microsomes, and rat and human hepatocytes. Also, two glutathione conjugates were observed in rat hepatocytes but not in hepatocytes from the other species. M4 was not detected in the hepatocyte incubations, but was observed in UDP-glucuronic acid-enriched dog liver microsomal incubations under a CO2-enriched environment. M1 was not detected in any of the in vitro incubations.

**Table 3: Dose recovery (percentage) in urine and feces after single dose administration of [14C]sitagliptin to rats.**

<table>
<thead>
<tr>
<th>Species</th>
<th>Dose</th>
<th>Urine (%)</th>
<th>Bile (%)</th>
<th>Feces (%)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>2 mg/kg i.v.</td>
<td>54 ± 5.8</td>
<td>40 ± 3.2</td>
<td>94 ± 9.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5 mg/kg p.o.</td>
<td>37 ± 2.6</td>
<td>N.D.</td>
<td>59 ± 2.5</td>
<td>96 ± 5.1</td>
</tr>
<tr>
<td>BDC Rat</td>
<td>2 mg/kg i.v.</td>
<td>55 ± 1.4</td>
<td>21 ± 1.2</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td></td>
<td>5 mg/kg p.o.</td>
<td>54 ± 7.3</td>
<td>20 ± 6.7</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td></td>
<td>20 mg/kg p.o.</td>
<td>53 ± 7.7</td>
<td>29 ± 5.8</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td>BDC Dog</td>
<td>0.5 mg/kg i.v.</td>
<td>75.0 ± 4.5</td>
<td>8.1 ± 4.2</td>
<td>9.3 ± 2.0</td>
<td>95 ± 4.9*</td>
</tr>
<tr>
<td></td>
<td>2 mg/kg p.o.</td>
<td>77.5 ± 1.7</td>
<td>7.8 ± 5.3</td>
<td>4.2 ± 2.1</td>
<td>94 ± 6.0*</td>
</tr>
</tbody>
</table>

N.D., not determined.

* Total recovery in dogs includes radioactivity in cage washes and debris.

**Discussion**

The present studies indicated that the disposition of sitagliptin in rats and dogs is similar to that in humans (Vincent et al., 2007) in that it is eliminated primarily unchanged into the urine. Biliary excretion was an important elimination pathway in rats but not dogs, whereas metabolism was minimal in both species. As reported in humans (Bergman et al., 2006), results from the present studies indicate that sitagliptin is subject to active renal secretion in rats, as well. The renal clearance of unbound drug, estimated from the total plasma clearance (~40–48 ml/min/kg in rats, ~9 ml/min/kg in dogs), the unbound fraction in plasma (~0.65 in both species), and the fraction of dose eliminated unchanged into urine (~0.54 in rats and 0.75 in dogs) was ~33 to 39 ml/min/kg in rats and ~10 ml/min/kg in dogs. Thus, the renal clearance in the rat but not dog was much higher than the glomerular filtration rate, ~5 to 15 ml/min/kg. Results from studies described elsewhere indicate that sitagliptin is a substrate of the human renal organic anion transporter, hOAT3, and P-glycoprotein, which may be involved in its uptake into the kidney and efflux into urine, respectively (X. Y. Chu, K. Bleasby, J. Yabut, X. Cai, S. Xu, A. J. Bergman, M. P. Braun, D. C. Dean, and R. Evers, manuscript submitted for publication). P-glycoprotein does not appear to limit the oral absorption of sitagliptin, inasmuch as ≥74% of radioactivity was recovered in bile and urine, primarily as parent drug, after administration of [14C]sitagliptin.
The lower recovery of radioactivity in bile from BDC rats than in feces from intact animals (21 ± 1.2 versus 40 ± 3.2% of an i.v. dose) is suggestive of intestinal secretion, possibly mediated by P-glycoprotein. As well, the small increase in the dose-normalized AUC of sitagliptin with oral dose (0.6 ± 0.1 at 2 mg/kg increasing to 1.2 ± 0.1 at 180 mg/kg) is consistent with this speculation, although this could be caused also by saturation of metabolism and renal secretion at the high oral doses in rats.

Although metabolism of [14C]sitagliptin was minimal in rats and dogs, with ~10 and 16% of the total dose recovered as metabolites in the excreta, respectively, a variety of metabolites (Fig. 1) were observed which were formed by phase I (M6, hydroxylation; M2 and M5, oxidative desaturation) and phase II pathways (M1, sulfation; M3, glucuronidation after hydroxylation; M4, carbamoyl glucuronidation). All six metabolites were observed in rats, whereas dogs generated four of these metabolites, namely, M2, M4, M5, and M6. A mixture of glutathione conjugates (Fig. 1) speculated to have been formed by epoxidation of the trifluorophenyl ring, followed by addition of reduced glutathione and loss of fluoride (Rietjens et al., 1997; Park et al., 2001) appeared to be rat-specific, in that they were not detected in humans (Vincent et al., 2007) or dogs. Another interesting species difference was the relative abundance of M2 and M5 in dog plasma especially at the later time points (~33 and 56% of the plasma radioactivity at 7 and 24 h, respectively), even though a similar fraction of the dose, less than 5%, was eliminated as M2 and M5 in all species. This observation suggested that these metabolites were cleared at a slower rate than sitagliptin in dogs.

Metabolism to a carbamoyl glucuronide was a minor elimination pathway, accounting for ~1, ~4, and ~2% of the dose in rats, dogs, and humans, respectively. Carbamoyl glucuronic acid conjugates are formed by reaction of the primary amine with carbon dioxide to form a carbamic acid with subsequent conjugation with glucuronic acid. In the presence of carbon dioxide, amino acids (Morrow et al., 1974) and other amines (Greenaway and Whatley, 1987; Delbressine et al., 1990; Schaefer, 1992) can undergo nonenzymatic, reversible reactions to form carbamic acids. Formation of a carbamoyl glucuronide of a
primary amine was reported for the dipeptidyl peptidase inhibitors t-threo isoleucine thiazolidide and its allo stereoisomer (Beconi et al., 2003) and seems to be common for a group of low molecular weight amino amides.

Another interesting biotransformation pathway of sitagliptin in rats and humans, also minor, is sulfation of the primary amine. To our knowledge, N-sulfation of aliphatic amine groups has not been reported in the literature for xenobiotics, although it is a critical step in heparan sulfate/heparin biosynthesis. The latter is catalyzed by heparan sulfate/heparin N-deacetylase/N-sulfotransferase-1, a bifunctional enzyme that removes N-acetyl groups from selected N-acetyl-D-glucosamine units follow by N-sulfation of the generated free amino groups (Sugahara and Kitagawa, 2002).

In conclusion, the systemic clearance of sitagliptin in rats and dogs is driven primarily by renal elimination of intact parent drug, with contribution from biliary excretion (mostly in rats) and metabolism (minor in both species). Our data also suggest that active transport mechanisms are probably involved in the renal elimination of sitagliptin in rats but not dogs.

Acknowledgments. We thank the following individuals, all of MRL: Drs. Ashok Chaudhary, David Schenk, Dennis Dean, Conrad Raab, Allen Jones, and Adria Colletti, and Ann Mao, Randy Miller, Courtney Nugent, Yolanda Jakubowski, Chris Freedon, and John Strauss for their contribution in these studies, and Drs. Eugene Tan and Jiunn Lin for helpful discussions. Also, we acknowledge the contribution of Paul Zavorskas and other Charles River Laboratories personnel in the BDC dog study.

References

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