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Absorption, Metabolism, and Excretion of [^{14}C]Imidafenacin, a New Compound for Treatment of Overactive Bladder, After Oral Administration to Healthy Male Subjects

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ABSTRACT:

The absorption, metabolism, and excretion of imidafenacin [KRP-197/ONO-8025, 4-(2-methyl-1*H*-imidazol-1-yl)-2,2-diphenylbutanamide], a new antimuscarinic drug developed for treatment of overactive bladder, were assessed in six healthy male subjects after a single oral administration of 0.25 mg of [^{14}C]imidafenacin (approximately 46 μCi). The highest radioactivity in the plasma was observed at 1.5 h after administration. The apparent terminal elimination half-life of the total radioactivity was 72 h. Approximately 65.6 and 29.4% of the administered radioactivity were recovered in the urine and feces, respectively, within 192 h after administration. The metabolite profiling by high-performance liquid chromatography-radiodetector and liquid chromatography/tandem mass spectrometry demonstrated that the main component of radioactivity was unchanged imidafenacin in the 2-h plasma. The *N*-glucuronide conjugate (M-9) was found as the major metabolite and the oxidized form of the 2-methylimidazole moiety (M-2) and the ring-cleavage form (M-4) were detected as the minor metabolites in the 2-h plasma, but M-4 was found to be the main component in the 12-h plasma. Unchanged imidafenacin, M-9, M-2, and other oxidized metabolites were excreted in the urine, but the unchanged imidafenacin and M-9 were not found in the feces. Two unique metabolites were found in the urine and feces, which were identified as the interchangeable *cis*- and *trans*-isomers of 4,5-dihydrodiol forms of the 2-methylimidazole moiety. These findings indicate that imidafenacin is rapidly and well absorbed (at least 65% of dose recovered in urine) after oral administration, circulates in human plasma as the unchanged form, its glucuronide, and other metabolites, and is then excreted in urine and feces as the oxidized metabolites of 2-methylimidazole moiety.

Imidafenacin [KRP-197/ONO-8025, 4-(2-methyl-1*H*-imidazol-1-yl)-2,2-diphenylbutanamide] (Fig. 1) is a newly synthesized antimuscarinic drug developed for treatment of overactive bladder. Acetylcholine is well known for playing a major role in contracting the bladder through activation of muscarinic receptors (Somogyi and de Groat, 1992; Wang et al., 1995; Braverman et al., 1998). Compounds with high affinity for the muscarinic acetylcholine receptor, including propiverine, tolterodine, oxybutynin, darifenacin, and solifenacin, have been used in management of overactive bladder (Chapple et al., 2002; Andersson and Yoshida, 2003; Andersson, 2004; Robinson and Cardozo, 2005). Imidafenacin showed high in vitro affinity for muscarinic receptor subtypes M_1 and M_3 in the functional assay using isolated animal tissues and in the binding assay using recombinant human receptors (Miyachi et al., 1999; Kobayashi et al., 2007a). In addition, imidafenacin inhibited carbachol-induced contraction of isolated guinea pig and human bladder mediated by the M_3 receptor and acetylcholine release from isolated rat and human bladder mediated by the prejunctional M_1 receptor (Murakami et al., 2003; Kobayashi et al., 2007a). A carbachol-induced reduction in bladder capacity and distention-induced rhythmic bladder contraction were prevented by imidafenacin dose dependently in conscious rats (Miyachi et al., 1999; Kobayashi et al., 2007b). On the other hand, the effects of imidafenacin on carbachol-induced salivary gland secretion mediated by M_3 receptor alone were less potent than those on bladder contraction in rats (Miyachi et al., 1999; Kobayashi et al., 2007b).

In the pharmacokinetic assays in the preclinical toxicology studies, imidafenacin was absorbed rapidly with absolute bioavailability of 5.6% in rats and 36.1% in dogs after oral administration (Masuda et al., unpublished observations). Orally administered [^{14}C]imidafenacin was excreted as many metabolites in the feces, and the total recovery in the urine and feces were more than 95% of the administered dose in rats and dogs (Sato et al., unpublished observations). In phase I clinical trials, the plasma concentration and urinary excretion of imidafenacin increased dose dependently in the range from 0.025 to 0.5 mg of single oral dose, and the apparent elimination half-life ranged from 2.6 to 3.0 h (Shimada et al., 2007b). Accumulation of imidafenacin in the plasma and urine was insignificant during multiple oral administration of 0.25 mg twice a day (Shimada et al., 2007a).

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ABBREVIATIONS: LSC, liquid scintillation counter; HPLC, high-performance liquid chromatography; RAD, radiodetector; MS/MS, tandem mass spectrometry; ESI, electron spray ionization; SRM, selected reaction monitoring; DEPT, distortionless enhancement by polarization transfer; ^{1}H-^{1}H COSY, proton-proton correlated spectroscopy; HMQC, heteronuclear multiple quantum coherence; HMBC, heteronuclear multiple bond coherence; UGT, uridine diphosphate glucuronosyltransferase.

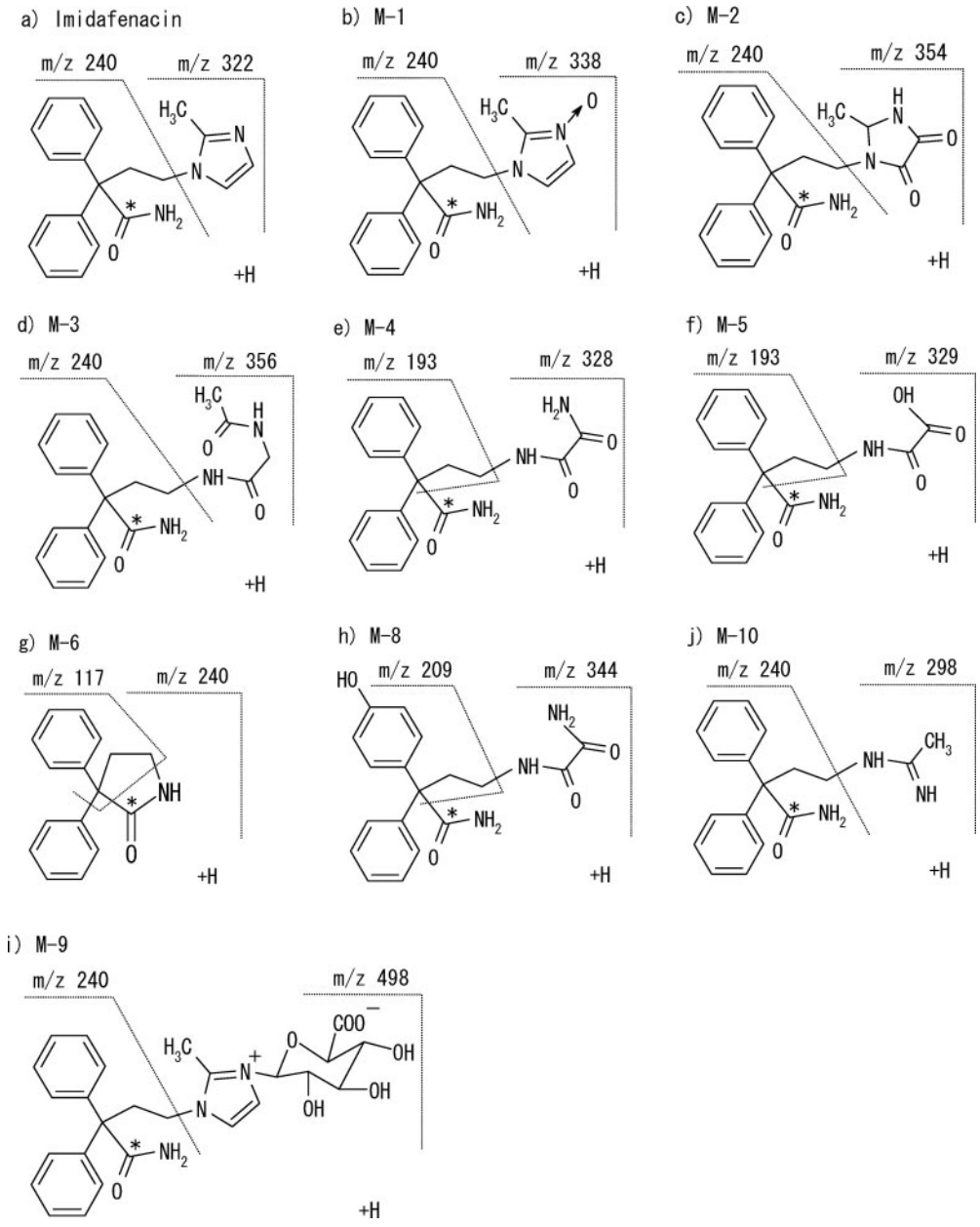


FIG. 1. Chemical structures of [^{14}C]imidafenacin and postulated metabolites, and their fragment ions. *, ^{14}C labeled position; broken line, precursor and product ions obtained by collision-induced dissociation in LC/MS/MS.

The purposes of this study were to determine the absorption and excretion kinetics of imidafenacin in humans after oral administration of [^{14}C]imidafenacin and to investigate the metabolite profile of imidafenacin in the plasma and excreta. Imidafenacin is supposed to be transformed to many metabolites in humans; however, clinical dosage is very low. In this case, a human mass-balance assay using radiolabeled drugs is a valuable measure to elucidate the fate of therapeutic drug in the body (Cox et al., 2000; Patrick et al., 2002; Lantz et al., 2003; Minematsu et al., 2005). Accordingly, ^{14}C-labeled imidafenacin ([^{14}C]imidafenacin, ^{14}C-labeled position is shown in Fig. 1) was administered oral at a dose of 0.25 mg (approximately 46 μCi) to six healthy male subjects, and the total radioactivity and metabolite profile in plasma, urine, and feces were determined by a LSC or HPLC connected to a RAD and MS/MS detectors. In addition, chemical structures of two unique diol metabolites found in the excreta were identified by comparison with those of the reference product obtained from imidafenacin with the cupro-ascorbate oxidation system.

Materials and Methods

Radiolabeled Material and Other Materials. [^{14}C]Imidafenacin (Fig. 1) was synthesized at GE Healthcare UK Ltd. (Little Chalfont, Buckinghamshire, UK), with a certificate of analysis of the radiochemical purity (99.4%) and specific activity (178 μCi/mg). [^{14}C]Imidafenacin was stored at −80°C in the dark. The radiochemical purity (more than 99.4%) and chemical authenticity of [^{14}C]imidafenacin was confirmed by HPLC before the study, after mock dose preparation and administration to subjects. Authentic standards of imidafenacin and its metabolites M-1, M-2, M-3, M-4, M-5, M-6, M-8, M-9, and M-10 (Fig. 1) were prepared by chemical synthesis or isolation from biological samples in our laboratory. All other reagents were of HPLC grade or analytical grade and were obtained from commercial sources.

Dose Preparation. [^{14}C]Imidafenacin was dissolved in ethanol, and the radioactive concentration was determined. A certain volume of the [^{14}C]imidafenacin ethanolic solution was dispensed into the hard gelatin capsules (equivalent to 0.25 mg and 46 μCi per capsule). Ethanol was evaporated under a steady stream of nitrogen.

The radioactive dose was set according to human dosimetry calculations established by the National Radiological Protection Board (Oxon, UK). Ad-

ministration of [^{14}C]imidafenacin for the radioactive medical product was authorized by the Administration of Radioactive Substances Advisory Committee (Oxon, UK). The radiation exposure in this study, approximately 0.34 mSv, fell into category IIa studies (0.1–1 mSv) in the International Commission on Radiological Protection guidelines (1992). A single oral dose of 0.25 mg of imidafenacin has been known to be well tolerated in the phase I clinical trials of nonradiolabeled imidafenacin.

Study Design. This study was an open-label and nonrandomized study involving six healthy Caucasian male subjects, all of whom were to receive a single oral dose of [^{14}C]imidafenacin. The clinical phase of this study was conducted at the clinical unit of Inveresk Research (Edinburgh, UK). This study was performed in accordance with good clinical practice guidelines and the Declaration of Helsinki. Before the start of study, the study protocol and the informed consent documents were approved by an independent ethics review committee. All of the subjects provided written informed consent before the study.

The subjects were screened within 21 days before administration of [^{14}C]imidafenacin. The mean age of the subjects was 44.0 (from 32 to 53) years, the mean height was 177.7 (from 168 to 185) cm, the mean weight was 82.43 (from 71.2 to 97.1) kg, and the mean body mass index was 26.08 (from 22.0 to 29.0) kg/m^2. The subjects were admitted to the clinical unit on the day before administration of [^{14}C]imidafenacin, and a light supper was served at approximately 11:00 PM. Neither food nor beverage was permitted after the supper except for water. At approximately 8:30 AM on day 1 each subject was administered a single oral dose of 0.25 mg of [^{14}C]imidafenacin with approximately 200 ml of water. Subjects fasted for approximately 4 h after administration, and then a light lunch was served. Safety evaluations including physical examination, hematology and clinical chemistry analyses, urinalysis, 12-lead electrocardiogram, and vital signs were performed throughout the admission period

Sample Collection. Blood samples were collected from an in situ venous cannula or by venipuncture into lithium heparin tubes. From 10 to 20 ml of blood samples were taken at the following times: 0 (before administration), 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 48, 72, 96, 120, 144, and 168 h after administration. Blood samples were centrifuged for 10 min at approximately 4°C, and separated plasma was transferred into polypropylene tubes. Total radioactivities in the samples were analyzed as soon as possible, and the remainders of the samples were stored at approximately −80°C for metabolite profiling.

Urine samples were collected for the following intervals: 0 (before administration), 0 to 4, 4 to 8, 8 to 12, 12 to 24, 24 to 48, 48 to 72, 72 to 96, 96 to 120, 120 to 144, and 144 to 168 h after administration. For one subject, urine samples were collected until 192 h after administration because excretion of radioactivity continued in the urine. Aliquots of each sample were transferred into separate polypropylene tubes for analysis of the total radioactivity and metabolite profiling, and then the samples were stored at approximately −20°C and at approximately −80°C, respectively.

Fecal samples were collected for the following intervals: 0 (before administration), 0 to 24, 24 to 48, 48 to 72, 72 to 96, 96 to 120, 120 to 144, and 144 to 168 h after administration. For one subject, fecal samples were collected until 192 h after administration because excretion of radioactivity continued in the feces. Fecal samples were homogenized in water, aliquots of each homogenate were retained in separate closed containers for analysis of the total radioactivity and metabolite profiling, and the samples were stored at approximately −20°C and at approximately −80°C, respectively.

Analysis of Total Radioactivities in Samples. Volumes or weights of all samples were measured. Duplicate portions of plasma and urine samples were diluted with water or compatible solvent and dissolved in liquid scintillation fluid, Quickszint 1 (Zinsser Analytic, Maidenhead, UK). Duplicate portions of fecal homogenate samples (approximately 0.3 g) were combusted using a Tri-Carb 307 automatic sample oxidizer (Packard Biosciences Ltd., Pangbourne, UK). The $^{14}CO_2$ generated was collected in the absorbing fluid, Carbo-Sorb CO_2 and scintillation fluid, Permafluor E$^+$ (Packard Biosciences Ltd.).

All of the samples in the scintillation fluid were subjected to liquid scintillation counting for 5 min, together with representative blank samples, using an LSC (Tri-Carb 2500TR; Packard Biosciences Ltd.) with automatic quench correction by an external standard method. The limit of reliable measurement was defined as 30 dpm above the background.

Calculation of Urinary and Fecal Excretion. The total radioactivities excreted in the urine and feces were calculated by the time points after administration of [^{14}C]imidafenacin and are expressed as a percentage of the administered radioactivity (percentage of dose).

Estimation of Pharmacokinetic Parameters. Pharmacokinetic parameters were estimated using WinNonlin Professional (version 3.1; Pharsight, Mountain View, CA). A noncompartmental approach was used to generate parameter estimates, using WinNonlin model 200 (extravascular input). All of the total radioactivities calculated from data below the limit of reliable detection (<30 dpm above the background) were considered to be zero. The apparent terminal elimination phase was identified by regression analysis with WinNonlin, using data points from 24 to 168 h after administration.

The observed maximum concentration (C_{max}) of the total radioactivity and time at maximum concentration (T_{max}) after administration were identified by inspection of the total radioactivity versus time data. The area under the concentration versus time curve from time 0 to t h (AUC_{0-t}), where t indicates the time point for the last sample in which the total radioactivity was reliably quantified, was calculated using the linear trapezoidal method. The AUC_{0-inf}, area under the concentration versus time curve from time 0 to infinity, was calculated as the sum of AUC_{0-t} and C_t/K_{el}, where C_t indicates the total radioactivity at t hours as mentioned above and K_{el} indicates the terminal elimination rate constant determined from the slope of the terminal elimination phase. The half-life of the terminal elimination phase ($t_{1/2el}$) was calculated as $0.693/K_{el}$.

Metabolite Profiling. *Pretreatment of plasma.* Plasma samples at 2 h (approximately T_{max}) and at 12 h (elimination phase) were selected as the samples to assess the metabolic profile of imidafenacin. The radioactivity in the latter sample was on the brink of the detection limit. The cryopreserved plasma samples (2 and 12 h) were thawed, and designated amounts of the plasma samples obtained from six subjects after administration of [^{14}C]imidafenacin were pooled. The pooled plasma was mixed with a 3-fold volume of acetonitrile containing 1% acetic acid, sonicated for 5 min, and centrifuged at 1800g for 15 min at 4°C to separate the supernatant. The residue was reextracted with the same solvent, followed by shaking and centrifugation. The extraction recoveries were 84.5% for 2-h plasma and 73.5% for 12-h plasma.

Entire supernatants were combined and evaporated to dryness under reduced pressure, and the residue was reconstituted in 0.3% formic acid in water-acetonitrile (90:10, v/v) and centrifuged at 1800g for 5 min at 4°C. The supernatant was filtered (0.45 μm), and an aliquot of the filtrate was analyzed under the conditions described below.

Pretreatment of urine and feces. The cryopreserved urine samples (0–4 and 4–24 h) were thawed, and the samples obtained from six subjects after administration of [^{14}C]imidafenacin were pooled at the sample volume ratio. The cryopreserved fecal homogenates (0–48 and 48–96 h) were thawed, and the homogenates containing radioactivity of 2% or more of the administered dose, prepared from six subjects after administration of [^{14}C]imidafenacin, were pooled at the sample weight ratio. An aliquot of the pooled sample was mixed with a 3-fold volume of methanol, shaken for 10 min, and centrifuged at 1800g for 10 min at 4°C to separate the supernatant. The residue was reextracted with methanol, followed by shaking and centrifugation. The extraction recoveries were 93.1% for 0- to 4-h urine, 88.6% for 4- to 24-h urine, 77.2% for 0- to 48-h feces, and 69.2% for 48- to 96-h feces.

Entire supernatants were combined and evaporated to dryness under reduced pressure. The residue was reconstituted in 0.3% formic acid in water-acetonitrile (90:10, v/v), and an aliquot of the solution was analyzed under the conditions described below. The reconstituted fecal solution was centrifuged at 1800g for 5 min at 4°C. The supernatant was filtered (0.45 μm), and an aliquot of the filtrate was analyzed under the conditions described below.

Analysis of metabolites in samples. Relative amounts of metabolites in the plasma, urine, and feces were determined by HPLC connected to a RAD or by a LSC after collection of HPLC eluates. The metabolites were identified by comparison of retention times between radioactive peaks and ion peaks on the high-performance liquid chromatograph connected to the MS/MS system.

An TSKgel Octyl-80Ts column (5 μm, 4.6 mm i.d. × 150 mm in length; Tosoh Corp., Tokyo, Japan) connected to a guard column was used as the LC column. As the mobile phase, the mixture of 0.3% formic acid in water-

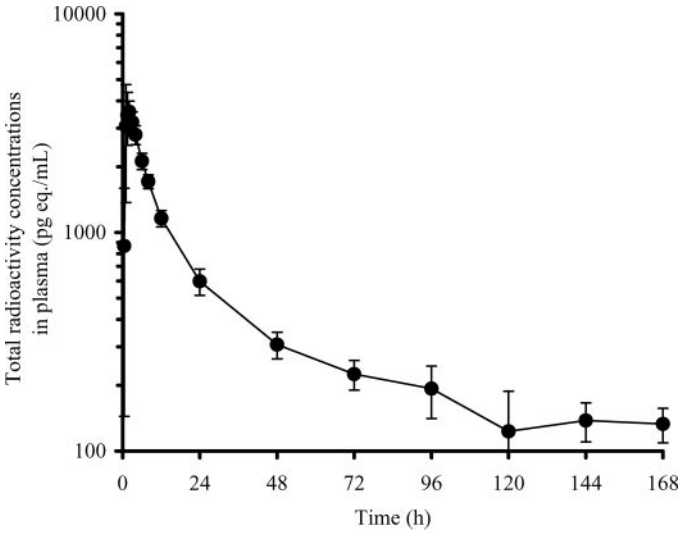


FIG. 2. Total radioactivity in plasma after a single oral administration of 0.25 mg of [^{14}C]imidafenacin in six healthy male subjects. Each point represents the mean ± S.D. of six individuals.

acetonitrile (90:10, v/v) (A) and 0.7% formic acid in acetonitrile (B) was flowed at 1 ml/min in the following linear gradient mode: starting with 0% of B composition to 5 min, increasing to 10% in 5 to 10 min, increasing to 18% in 10 to 30 min, and finally increasing to 100% in 30 to 35 min and maintaining 100% in 35 to 40 min. The column was maintained at room temperature. The column eluate was split in the ratio of approximately 4:1 and introduced to the RAD, (FLO-ONE/525TR; Packard Biosciences Ltd.) and the mass spectrometer (TSQ7000; Thermo Electron Corporation, Waltham, MA), respectively. As scintillation fluid for the RAD, Flo-Scint II (Packard Biosciences Ltd.) was delivered to the HPLC eluate at a 3-fold flow rate of the mobile phase, and the radioactivity was monitored using the RAD with 6-s integration. For the sensitive detection of radioactivity in plasma, the HPLC eluates were collected every 18 s and dissolved in liquid scintillation fluid (Hionic-Fluor; Packard Biosciences Ltd.), and then the radioactivity in each fraction was detected for 2 min using the LSC (Tri-Carb 2500TR; Packard Biosciences Ltd.). Detection limits of radioactivity in metabolite peaks in the RAD and LSC assays were defined as 3 and 2 times the background values, respectively.

For ionization and detection of all analytes, the conditions of the TSQ7000 equipped with ESI interface were positive ionization mode, spray voltage at 4.5 kV, capillary temperature at 290°C, multiplier voltage at 1500 V, sheath gas pressure at 70 psi (N_2), auxiliary gas flow at 10 arbitrary units (N_2), collision gas pressure at 2.0 mTorr (Ar), and collision energy at 18 to 30 eV. The precursor and product ions monitored in SRM, as shown in Fig. 1, were *m/z* 322→240 for [^{14}C]imidafenacin, 338→240 for [^{14}C]M-1, 354→240 for [^{14}C]M-2, 356→240 for [^{14}C]M-3, 328→193 for [^{14}C]M-4, 329→193 for [^{14}C]M-5, 240→117 for [^{14}C]M-6, 344→209 for [^{14}C]M-8, 498→240 for [^{14}C]M-9, 298→240 for [^{14}C]M-10, and 514→240 for postulated metabolites.

Data processing. The ratio of counts of each radioactive peak to the total radioactivity counts over run time (percentage on HPLC) was obtained and multiplied by the extraction recovery through the pretreatment of samples to determine the compositions of metabolites to the radioactivities in the plasma, urine, and feces (percentage in sample). The metabolite concentrations in the plasma were calculated from the mean values of the total radioactivity in six subjects and are expressed as equivalents of imidafenacin (nanogram equivalents of imidafenacin per milliliter). The radioactivities of the metabolites excreted in the urine and feces were calculated from the mean values of the total radioactivities excreted in six subjects and are expressed as a percentage of the mean radioactivity administered (% dose).

Identification of M-11 (M-11a and M-11b). *Synthesis and purification of reference products.* Imidafenacin was chemically oxidized in the cupro-ascorbate system to obtain the reference product for two unknown metabolites found in the urine and feces. To 20 ml each of 100 mM imidafenacin methanolic solution, 0.5 M L-(+)-ascorbate solution, and 5 mM copper chloride solution were added 2 liters of 0.1 M phosphate buffer solution (pH 7.2), and the mixture was stirred for 24 h at room temperature. After washing three times with 1 liter of chloroform, an aqueous layer was lyophilized and concentrated. The reference products were semipurified as the major constituent-rich fraction and the minor constituent-rich fraction by sequential column chromatography with Chromatorex ODS (Fuji Silysia Chemical Ltd., Aichi, Japan) and Wakosil 25C18 (Wako Pure Chemical Industries Ltd., Osaka, Japan) columns, eluting with the mixture of water and acetonitrile containing 0.05 or 0.1% formic acid.

TABLE 1

Pharmacokinetic parameters of total radioactivity in plasma after a single p.o. administration of 0.25 mg of [^{14}C]imidafenacin in six healthy male subjects

Parameters	Mean	S.D.
C_{max} (pg Eq/ml)	3860	723
T_{max} (h)	1.50	0.45
$t_{1/2el}$ (h)	72.11	6.54
AUC_{0-t} (ng Eq · h/ml)	69.09	7.63
AUC_{0-inf} (ng Eq · h/ml)	83.06	10.18

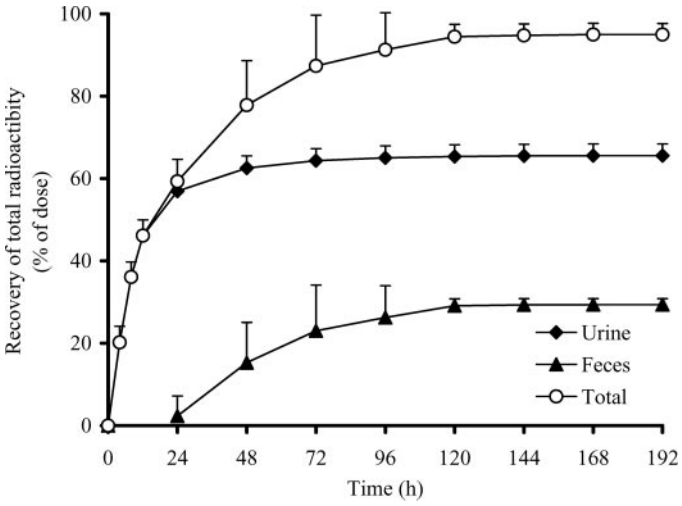


FIG. 3. Urinary and fecal recovery of total radioactivity in urine and feces after a single oral administration of 0.25 mg of [^{14}C]imidafenacin in six healthy male subjects. Each point represents the mean ± S.D. of six individuals.

Structural elucidation of reference products. The reference products were dissolved in NMR grade D_2O (Sigma-Aldrich, St. Louis, MO) spiked with 3-(trimethylsilyl)-propionic-2,2,3,3-d_4 acid, sodium salt (Sigma-Aldrich) as an internal reference for the chemical shift. The ^{1}H NMR and ^{13}C NMR spectra were measured on the JNM EX-400 spectrophotometer (JEOL, Tokyo, Japan) for the structural elucidation of the reference products. In addition, the nuclear Overhauser effect, DEPT, ^{1}H-^{1}H COSY, HMQC, and HMBC spectra were also acquired for further assignment of proton and carbon signals.

The reference products were dissolved in the mixture of water and acetonitrile or D_2O and acetonitrile. Then the solution was injected into the MS/MS system comprising an API-4000 mass spectrometer equipped with an ESI interface (Applied Biosystems/MDS Sciex, Foster City, CA) at the constant rate of 3 μl/min to determine precursor and product ion spectra of the reference products.

Identification of M-11 in human urine. Using a high-performance liquid chromatograph connected to the MS/MS system comprising the API-4000 mass spectrometer equipped with an ESI interface, the retention time and product ion spectra of reference products were compared with those of unknown metabolites in human urine collected at 2 to 4 h after a single oral administration of nonradiolabeled imidafenacin. A TSKgel ODS-80Ts column (5 μm, 2.0 mm i.d. × 150 mm in length, Tosoh Corp.) connected to a guard column was used as the semimicro LC column. As the mobile phase, the mixture of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B) was flowed at 0.2 ml/min in the following linear gradient mode: starting with 10% of B composition to 5 min, increasing to 19% in 5 to 10 min, increasing to 26% in 10 to 30 min, and finally increasing to 91% in 30 to 35

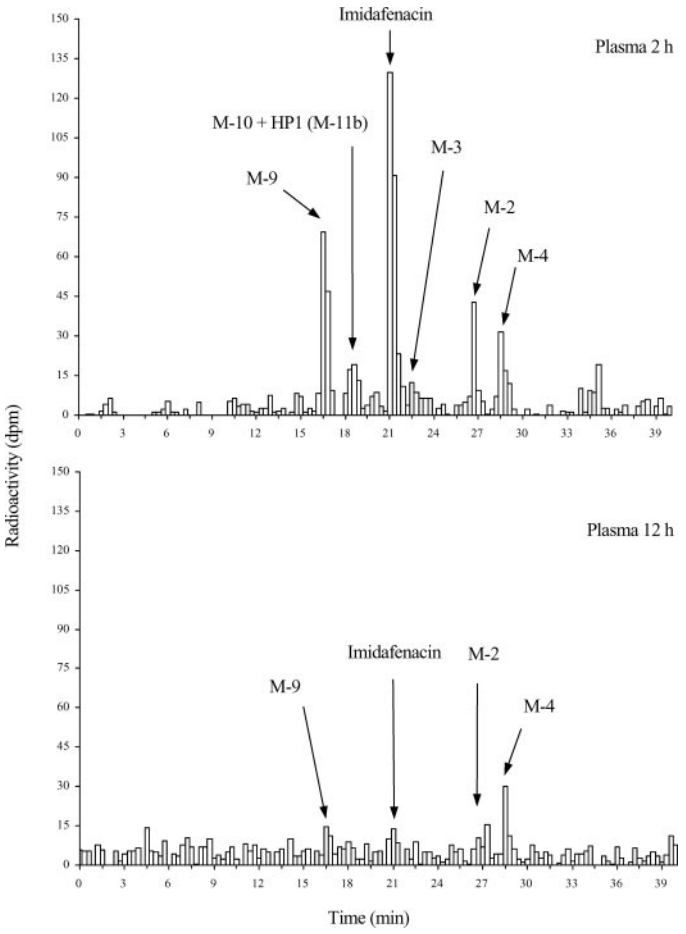


FIG. 4. HPLC radiochromatograms of imidafenacin and its metabolites in pooled plasma after a single oral administration of 0.25 mg of [^{14}C]imidafenacin in six healthy male subjects.

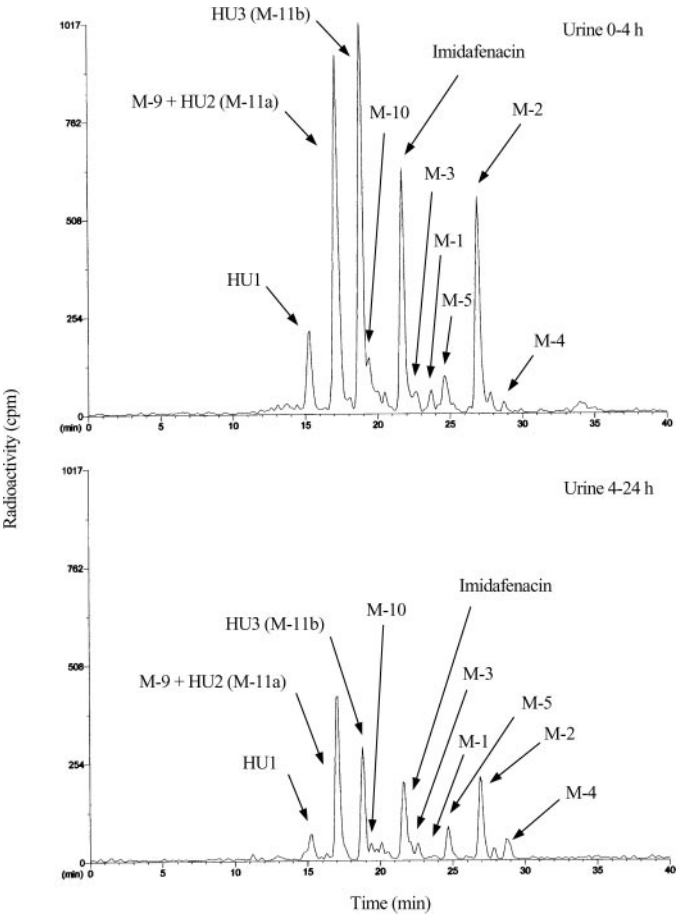


FIG. 5. HPLC radiochromatograms of imidafenacin and its metabolites in pooled urine after a single oral administration of 0.25 mg of [^{14}C]imidafenacin in six healthy male subjects.

min and maintaining 91% in 35 to 40 min. The column was maintained at room temperature.

Stability of reference products. The reference products were dissolved in water or 100 mM phosphate buffer (pH 7.2) and then were allowed to stand at room temperature or incubated at 50°C for 5 h. After incubation, the ratio of the major and minor constituents of the reference products or their decomposed product, amidine form M-10, was measured by HPLC. A TSKgel ODS-80T$_M$ column (5 μm, 4.6 mm i.d. $\times$ 150 mm in length, Tosoh Corp.) connected to a guard column was used as the LC column. As the mobile phase, 0.1% formic acid in water-0.1% formic acid in acetonitrile (73:27, v/v) was flowed at 1 ml/min. The column was maintained at room temperature. The analytes were detected by ultraviolet absorption at 210 nm.

Results

Safety Assessment. Six subjects were given a single oral dose of 0.25 mg of [^{14}C]imidafenacin. No serious adverse events were reported, and no subjects were withdrawn from the study because of adverse events.

Radioactivity in Plasma. The total radioactivity in the plasma is shown in Fig. 2, and the pharmacokinetic parameters are summarized in Table 1. After oral administration of 0.25 mg of [^{14}C]imidafenacin, C_{max}, the mean of the maximum concentration of the total radioactivity in the plasma, was 3860 pg Eq/ml (from 3230 to 5210 pg Eq/ml) at 1.50 h after administration (T_{max}, from 1.00 to 2.00 h). Plasma concentrations decreased rapidly to the mean of 598 pg Eq/ml by 24 h and then gradually decreased to 133 pg Eq/ml by 168 h after administration. The mean of $t_{1/2el}$, estimated from 24 to 168 h after administration, was 72.11 h. The means of AUC_{0-t} and AUC_{0-inf} were 69.09 ng Eq · h/ml (from 60.59 to 81.11 ng Eq · h/ml) and 83.06 ng Eq · h/ml (from 73.45 to 101.2 ng Eq · h/ml), respectively.

Urinary and Fecal Excretion of Radioactivity. Urinary and fecal recoveries of the total radioactivity in the urine and feces are shown in Fig. 3. After oral administration of 0.25 mg of [^{14}C]imidafenacin, urinary excretion accounted for 62.5% of the administered dose by 48 h after administration and the mean of 65.6% (from 62.6 to 69.3%) of the administered dose by the end of the collection period, 192 h after administration, demonstrating that radioactivity was excreted primarily in the urine. Fecal excretion accounted for the mean of 29.4% (from 27.6 to 31.3%) of the administered dose. Recovery of the total radioactivity was quantitative with the mean of 95.0% (from 91.3 to 98.3%) of the administered dose by 192 h after administration.

Metabolite Profiles. HPLC radiochromatograms of imidafenacin and its metabolites in the plasma, urine, and feces are shown in Figs. 4, 5, and 6. The metabolites were identified by comparison of retention times between radioactive peaks and ion peaks on the LC/MS/MS chromatogram. Relative amounts of metabolites in the plasma, urine, and feces are summarized in Table 2. M-6 and M-8 were not found in all samples determined.

In the 2- and 12-h plasma (Fig. 4; Table 2), the concentrations of unchanged imidafenacin were 1.19 ng Eq/ml (equivalent to 33.3% of the radioactivity in the plasma) and 0.12 ng Eq/ml (10.7%), respectively. In the 2-h plasma, a major metabolite M-9 was detected, and then M-2 and M-4 and a minor metabolite M-3 and a mixture of a minor metabolite M-10 and unknown metabolite HP1 (identified as

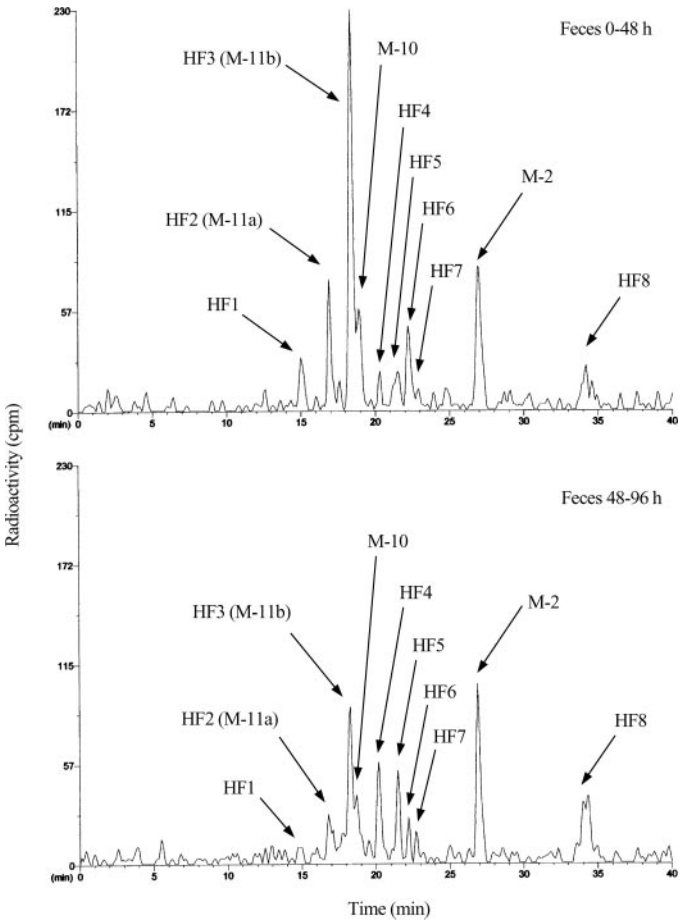


FIG. 6. HPLC radiochromatograms of imidafenacin and its metabolites in pooled feces after a single oral administration of 0.25 mg of [^{14}C]imidafenacin in six healthy male subjects.

M-11b) were detected. In the 12-h plasma, a major metabolite M-4 and minor metabolites M-2 and M-9 were detected.

In the 0- to 4- and 4- to 24-h urine (Fig. 5; Table 2), unchanged imidafenacin accounted for 2.9% of the administered dose (equivalent to 14.2% of the radioactivity in the urine) and 5.1% (13.9%), respectively. In the 0- to 4-h urine, M-2, a mixture of M-9 and an unknown metabolite HU2 (identified as M-11a), and an unknown metabolite HU3 (identified as M-11b) were mainly detected, accounting for 2.6 to 4.8% of the dose (equivalent to 13.0–23.6% of the radioactivity in urine). In the 4- to 24-h urine, M-2, a mixture of M-9 and HU2, and HU3 were also mainly detected, accounting for 4.6 to 9.1% of the dose (12.4–24.9%). Minor unknown metabolite HU1 detected by SRM would be a glucuronide of mono-oxygenated imidafenacin (*m/z* 514→240).

In the 0- to 48- and 48- to 96-h feces (Fig. 6; Table 2), unchanged imidafenacin was not detected. In the 0- to 48-h feces, M-2, M-10, an unknown metabolite HF2 (identified as M-11a), and an unknown metabolite HF3 (identified as M-11b) were mainly detected, accounting for 1.0 to 3.9% of the administered dose (equivalent to 5.7–21.9% of the radioactivity in the feces). In the 48- to 96-h feces, M-2 and HF3 accounted for 1.3 and 1.2%, respectively. There were several unknown metabolite peaks in the 0- to 48- and 48- to 96-h feces, accounting for 1.2% or less of the administered dose (9.5% or less).

Identification of M-11 (M-11a and M-11b). Two major unknown metabolites at 16.8 to 17.1 min (HU2 and HF2) and 18.3 to 18.9 min (HP1, HU3, and HF3) were found in the urine and feces and were detected by SRM as identical *m/z* 356→240 transitions (Table 2). The product ion (*m/z* 240) indicated a ^{14}C-labeled 2,2-diphenylbutanamide moiety, which was identical to that of [^{14}C]imidafenacin; therefore, according to the precursor ion (*m/z* 356), the 2-methylimidazole moiety of [^{14}C]imidafenacin would be modified by adding 34 Da to form these unknown metabolites. However, absolute amounts of these metabolites in human urine and feces were not enough to elucidate their chemical structures. Accordingly, imidafenacin was oxidized in the cupro-ascorbate system to obtain the reference products, its oxides

TABLE 2

Compositions of imidafenacin and its metabolites in plasma, urine, and feces after a single p.o. administration of 0.25 mg of [^{14}C]imidafenacin in healthy male subjects

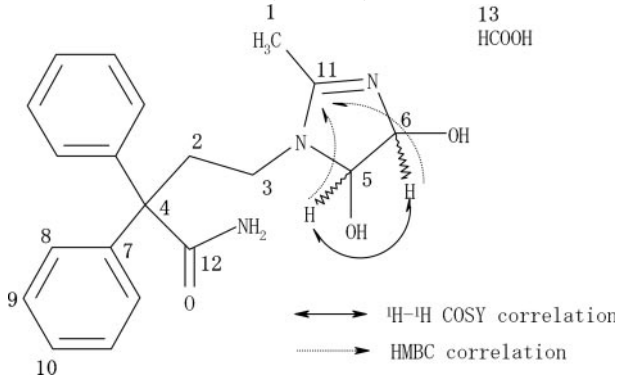
Numbers in parentheses denote percentage of total radioactivity in samples.

Metabolites	Mass Transition	Retention Time	Plasma		Urine		Feces	
			2 h	12 h	0–4 h	4–24 h	0–48 h	48–96 h
	m/z	*min*	*ng Eq/ml*		*% dose*			
Imidafenacin	322→240	21.3–21.7	1.19 (33.3)	0.12 (10.7)	2.9 (14.2)	5.1 (13.9)	—	—
M-1	338→240	23.7	—	—	0.3 (1.4)	0.2 (0.5)	—	—
M-2	354→240	26.9–27.0	0.34 (9.5)	0.09 (7.6)	2.6 (13.0)	4.6 (12.4)	1.8 (9.9)	1.3 (10.9)
M-3	356→240	22.6–22.8	0.15 (4.1)	—	0.3 (1.3)	0.7 (1.9)	—	—
M-4	328→193	28.7–28.8	0.34 (9.4)	0.21 (18.0)	0.1 (0.5)	1.3 (3.6)	—	—
M-5	329→193	24.6–24.7	—	—	0.5 (2.6)	1.8 (5.0)	—	—
M-9	498→240	16.8–17.1	0.63 (17.6)	0.11 (9.9)	4.8 (23.6)[a]	9.1 (24.9)[a]	—	—
M-10	298→240	18.7–19.4	0.28 (7.9)[b]	—	0.7 (3.7)	0.7 (1.9)	1.0 (5.7)	0.6 (4.7)
HU2, HF2 (M-11a)	356→240	16.8–17.1	—	—	4.8 (23.6)[a]	9.1 (24.9)[a]	1.1 (6.2)	0.3 (2.8)
HP1, HU3, HF3 (M-11b)	356→240	18.3–18.9	0.28 (7.9)[b]	—	4.6 (22.6)	5.5 (14.9)	3.9 (21.9)	1.2 (9.7)
HU1	514→240	15.2–15.3	—	—	1.1 (5.6)	2.0 (5.4)	—	—
HF1	—	14.7–15.0	—	—	—	—	0.6 (3.3)	0.2 (1.4)
HF4	—	20.2–20.3	—	—	—	—	0.3 (1.7)	0.8 (6.4)
HF5	—	21.5	—	—	—	—	0.6 (3.3)	0.6 (4.8)
HF6	—	22.2	—	—	—	—	0.9 (5.1)	0.2 (1.9)
HF7	—	22.7–22.9	—	—	—	—	0.2 (0.9)	0.2 (1.5)
HF8	—	34.2–34.3	—	—	—	—	0.9 (4.8)	1.2 (9.5)
Others			N.C. (2.7)	N.C. (27.3)	2.3 (11.5)	5.7 (15.6)	6.6 (22.8)	5.7 (30.8)
Total			3.57 (84.5)	1.16 (73.5)	20.2 (100)	36.7 (100)	17.9 (85.6)	12.3 (84.4)

—, not detected; N.C., not calculated.
[a] Total amount of M-9 and HU2.
[b] Total amount of M-10 and HP1.

TABLE 3

Chemical shifts of protons and carbons in ^{1}H NMR and ^{13}C NMR spectra of major (M-11b) and minor (M-11a) constituents of reference products obtained from imidafenacin



Position of Proton	^{1}H NMR Data (in D_2O)	
	Major Constituent (M-11b)	Minor Constituent (M-11a)
1	2.18[a] (3H[b], s[c])	2.11[a] (3H[b], s[c])
2	2.82 (2H, m)	2.79 (2H, m)
3	3.45 (2H, m)	3.41 (2H, m)
5	5.26 (1H, s)	5.43–5.47[d] (1H, d, J = 8.1[e])
6	5.33 (1H, s)	5.43–5.47[d] (1H, d, *J* = 8.1[e])
8, 9, and 10	7.39–7.49 (10H, m)	7.40–7.48 (10H, m)
13	8.45 (1.3H, s)	8.45 (2H, s)

Position of Carbon	^{13}C-NMR Data (in D_2O)xc	
	Major Constituent (M-11b)	Minor Constituent (M-11a)
1	14.61[a]	14.48[a]
2	39.04	38.49
3	43.49	42.90
4	61.95–61.99[f]	61.95–61.99[f]
5	87.61	80.22 or 85.78[f]
6	93.10	80.22 or 85.78[f]
7	144.2–144.4[f]	144.2–144.4[f]
8, 9, and 10	130.7–131.8[f]	130.7–131.8[f]
11	170.8	169.5
12	181.9–182.2[f]	181.9–182.2[f]
13	173.8	173.8

[a] Chemical shifts are reported in parts per million.
[b] Intensities are represented as number of protons.
[c] Multiplicity: s, singlet; d, doublet; m, multiplet.
[d] These proton signals could not be distinguished.
[e] Coupling constants (*J*) are given in Hertz.
[f] These carbon signals could not be distinguished.

of the 2-methylimidazole moiety as reported by Ohta et al. (1998). Then, the mass spectra of the reference products were compared with those of the unknown metabolites in human urine.

Structural elucidation of the reference products was achieved by analyses of one- and two-dimensional NMR spectra including ^{1}H-^{1}H COSY, HMQC, HMBC, DEPT, and nuclear Overhauser effect. ^{1}H and ^{13}C NMR assignments for the reference products are shown in Table 3. Because those reference products were mixtures of the major and minor constituents in each fraction, signals of H-1, H-5, and H-6 protons and C-1, C-2, C-3, C-5, C-6, and C-11 carbons in the major and minor constituents were observed in the spectra of both fractions. The oxygenated 2-methylimidazole moiety was analyzed as follows. The presence of two oxygenated methine carbons (87.61 and 93.10 ppm) was supported by DEPT spectra. The proton signals (5.26 and 5.33 ppm) on these oxy-methine carbons were deduced from HMQC analysis, and the neighboring connectivity of these two oxy-methines was analyzed by direct coupling between protons at 5.26 and 5.33 ppm from ^{1}H-^{1}H COSY spectra. Furthermore, the HMBC correlations between three protons (H-1, H-5, and H-6) and the sp^2 carbon signal

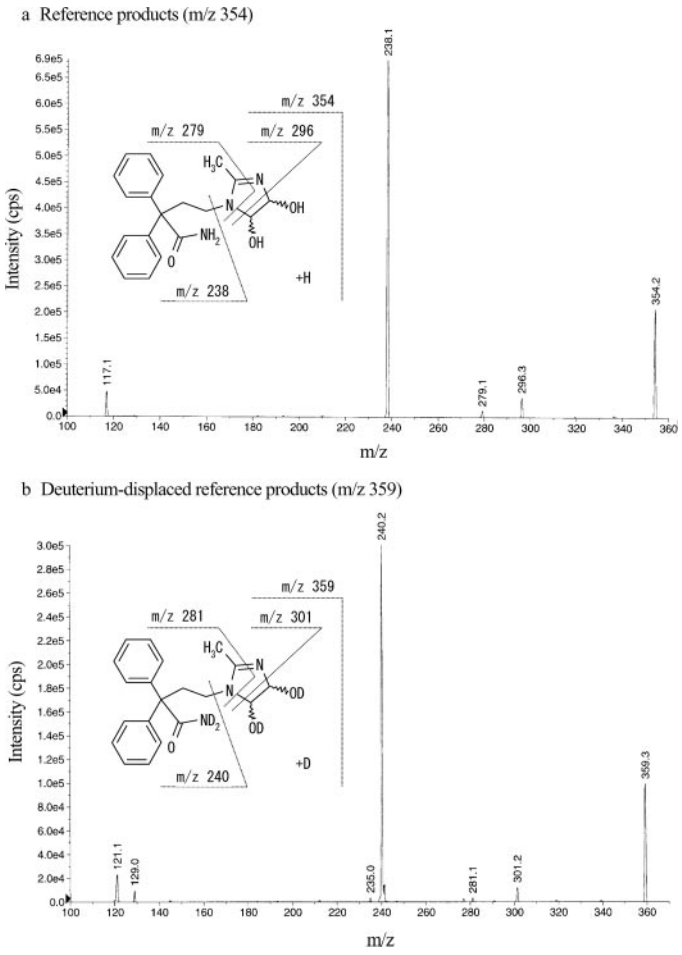


FIG. 7. Product ion spectra of reference products obtained by the cupro-ascorbate oxidation system with or without deuterium displacement.

at 170.8 ppm (C-11) suggested the presence of a five-membered ring. These findings corroborated the hypothesis that the reference products were obtained by the oxidation of C-5 and C-6 carbons in imidafenacin.

Product ion spectra of reference products dissolved in water and acetonitrile or D_2O and acetonitrile are shown in Fig. 7. Product ion spectra of reference products (*m/z* 354) and deuterium-displaced reference products (*m/z* 359) suggested that the reference products had two deuterium-displaceable hydrogens, such as hydroxyl groups on C-5 and C-6 carbons in the 2-methylimidazole moiety of imidafenacin, as indicated in Fig. 7.

^{1}H NMR spectra showed vicinal proton-proton coupling (J = 8.1 Hz) on doublet signals of H-5 and H-6 protons in the minor constituent. According to the Karplus (1963) equation, the dihedral angle between vicinal protons attached to neighboring C-5 and C-6 carbons was approximately 0°. In contrast, H-5 and H-6 protons in the major constituent were singlet signals, indicating orthogonal protons attached to C-5 and C-6 carbons. Consequently, structures of the major and minor constituents of the reference products were estimated to be *trans*- and *cis*-isomers of diol forms, respectively.

The LC/MS/MS chromatogram on SRM at *m/z* 354→238 of human urine and the reference products are shown in Fig. 8. Product ion spectra of *m/z* 354 derived from two unknown metabolites, HU2 and HU3, in human urine, and the major and minor constituents of reference products are shown in Fig. 9. The LC/MS/MS retention time and product ion spectra of HU2 and HU3 in the urine collected at 2 to

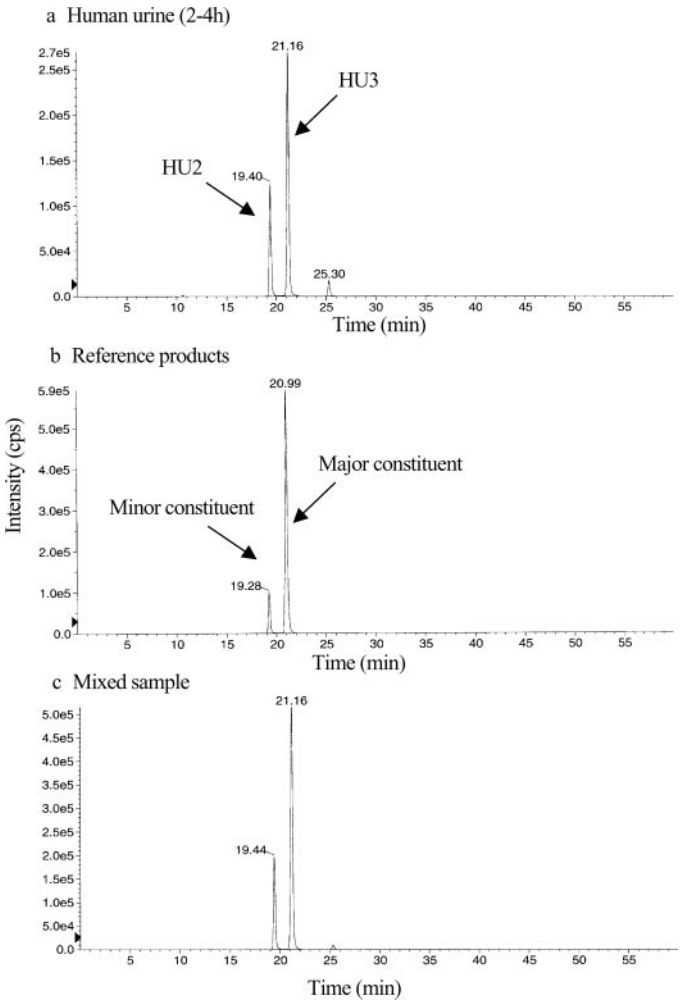


FIG. 8. LC/MS/MS chromatograms on SRM at *m/z* 354→238 of human urine and reference products obtained from imidafenacin.

4 h after a single oral administration of imidafenacin corresponded to those of the minor and major constituents of reference products, respectively. From these results, HU2 and HF2 were identified as the *cis*-isomer (M-11a), and HP1, HU3, and HF3 were identified as the *trans*-isomer (M-11b) of the diol form.

After the reference products were allowed to stand at room temperature for 5 h in water or 100 mM phosphate buffer (pH 7.2), the ratio of the minor to major constituents remained unchanged, that is, approximately 30% based on the calculation of peak areas on the HPLC chromatogram. After incubation at 50°C for 5 h, however, peak areas of both constituents decreased and the decomposed product, amidine form M-10, increased to more than 30% of the initial peak area of both constituents. After NMR measurements, the ratio of the major constituent increased even in the minor constituent-rich fraction. These findings suggested that the major and minor constituents might be interchangeable and gradually decomposed to the amidine form under heating conditions.

Discussion

In the present study, the absorption and excretion kinetics and metabolic profile of imidafenacin were investigated in six healthy male subjects after a single oral administration of 0.25 mg of [^{14}C]imidafenacin. Unknown metabolites found in the excreta were identified by LC/MS/MS and NMR analyses.

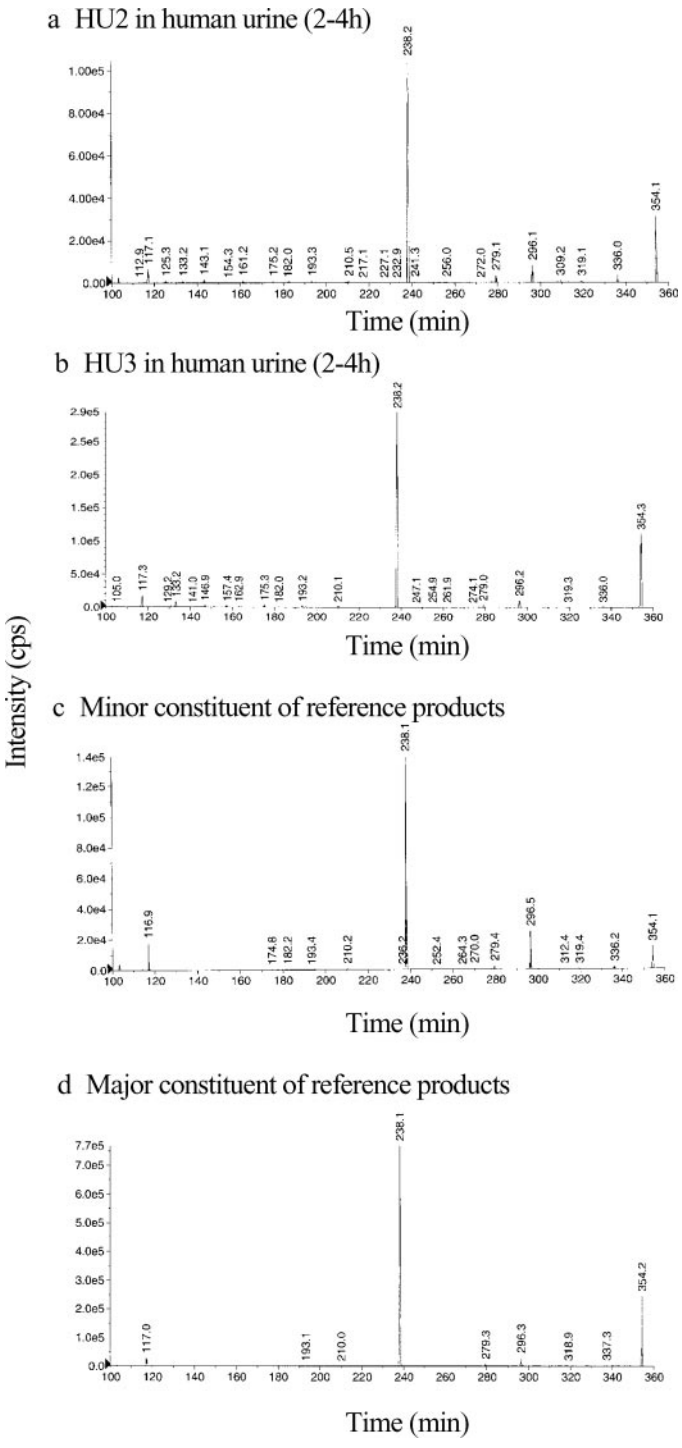


FIG. 9. Product ion spectra of *m/z* 354 derived from unknown metabolites, HU2 and HU3, in human urine, and major and minor constituents of reference products obtained from imidafenacin.

The highest total radioactivity in the plasma was observed at 1.50 h after administration, which was consistent with rapid absorption of the oral dose of imidafenacin in humans (Fig. 2; Table 1). These findings are supported by the results in the preclinical toxicological studies in rats and dogs, demonstrating that the total radioactivity in the plasma peaked within 2.0 h after oral administration of [^{14}C]imidafenacin. Furthermore, 65.6% of the administered dose of radioactivity was detected in the urine (Fig. 3), suggesting that no less than a 65.6% dose of imidafenacin would be absorbed from the gut. This finding is

consistent with the absolute bioavailability of 57.8% after intravenous administration of imidafenacin in humans (Ohno et al., 2007). The bioavailability of humans was larger than that of rat (5.3%) and dog (36.1%) (Masuda et al., unpublished observations).

In contrast with the rapid increase in radioactivity in the plasma, the radioactivity gradually decreased from 24 to 168 h after administration, with a relatively long $t_{1/2el}$ of 72.11 h (Fig. 2; Table 1). On the metabolite profiling, major components of radioactivity were unchanged imidafenacin and M-9 (50.9% in total) in the 2-h plasma and M-4 (18.0%) in the 12-h plasma (Fig. 4; Table 2). M-4 and related minor metabolites might contribute to the relatively long $t_{1/2el}$ of radioactivity in the plasma. Approximately 65.6 and 29.4% of the administered dose of [^{14}C]imidafenacin were excreted via urine and feces, respectively, within 192 h after administration (Fig. 3). The total recovery of radioactivity reached 95.0% of the administered dose. In the preclinical toxicological studies, urinary and fecal recoveries of oral administered [^{14}C]imidafenacin were 18.2 and 77.0%, respectively, in rats, and 44.8 and 51.6%, respectively, in dogs (Ooie et al., unpublished observations). From these data, oral administered imidafenacin was thoroughly excreted via urine and feces in all of the species studied, despite differences in the main excretion route among the species.

After oral administration to humans, imidafenacin underwent different metabolic transformations, including *N*-glucuronide conjugation (M-9) and oxidation of the 2-methylimidazole moiety (M-1, M-2, M-11a, and M-11b) or the ring cleavage (M-3, M-4, M-5, and M-10); the metabolic activity for *N*-glucuronidation, in particular, was found to be relatively high. The metabolic pathway of imidafenacin for *N*-glucuronidation, however, was uncommon in rats and dogs, and rats have the unique metabolic pathway for the oxidation on the phenyl moiety, generating M-8 and its related metabolites (Sato et al., unpublished observations). Species differences for *N*-glucuronidation were also observed in afloqualone in common with imidafenacin, indicating high *N*-glucuronidation activity in humans compared with activity in experimental animals except rabbits (Green and Tephly, 1998; Kaji and Kume, 2005).

On the basis of the metabolites found in the plasma, major metabolites of imidafenacin were M-2, M-4, and M-9, accounting for approximately 10% or more of the total radioactivity in the 2- or 12-h plasma (Fig. 4; Table 2). It has been reported that M-2, M-4, and M-9 had low affinities for muscarinic receptors, whereas unchanged imidafenacin had high affinity for muscarinic receptor subtypes M_1 and M_3 (Kobayashi et al., 2007a). These findings suggest that the in vivo anticholinergic effects of oral administered imidafenacin would result from the unchanged form alone. In addition, toxicological symptoms attributable to M-2 and M-4 after oral administration of imidafenacin and to M-9 after intravenous administration of M-9 were insignificant in the preclinical studies (Kasai et al., unpublished observations), and the inhibitory potentials of these metabolites on cytochrome 450 isozymes were extremely low (Kanayama et al., 2007). In the meantime, because imidafenacin was metabolized by UGT1A4 to form M-9 and by CYP3A4 to form M-2 and M-4, metabolism of imidafenacin might be inhibited by concomitant administration of drugs with an inhibitory potential on CYP3A4 or UGT1A4 (Kanayama et al., 2007).

We identified interchangeable diol-form metabolites, M-11a and M-11b, in the urine and feces (Fig. 7). Because the minor constituent of the diol-form metabolite, *cis*-isomer (M-11a), was readily interchangeable to the major constituent, *trans*-isomer (M-11b), up to approximately 30% of the *trans*-isomer was found in the minor constituent-rich fraction. Furthermore, M-11a and M-11b decomposed to the amidine form M-10 under heating conditions. However, M-10 was rarely detected in human plasma, urine, and feces, probably because the samples for metabolite profiling were treated under cooling conditions during storage and pretreatment throughout the study. A similar metabolic pathway has been reported in a hypoglycemic argent, midaglizole. That is, midaglizole was oxidized and hydrated at a 2-substituted-imidazole moiety to form a 4,5-dihydro-diol intermediate, although its stereochemistry was unknown, and then amidine metabolites were obtained by a ring-opening reaction (Nakaoka and Hakusui, 1987). However, this intermediate metabolite was not isolated and identified owing to its instability. In contrast, the 4,5-dihydro-diol metabolites of imidafenacin, M-11a and M-11b, were relatively stable in the biological samples during storage and pretreatment, whereas their absolute amounts were too small to isolate for structural elucidation. Therefore, M-11a and M-11b were successfully identified by obtaining the reference product from imidafenacin with oxidation in the cupro-ascorbate system.

In conclusion, the present study clarified the absorption and excretion kinetics of imidafenacin and the characteristics of metabolites in the plasma and excreta in six healthy male subjects after a single oral administration of 0.25 mg of [^{14}C]imidafenacin. The results indicate that imidafenacin is rapidly and well absorbed after oral administration and circulates in the plasma as the unchanged form, its glucuronide conjugate, and other metabolites, and then is excreted primarily in the urine and feces as the oxidized metabolites of the 2-methylimidazole moiety. For treatment of overactive bladder, imidafenacin is expected to provide suitable anticholinergic effects through its excellent pharmacokinetic properties in humans verified in the present study.

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