

Disposition and Metabolism of [¹⁴C]Brasofensine in Rats, Monkeys, and Humans

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ABSTRACT:

Brasofensine is an inhibitor of the synaptic dopamine transporter. These studies were conducted to characterize the pharmacokinetics, absolute bioavailability, disposition, and metabolism of brasofensine after i.v. and/or p.o. administrations of [¹⁴C]brasofensine in rats (1.5 mg/kg i.v., 4 mg/kg p.o.) and monkeys (4 mg i.v., 12 mg p.o.) and humans (50 mg p.o.). Brasofensine was rapidly absorbed after p.o. administration in rats and monkeys, with peak plasma concentrations occurring 0.5 to 1 h but 3 to 8 h for brasofensine in humans. Plasma terminal elimination half-lives were ~2 h in rats, ~4 h in monkeys, and ~24 h in humans. Total body clearance and steady-state volume of distribution values were 199 ml/min/kg and 24 l/kg, respectively, in the rat and 32 ml/min/kg and 46 l/kg,

respectively, in the monkey. Absolute bioavailability was 7% in rats and 0.8% in monkeys. After a single p.o. dose, urinary excretion of radioactivity accounted for 20% of the administered dose in rats, 70% in monkeys, and 86% in humans, with the remainder excreted into the feces. Brasofensine had extensive first-pass metabolism following p.o. administration in humans, monkeys, and rats. It primarily underwent *O*- and *N*-demethylation and isomerization. Some of the desmethyl metabolites were further converted to glucuronides. These primary metabolites and glucuronides of demethyl brasofensine (M1 and M2) were major circulating metabolites in humans and were also observed in rat and monkey plasma.

Brasofensine (BMS-204756) is a novel inhibitor of the synaptic dopamine transporter and has potential use in Parkinson's disease (Johnston and Brotchie, 2004), a disease estimated to affect approximately 500,000 persons in the United States alone (Battistin et al., 1996; Brooks, 1997). It is known that nigrostriatal dopamine loss is the major factor in Parkinson's disease pathogenesis, with clinical symptoms of parkinsonism beginning when there is approximately 80% depletion of the striatal dopamine (Kish et al., 1988). Over time, those with Parkinson's disease have a degeneration of the dopaminergic neurons, causing a continued depletion of the amount of dopamine released. This exaggerates the symptoms of parkinsonism, and it is thought that this progression of symptoms could be slowed by inhibition of the dopamine transporter (Mouradian et al., 1987). The dopamine transporter actively transports released dopamine from the synaptic cleft back into the presynaptic nerve terminal where it is metabolized by monoamine oxidase or extracellularly by catechol-*O*-

methyltransferase. Inhibition of the dopamine transporter (dopamine reuptake inhibition) enhances the effectiveness of the diminishing amounts of dopamine that are released into the synaptic cleft. Thus, the primary role of brasofensine is to conserve the greatly reduced level of endogenous dopamine within the synaptic cleft and also to extend the potency and duration of action of exogenous dopamine from the administration of levodopa. This appears to be true in a marmoset model of Parkinson's disease (Pearce et al., 2002) but has not yet been shown in the clinic (Frackiewicz et al., 2002).

The main objectives of these studies were to 1) assess the pharmacokinetics and absolute bioavailability of brasofensine in rats, monkeys, and humans; 2) investigate biotransformation of brasofensine in rats and monkeys; and 3) compare the disposition and plasma metabolite profiles of brasofensine in rats, monkeys, and humans after p.o. and/or i.v. administrations of radiolabeled brasofensine.

Materials and Methods

Chemicals. Radiolabeled [¹⁴C]brasofensine (Fig. 1) as the maleate salt had a radiochemical purity of 97.6% and specific activity of 50 μCi/mg free base. [¹⁴C]Brasofensine, unlabeled brasofensine, unlabeled BMS-205912, and metabolite standards [*O*-desmethyl brasofensine (ODME¹), *O*-desmethyl BMS-

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ABBREVIATIONS: brasofensine, BMS-204756, (+)-(E)-(1*R*,2*R*,3*S*)-3-(3,4-dichlorophenyl)-8-methyl-8-azabicyclo[3.2.1]octane-2-carbaldehyde *O*-methyloxime; BMS-205912, (+)-(Z)-(1*R*,2*R*,3*S*)-3-(3,4-dichlorophenyl)-8-methyl-8-azabicyclo[3.2.1]octane-2-carbaldehyde *O*-methyloxime; BMS-180448, (3*S*-trans)-*N*-(4-chlorophenyl)-*N'*-cyano-*N''*-(6-cyano-3,4-dihydro-3-hydroxy-2,2-dimethyl-2*H*-1-benzopyran-4-yl)guanidine; BMS-217380, *N,N*-diethyl-2-[4-(phenylmethyl-¹⁴C)phenoxy]ethanamine, monohydrochloride; BMS-181101, 5-fluoro-3-[3-[4-(5-methoxy-4-pyrimidinyl)-1-piperazinyl]propyl]-1*H*-indole; ODME, *O*-desmethyl brasofensine; ODMZ, *O*-desmethyl BMS-205912; NDME, *N*-desmethyl brasofensine; BMS, Bristol-Myers Squibb; LE, Long Evans; BDC, bile duct-cannulated; LC/MS, liquid chromatography/mass spectrometry; MS/MS, tandem mass spectrometry; QC, quality control; LLQ, lower limit of quantitation; CSF, cerebrospinal fluid; HPLC, high-performance liquid chromatography; AUC_{INF}, area under the plasma concentration-time curve from 0 to infinity; CL_T, total body clearance; Vd_{SS}, steady-state volume of distribution; AUMC, area under the first moment curve; MRT, mean residence time(s); amu, atomic mass unit(s).

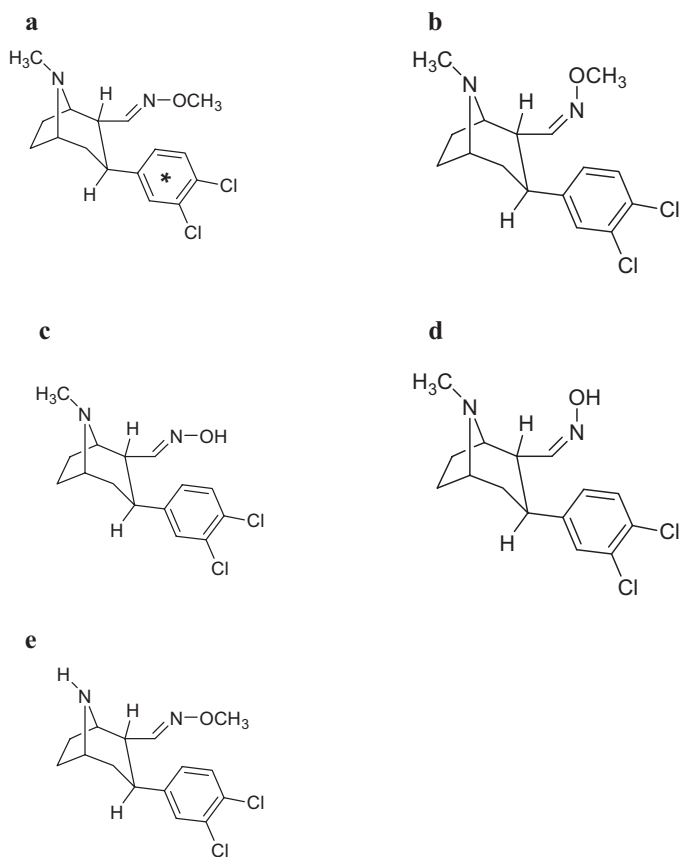


FIG. 1. Chemical structures of brasofensine (E-isomer) (a), BMS-205912 (Z-isomer) (b), ODME (c), ODMZ (d), and NDME (e). The asterisk (*) indicates the position of ^{14}C on brasofensine (all six carbons were labeled).

205912 (ODMZ), and *N*-desmethyl brasofensine (NDME)] were synthesized at Bristol-Myers Squibb (BMS) Pharmaceutical Research Institute (Princeton, NJ and New Brunswick, NJ).

Rat Study. Male Long Evans (LE) rats (173–281 g) obtained from Charles River Laboratories, Inc. (Wilmington, MA) were used in this study after approval of the BMS Animal Care and Use Committee. There were three control animals (for blank plasma) and 61 rats dosed with [^{14}C]brasofensine. The rats were fasted overnight before dose administration and for approximately 4 h after dosing. The animals had free access to water at all times. Three bile duct-cannulated (BDC) rats were used for 24-h bile collection. Blood and selected tissues were collected, and animals were sacrificed via cardiac puncture exsanguination under halothane anesthesia.

The [^{14}C]brasofensine dosing solutions were prepared in 100 mM sodium phosphate buffer, pH 7.0. Each rat received 4 mg/kg (20 μCi) of the dosing solution by p.o. gavage or 1.5 mg/kg (20 μCi) i.v. administered as a bolus injection in the tail vein. There were three rats per sampling time except at 168 h postdose, where five rats were additionally used for collection of urine and feces. Blood samples (one per rat) were collected at 3 (i.v. only) and 30 min and 1, 3, 6, 12, 24, 48, 96, and 168 h after dosing. Blood (2–10 ml) was collected at time of sacrifice via cardiac puncture into tubes containing K_3EDTA . Urine and feces were collected quantitatively before dosing and then daily for up to 7 days after dosing. Plasma was prepared from blood by centrifugation. Plasma, urine, and feces were stored at or below -20°C until analyzed.

Monkey Study. Four male cynomolgus monkeys (5–6 kg) were used in this study after approval of the BMS Animal Care and Use Committee. The animals with indwelling vascular catheters for blood sample collection were fasted overnight and for 4 h after dosing, chaired for 12 h after dosing, and then housed individually in metabolic cages for the remainder of the study. Each monkey was dosed both i.v. and p.o. with [^{14}C]brasofensine in a nonrandomized crossover design with a 2-week washout period between dosings.

The [^{14}C]brasofensine dosing solutions were prepared in 100 mM sodium

phosphate buffer, pH 7.0. Each monkey received 12 mg (50 μCi) by p.o. gavage and 4 mg (50 μCi) by a 6-min i.v. infusion into the venous port using an infusion pump. Blood samples were collected before dosing and at 6 (i.v. only), 10 (i.v. only), 15, 30, and 45 (i.v. only) min and 1, 1.5 (p.o. only), 2, 3, 6, 8, 12, 24, 48, 72, 96, 120, 144, and 168 h (or until the level of radioactivity became less than or equal to twice the background radioactivity count). Blood (1.5 ml) was collected via the indwelling vascular catheters into tubes containing K_3EDTA . Urine and feces were collected quantitatively before dosing and then daily for up to 7 days after dosing. Plasma was prepared from blood by centrifugation. Plasma, urine, and feces were stored at or below -20°C until analyzed.

Human Study. The study was conducted as a single dose in six healthy male subjects. Each subject received 2 mg (2 ml, 100 μCi) of [^{14}C]brasofensine as a solution in water, administered p.o. following a 10-h fast. The subjects continued to fast until 4 h after dosing, at which time they were served lunch.

The study protocol was approved by the Institutional Review Board and Radiation Safety Committee at the investigational site. All the subjects gave consent to participate in the study by signing and dating an informed consent form after the study was completely explained to each person.

All the subjects were in good health based on medical history, prestudy physical examinations, and clinical laboratory testing. The mean \pm S.D. age of all the subjects who entered the study was 30 ± 8 years, with a range of 22 to 44 years.

Serial blood samples (7 ml) were collected at predose, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 16, 24, 30, 36, 48, 72, 96, 120, 144, 168, 192, 216, and 240 h postdose. Immediately after collection, each blood sample was gently inverted a few times for complete mixing with the anticoagulant (K_3EDTA) and placed in chilled ice. Within 1 h of collection, the blood sample was centrifuged to obtain plasma. The plasma was divided into two portions: one for total radioactivity measurement and the remainder for analysis of unchanged brasofensine and BMS-205912 using a specific liquid chromatography/mass spectrometry (LC/MS) assay. Blood and plasma were stored at or below -20°C until analyzed. Urine and feces were collected predose, 0 to 4, 4 to 8, 8 to 12, 12 to 24 h, and then daily for up to 22 days postdose. An aliquot of each urine and fecal homogenate sample was set aside for total radioactivity measurement. All the urine and feces samples were stored at or below -20°C until analyzed.

Analytical Methods. *Brasofensine and BMS-205912: rat plasma and urine, monkey plasma and urine, human plasma and urine using LC/MS.* The methods involved the addition of internal standard (BMS-217380 for human plasma and urine, BMS-180448 for rat and monkey plasma, and BMS-181101 for rat and monkey urine) to 0.5 ml of human plasma and urine, 0.2 ml of rat plasma and urine and monkey plasma, and 1.0 ml of monkey urine. For human plasma and urine, rat urine, and monkey urine the sample and internal standard were mixed with a 0.1 M sodium carbonate solution, hexanes added (for rat plasma and monkey plasma, the extracting solvent was methyl *t*-butyl ether), shaken for 40 min, and then centrifuged. The tube was placed in a dry ice/acetone bath, the lower layer frozen, and the supernatant decanted and evaporated to dryness at 30 to 35°C under nitrogen. The residue was reconstituted with a 50/50 mixture of methanol and 0.01 M ammonium acetate solution, pH 6.0, centrifuged, and injected into the chromatographic system. Peak separation was achieved on an YMC basic, 5- μm , $2.1 \times 100\text{-mm}$, analytical column (Waters, Milford, MA) at 40°C . For rat urine and monkey urine, detection was done on a SCIEX API-1 mass spectrometer (Applied Biosystems/MDS-Sciex, Foster City, CA), which was operated in the positive electrospray mode. The protonated $[\text{M}+\text{H}]^+$ ions at m/z 327 for brasofensine and BMS-205912 and m/z 370 for the internal standard BMS-181101 were monitored for quantitation. The isocratic mobile phase (0.3 ml/min) consisted of 47% methanol and 53% aqueous 0.01% formic acid. The retention times were about 2.3, 2.5, and 2.6 min for BMS-205912, internal standard BMS-181101, and brasofensine, respectively. For human plasma, human urine, rat plasma, and monkey plasma, positive ion electrospray LC/tandem MS (MS/MS) was used for detection and quantitation with a Finnigan TSQ 7000 mass spectrometer (Thermo Electron Corporation, Waltham, MA) equipped with an electrospray ionization source. The mass spectrometer was programmed to admit the protonated molecules $[\text{M}+\text{H}]^+$ at m/z 327 (brasofensine and BMS-205912), m/z 396 (BMS-180448 internal standard for rat and monkey plasma), and m/z 284 (BMS-217380

TABLE 1

Mean (S.D.) percentage cumulative recovery of total radioactivity^a postdose after i.v. and p.o. administration of [¹⁴C]brasofensine in male LE rats (n = 5), male cynomolgus monkeys (n = 4), and healthy male subjects (n = 6)

Species	Matrix	i.v. Solution	p.o. Solution
		Dose: 1.5 mg/kg	Dose: 4 mg/kg
Rat	Urine	22.8 (3.8)	21.2 (2.8)
	Feces	62.1 (3.3)	71.2 (2.7)
	Carcass ^b	5.4 (0.5)	5.1 (0.4)
	Total	90.3 (3.7)	97.5 (0.6)
		Dose: 4 mg	Dose: 12 mg
Monkey	Urine	66.8 (4.0)	64.7 (4.0)
	Feces	23.4 (2.6)	22.7 (3.8)
	Total	90.2 (2.7)	87.4 (2.9)
		Dose: 50 mg	
Human	Urine	N.D.	86.4 (4.5)
	Feces	N.D.	11.4 (2.4)
	Total	N.D.	97.7 (2.4)

N.D. = not determined.

^a Rat and monkey = 7 days postdose; human = 22 days postdose.

^b Whole animal.

internal standard for human plasma and urine) via the first quadrupole (Q1) with collision-induced fragmentation in Q2 (argon collision gas) and monitoring via the third quadrupole (Q3), the product ions at *m/z* 97 for brasofensine and BMS-205912, *m/z* 194 for the BMS-180448 internal standard, and *m/z* 100 for the BMS-217380 internal standard. A linear gradient mobile phase was used starting at 28% acetonitrile and 72% solvent A (75% aqueous 0.01 M ammonium acetate/25% methanol) to 60% acetonitrile and 40% solvent A in 2 min and holding this mixture for 1 min. The flow rate was 0.3 ml/min. The retention times were about 1.4, 2.0, 2.1, and 5.0 min for BMS-205912, brasofensine, BMS-217380, and BMS-180448, respectively.

Using peak area ratios, standard curves were obtained by quadratic regression weighted by $1/x^2$. Predicted concentrations were calculated using the regressed equation. Spiked quality control (QC) samples were prepared before

the initiation of the study in control plasma and urine using reference standards for brasofensine and BMS-205912 and stored with the study samples. QC samples were analyzed with study samples to establish stability and monitor acceptability of an analytical run.

The analytical runs were accepted based on a priori acceptance criteria as follows:

1. The back-calculated concentrations of at least three fourths of all the calibration standards were to be within $\pm 15\%$ of their individual nominal concentrations [$\pm 20\%$ at the lower limit of quantitation (LLQ)].

2. At least one replicate of the lowest concentration in the standard curve was to be within $\pm 20\%$ of the nominal concentration for that level to qualify as the LLQ. If this criterion was not met, the next level was subjected to the same test and the LLQ increased accordingly.

3. The back-calculated concentrations of all the analytical QC samples were to be within $\pm 15\%$ of their individual nominal concentrations, with at least one acceptable QC sample at each level.

The plasma standard curve range for brasofensine and BMS-205912 was 0.02 to 5 ng/ml in human plasma and urine, 0.05 to 10 ng/ml in rat and monkey plasma, 0.1 to 5 ng/ml in monkey urine, and 0.25 to 25 ng/ml in rat urine. The lowest end of the standard curve range represented the LLQ for brasofensine and BMS-205912. During analysis of study samples, the mean observed concentrations of the QC samples were within $\pm 7\%$ from nominal values. The between- and within-day coefficients of variation were within 14%. These results indicated that the assay methods were precise, accurate, and reproducible for the analyses of brasofensine and BMS-205912 in human plasma and urine, rat plasma and urine, and monkey plasma and urine. Brasofensine and BMS-205912 were stable under the assay conditions.

Total radioactivity. Total radioactivity in urine samples (1 ml of urine added to 15 ml of Hionic-Fluor, PerkinElmer, Waltham, MA) was measured without any processing. Fecal samples were first homogenized with approximately 3 volumes of 100 mM phosphate buffer or water and combusted for measurement of total radioactivity.

Samples of the following rat tissues or fluids were solubilized in the scintillation mixture directly or first combusted and then analyzed using liquid scintillation counting: adrenal glands, bladder, blood, bone, bone marrow

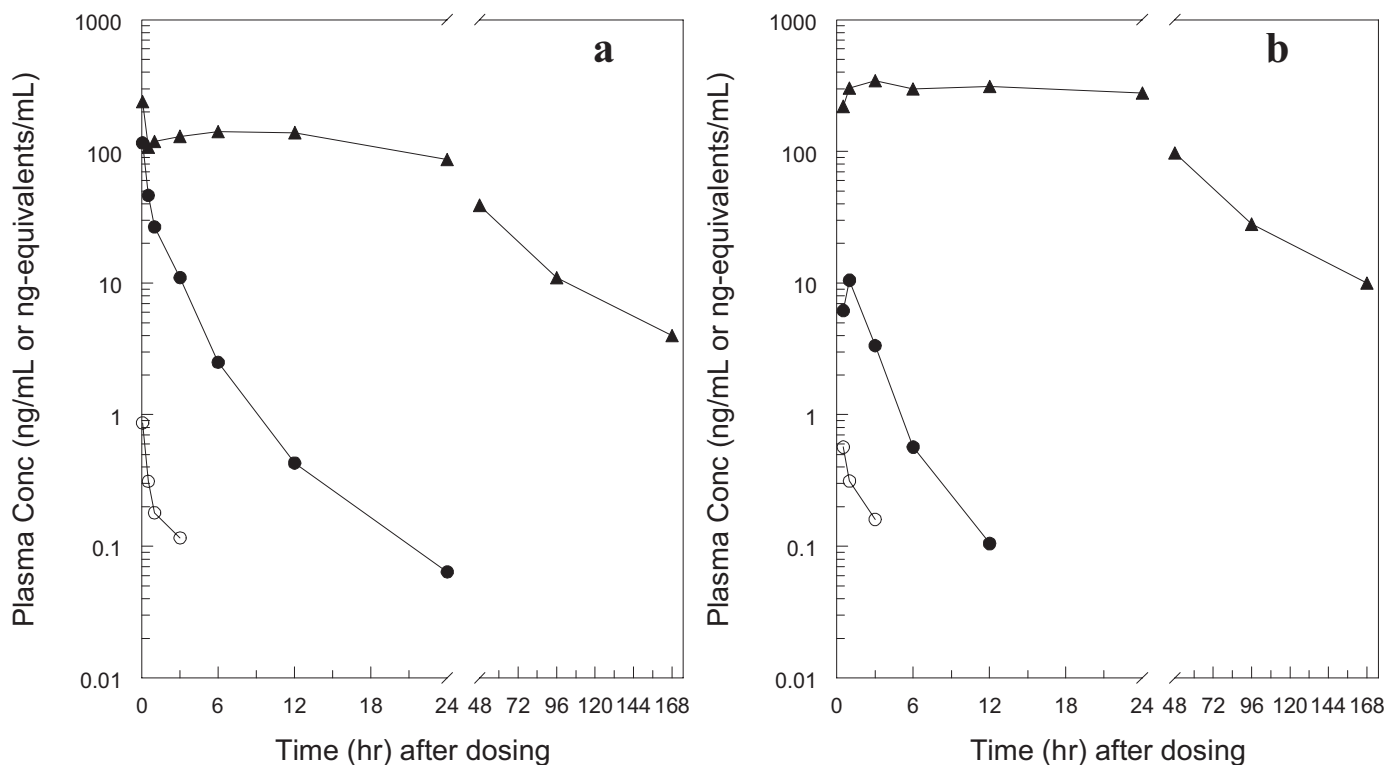


FIG. 2. Composite plasma concentration-time profiles of total radioactivity (-▲-), brasofensine (-●-), and BMS-205912 (-○-) after i.v. administration (1.5 mg/kg) (a) and p.o. administration (4 mg/kg) (b) of [¹⁴C]brasofensine in LE rats.

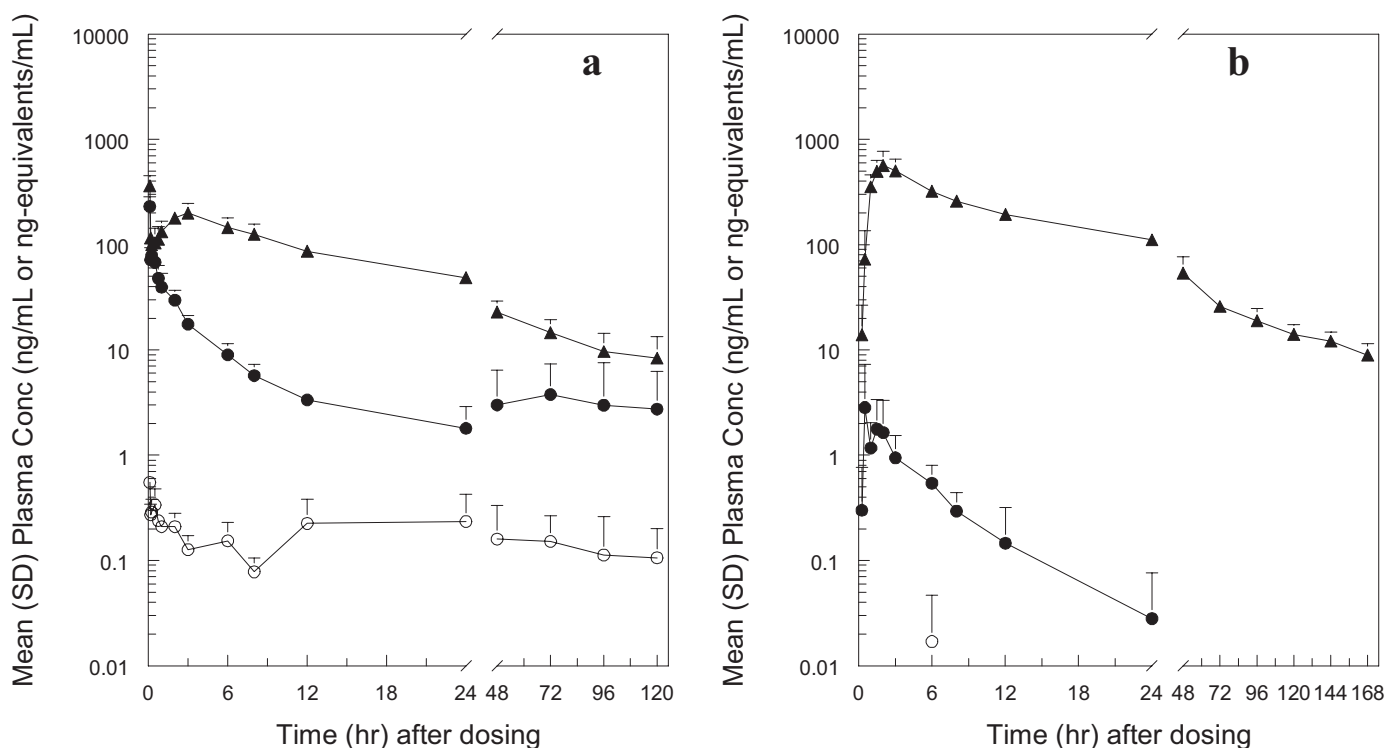


FIG. 3. Mean (S.D.) plasma concentration-time profiles of total radioactivity (-▲-), brasofensine (-●-), and BMS-205912 (-○-) after i.v. administration (4 mg) (a) and p.o. administration (12 mg) (b) of [^{14}C]brasofensine in cynomolgus monkeys.

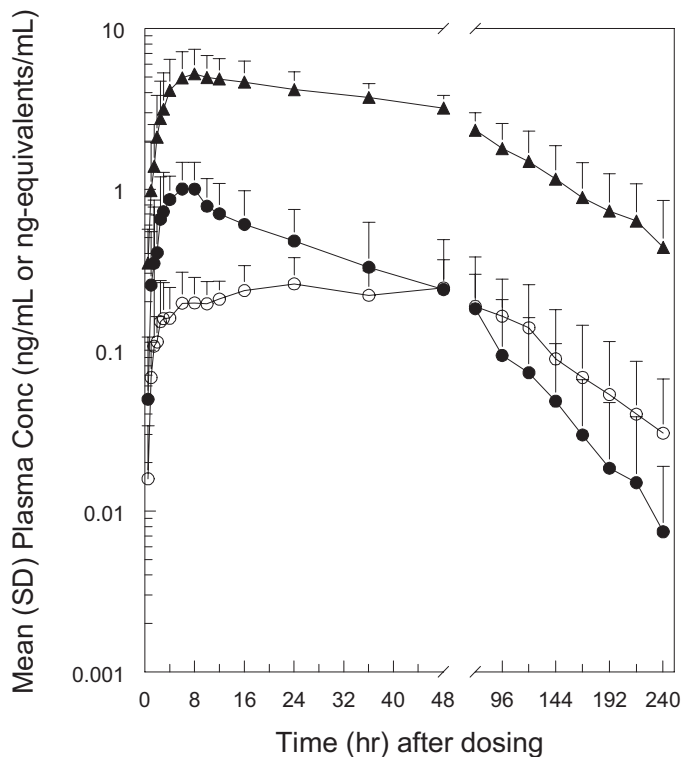


FIG. 4. Mean (S.D.) plasma concentration-time profiles of total radioactivity (-▲-), brasofensine (-●-), and BMS-205912 (-○-) after p.o. administrations (50 mg) of [^{14}C]brasofensine in humans.

(femur), brain, cerebrospinal fluid (CSF), eyes, heart, kidneys, large intestine, large intestinal contents, liver, lungs, muscle (thigh), plasma, skin (nonpigmented and pigmented), small intestine, small intestinal contents, spleen, stomach, stomach contents, testes, thyroid, carcass, and urine. The total organ

TABLE 2

Mean (S.D.) pharmacokinetic parameters for total radioactivity in LE rats, cynomolgus monkeys, and humans after i.v. and p.o. administration of [^{14}C]brasofensine

Species	Dose, Route	C_{\max}	T_{\max}^a	$\text{AUC}_{\text{INF}}^b$	Terminal Plasma $t_{1/2}^c$
		ng-Eq/ml	h	ng-Eq · h/ml	h
Rat ^c	1.5 mg/kg, i.v.			6143	31
	4 mg/kg, p.o.	344	3	15,631	31
Monkey	4 mg, i.v.			4254 (764)	52 (27)
	12 mg, p.o.	577 (187)	2	10,108 (1968)	88 (40)
Human	50 mg, p.o.	5.4 (2.1)	8	512 (142)	79 (19)

^a Median.

^b AUC for monkey is AUC_{0-T} , where T is the last measurable sampling time

^c Pharmacokinetic parameters derived from a composite profile.

or a tissue sample was accurately weighed and digested with an appropriate amount of 1N sodium hydroxide. An accurately weighed amount of the digested sample was then mixed with 15 ml of Ultima-Gold scintillation mixture. All the sample combustions were done in a model 306 or 307 Sample Oxidizer (PerkinElmer).

Metabolite profiling. For metabolite profiling, a portion (0.45–1.0 ml) of the pooled plasma or urine sample was applied to an activated Oasis Extraction Cartridge (Waters). The cartridge was eluted with 2 ml of water followed by 2 ml of methanol. The methanol fractions were dried under nitrogen and reconstituted with 0.5 ml of methanol (20%)/water (80%). The extraction efficiency of samples prepared for metabolic profiling was greater than 90%.

For plasma metabolite profiling by off-line high-performance liquid chromatography (HPLC)/liquid scintillation counting, two reverse-phase HPLC systems were utilized. One (method A) used a PKB-100 column [Supelco (Sigma-Aldrich, St. Louis, MO); 4.6×250 mm] and a linear stepwise gradient system with solvent A (0.06% trifluoroacetic acid in water, adjusted to pH 3.0 with NH_4OH) and solvent B (methanol). Solvent B in the gradient started at 27% and then increased as follows: 42% (30 min), 70% (35 min), 75% (40 min), 80% (50 min), and 90% (55 min). The second HPLC system (method B) used a Zorbax Rx-C₁₈ column (MAC-MOD Analytical, Chadds Ford, PA; 4.6×250 mm) and a linear stepwise gradient systems with solvent A (0.06%

TABLE 3

Mean (S.D.) pharmacokinetic parameters for brasofensine and BMS-205912 in LE rats, cynomolgus monkeys, and humans after i.v. and p.o. administration of brasofensine

Species	Dose, Route	C_{max}		T_{max}^a	AUC_{INF}^b		Terminal Plasma $t_{1/2}$	MRT	CL_T	$V_{d_{ss}}$	F
		Brasofensine	BMS-205912		Brasofensine	BMS-205912					
		ng/ml	ng/ml	h	ng · h/ml	ng · h/ml	h	h	ml/min/kg	l/kg	%
Rat ^c	1.5 mg/kg, i.v.				125	1.04	3.5	2.1	199	24	
	4 mg/kg, p.o.	10.5	0.57	1	24.9	1.16	1.7	2.5			7.4
Monkey	4 mg, i.v.				507 (367)	20.4 (19.3)	N.A. ^d	N.A. ^e	32 (17)	46 (5)	
	12 mg, p.o.	3.3 (4.1)	N.D. ^f	1	9.0 (8.4)	N.D. ^f	3.9 (2.2)	5.4 (0.7)			0.8
Human	50 mg, p.o.	1.2 (0.5)	0.31 (0.11)	7	39.0 (28.5)	35.5 (21.4)	24 (13) ^g	39 (23) ^h			

^a Median.

^b AUC for monkey is AUC_{0-T} , where T is the last measurable sampling time.

^c Pharmacokinetic parameters derived from a composite profile.

^d The terminal slope could not be determined.

^e AUC_{INF} could not be determined.

^f No detectable concentrations.

^g Terminal plasma $t_{1/2}$ for BMS-205912 was 55 (37) h.

^h MRT for BMS-205912 was 96 (43) h.

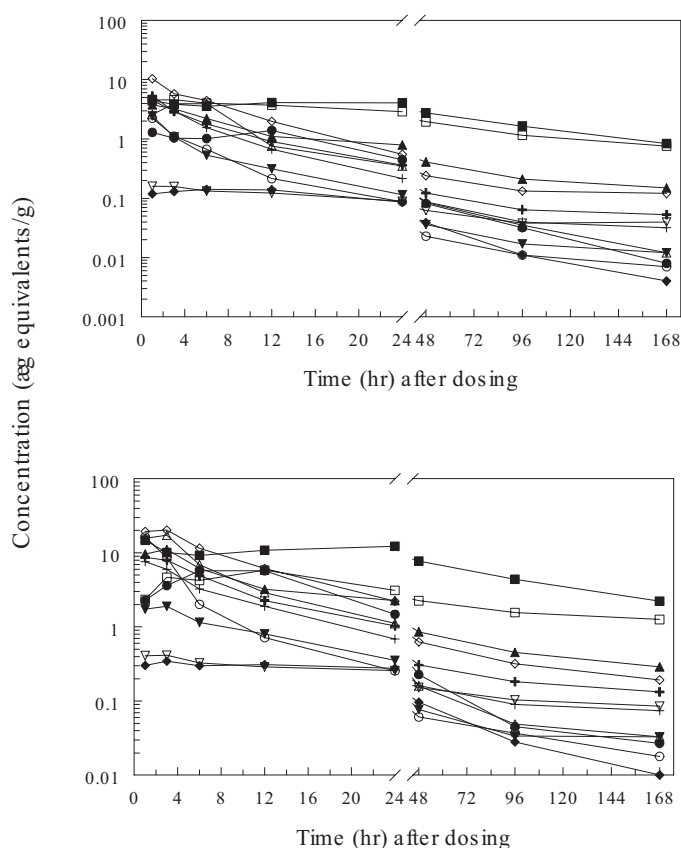


Fig. 5. Mean concentrations of total radioactivity in selected tissues from LE rats after i.v. (top) and p.o. (bottom) administration of [¹⁴C]brasofensine (●, large intestine; △, small intestine; ○, stomach; ■, liver; ▲, kidney; ◇, plasma; 197, blood; ▼, brain; ▽, eye; 224, lung; +, adrenal gland; +, spleen).

trifluoroacetic acid in water, pH 3.0) and solvent B (acetonitrile). Solvent B in the gradient was held at 27% for the first 3 min and then increased as follows: 35% (35 min), 70% (45 min), maintained at 70% (45–47 min), 90% (49 min), and maintained at 90% (49–55 min). HPLC analyses were performed on a Shimadzu Class VP System equipped with two pumps (model CL-10AT), an autoinjector (SIL 10AD), and a diode array detector (SPD-M10A) at a flow rate of 1 ml/min. The UV absorption of the effluent was monitored at a wavelength of 216 nm. The HPLC eluate was collected by a fraction collector (0.5 min/tube). Each fraction was mixed with 4.5 ml of Exolite liquid scintillation mixture and counted for 20 to 60 min in a Packard liquid scintillation

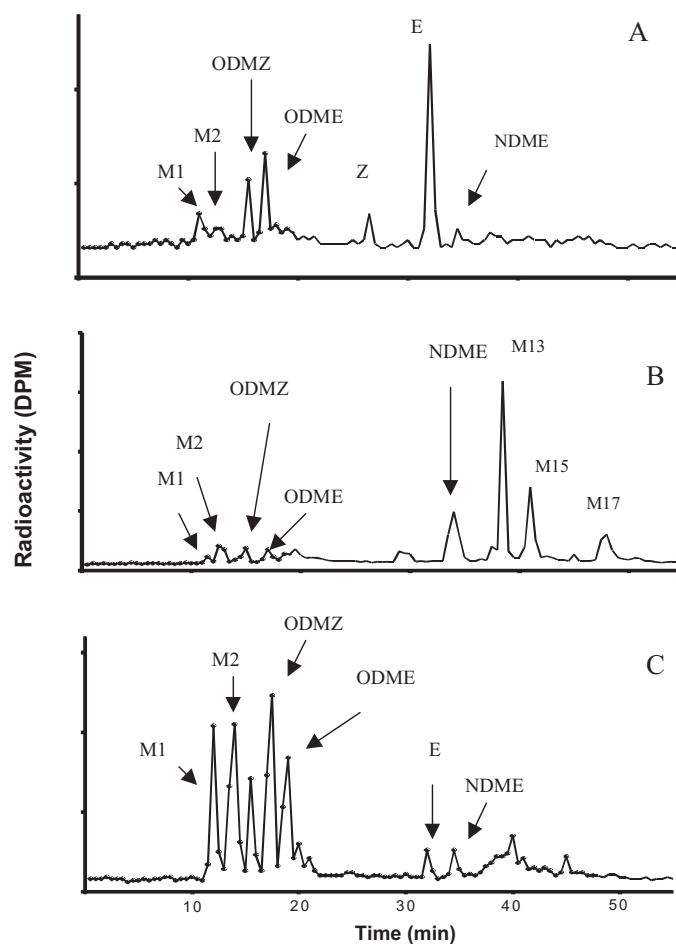


Fig. 6. Metabolite profiles of [¹⁴C]brasofensine in human (A, pooled from 4–24 h), monkey (B, pooled from 1.5–3 h), and rat (C, 2 h) plasma.

analyzer (PerkinElmer). For urine metabolite profiling, HPLC method A was used, and radioactivity in the HPLC eluate was determined with an on-line radioactivity detector (β -Ram IN/US Systems, Tampa, FL). HPLC eluate was mixed with IN-Flow 3 mixture (IN/US System) at a ratio of 1 to 3, and the mixture was passed through a 500- μ l liquid detection cell.

Metabolite identification. Radioactive metabolites in rat and monkey urine were identified by HPLC/radio-flow detection/MS. HPLC eluate (method A) from the diode array detector was split and transferred into a radio-flow detector (0.8 ml/min) and an ion trap MS (0.2 ml/min) (LCQ, Thermo Electron

Corporation). Mass spectrometric analysis was conducted using electrospray positive mode at a capillary temperature of 200°C. MSⁿ ($n = 1-3$) spectra of metabolites were obtained with collision energies of 15 to 20 eV. Plasma metabolites were identified by comparison of their HPLC retention times with those of reference standards and the metabolites identified in rat urine. If the retention times of an unknown metabolite in radiochromatograms from analyses by both HPLC methods (PKB and Zorbax) were the same as those of a reference standard or a known urinary metabolite, the structure of the unknown metabolite was assigned.

Pharmacokinetic analysis. Plasma concentration versus time data for brasofensine and BMS-205912 were analyzed by noncompartmental methods (Gibaldi and Perrier, 1982). The terminal log-linear phase of the plasma concentration versus time curve was identified by least-squares linear regression of data points that yielded a minimum mean square error. The area under the plasma concentration-time curve from 0 to infinity (AUC_{INF}) was determined by a combination of trapezoidal and log-trapezoidal methods plus the extrapolated area. The extrapolated area was determined by dividing the observed concentration at the time of the last nonzero plasma concentration by the slope (β) of the terminal log-linear phase. The $t_{1/2}$ of the terminal log-linear phase was calculated as $\ln(2)$ divided by the absolute value of β . The peak plasma concentration, C_{max} , and the time at which C_{max} occurred, T_{max} , were obtained from the observed data. Total body clearance (CL_T) and steady-state volume of distribution (Vd_{ss}) for brasofensine were calculated as follows:

$$CL_T = \text{Dose}_{(iv)}/AUC_{(INF)}$$

and

$$Vd_{ss} = \text{Dose}_{(iv)} \cdot AUMC/[AUC_{(INF)}]^2$$

where AUMC is the area under the first moment of plasma concentration versus time curve. The absolute bioavailability (F) of brasofensine and BMS-205912 was estimated as a ratio of the plasma AUC_{INF} of unchanged brasofensine or BMS-205912 after p.o. to that after i.v. administration. Mean residence time (MRT) was calculated as $AUMC/AUC_{INF}$.

Results

Excretion of Radioactivity. Mean (S.D.) percentage recovery of total radioactivity in urine and feces of rats, monkeys, and humans is summarized in Table 1. In all the species studied, the percentage of administered radioactivity recovered was comparable between i.v. and p.o. administration. After either i.v. or p.o. dosing in rats and monkeys, approximately 20 and 70% of total radioactivity, respectively, was recovered in the urine. Fecal excretion accounted for approximately 70 and 20% in the rat and monkey, respectively. The majority of the administered dose was recovered in the first 24 h postdose. Overall, approximately 90% of the total i.v. and p.o. dose was excreted up to 168 h postdose in the rat and monkey. Biliary excretion of total radioactivity in three BDC rats after p.o. dosing of [¹⁴C]brasofensine accounted for 64% of the total dose in 24 h.

In humans, the mean cumulative excretion of total radioactivity in urine over 22 days postdose was 86% following p.o. dosing of [¹⁴C]brasofensine. Approximately 90% of the radioactive dose recovered in urine was excreted over the 1st week postdose. Fecal excretion in the human accounted for 11% of the p.o. dose. Overall, 97% of the total radioactivity administered p.o. was recovered in urine and feces over 22 days post-oral dosing.

Plasma Time Course of Total Radioactivity and Pharmacokinetics of Brasofensine. Mean (S.D.) plasma concentration-time profiles of total radioactivity, brasofensine, and BMS-205912 after i.v. and p.o. dosing of brasofensine in rats and monkeys and after p.o. dosing in humans are shown in Figs. 2 through 4. Mean (S.D.) pharmacokinetic parameters of total radioactivity and brasofensine and BMS-205912 for rats, monkeys, and humans are summarized in Tables 2 and 3, respectively.

The AUC values of total radioactivity compared with the AUC

TABLE 4
Percentage distribution of brasofensine in human, monkey, and rat plasma^a

Species	Radioactivity in Plasma Corresponding to Human Metabolites							Total
	M1	M2	ODMZ	ODME	Z	E	NDME	
	%	%	%	%	%	%	%	
Human	9	6	11	20	6	45	3	100
Monkey		4	2	3	N.D.	N.D.	Trace	9
Rat		5	9	19	N.D.	2	2	37

^a Radioactivity distribution in plasma was determined by using HPLC method B. The method cannot separate M1 from M2.

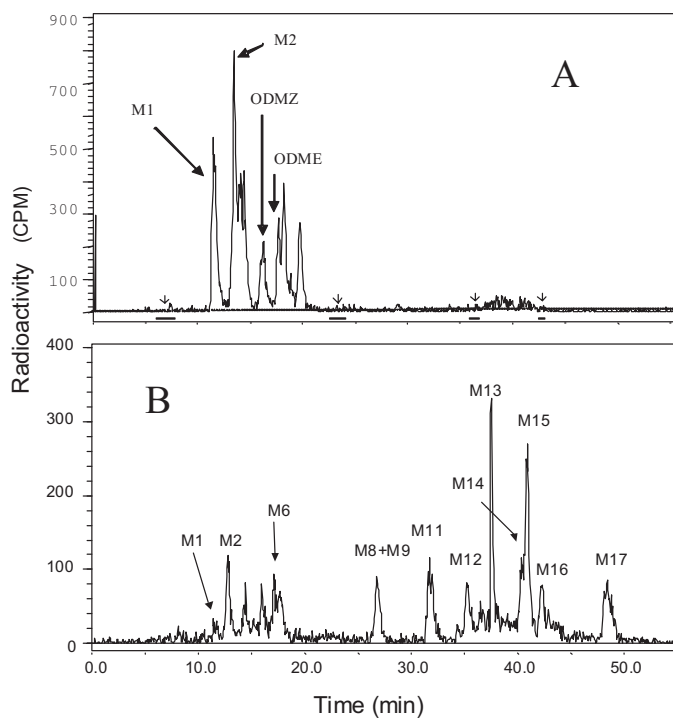


FIG. 7. Metabolite profile of brasofensine in rat urine (A, pooled 0-96 h) and monkey (B, pooled 0-24 h).

values of brasofensine following [¹⁴C]brasofensine administration were 49- and 628-fold greater following i.v. and p.o. dosing, respectively, in the rat, 8- and 1123-fold greater following i.v. and p.o. dosing, respectively, in the monkey, and 13-fold following p.o. dosing in humans. The half-life values of total radioactivity were comparable following i.v. and p.o. administration. The concentrations of brasofensine and BMS-205912 decreased much more rapidly than those for radioactivity. The exposure to BMS-205912 was <6% of the brasofensine exposure following administration of brasofensine in the rat and monkey. In contrast, the exposure to BMS-205912 in humans following p.o. brasofensine was approximately 90% of the brasofensine exposure, with mean BMS-205912 concentrations increasing more gradually than those of brasofensine and then exceeding brasofensine concentrations at 72 h postdose.

In the rat, brasofensine AUC_{INF} values were 125 ng · h/ml after i.v. dosing (1.5 mg/kg) and 25 ng · h/ml after p.o. dosing (4 mg/kg) with an absolute bioavailability of 7% (Table 3). The corresponding exposure to BMS-205912 was approximately 1 ng · h/ml following both routes of brasofensine administration. After p.o. dosing, brasofensine was absorbed rapidly with peak concentrations of 11 ng/ml achieved within 1 h of dosing. The decrease in plasma concentrations yielded

TABLE 5
Relative percent distribution, mass spectra data, and proposed structures of metabolites of [^{14}C]brasofensine in monkey urine

Metabolite	Percent of Radioactivity	MS ⁿ Spectral Data		Metabolite Identity
		M + H ⁺	MS ² and MS ³ Product Ions ^a	
M1	1	489	MS ² : 313, 295, 280, 266; MS ³ on 313: 295, 282, 266, 264, 159	Glucuronide of <i>O</i> -desmethyl brasofensine ^b
M2	10	475	MS ² : 299, 281, 266; MS ³ on 299: 281, 266, 264, 214	Glucuronide of <i>O,N</i> -didesmethyl brasofensine ^b
ODME	Trace ^c	313	MS ² : 295, 282, 269, 264, 159	<i>O</i> -desmethyl brasofensine (BMS-282697)
M6	3	473	MS ² : 197	Glucuronide conjugate of a phase I metabolite
M8	6	343	MS ² : 325, 295, 278, 268; MS ³ on 295: 278, 268, 264, 252, 123	Monohydroxylated brasofensine ^b
M9		505	MS ² : 487, 469, 458, 329; MS ³ on 329: 311, 298, 282, 280	Glucuronide of monohydroxylated <i>O</i> -desmethyl brasofensine ^b
NDME	Trace ^c	313	MS ² : 296, 281, 255, 159	<i>N</i> -desmethyl brasofensine (BMS-212702)
M11	10	491	MS ² : 473, 315, 297; MS ³ on 315: 297, 281, 280, 159	Glucuronide of monohydroxylated <i>O,N</i> -didesmethyl brasofensine ^b
M12	4	473	MS ² : 197	Glucuronide conjugate of a phase I metabolite
M13	14	505	MS ² : 455, 329, 311, 297; MS ³ on 329: 297, 281, 271, 238	Glucuronide of monohydroxylated <i>N</i> -desmethyl brasofensine ^b
M14	6	491	MS ² : 315, 297, 270; MS ³ on 297: 279, 270, 252	Glucuronide of monohydroxylated <i>O,N</i> -didesmethyl brasofensine ^b
M15	20	491	MS ² : 315, 297, 270; MS ³ on 297: 279, 270, 264, 252	Glucuronide of monohydroxylated <i>O,N</i> -didesmethyl brasofensine ^b
M16	5	473	MS ² : 197	Glucuronide conjugate of a phase I metabolite
M17	9	505	MS ² : 329, 311, 297; MS ³ on 329: 311, 297, 281, 271	Glucuronide of monohydroxylated <i>N</i> -desmethyl brasofensine ^b
Total	88 ^d			

^a Major or significant product ions.

^b This metabolite could be either the E-isomer or its Z-isomer.

^c Trace: Trace amount of the metabolite that was detected by LC/MS but not by radio-flow detector.

^d The remaining urinary radioactivity was distributed among several unidentified peaks, none of which individually accounted for more than 5% of the total urinary radioactivity.

a terminal plasma half-life of 1.7 to 3.5 h. MRT was 2.1 to 2.5 h. CL_T of brasofensine in rats was 199 ml/min/kg, and Vd_{ss} was 24 l/kg.

In the monkey, mean brasofensine AUC_{INF} values were 507 ng · h/ml after i.v. dosing (4 mg) and 9 ng · h/ml after p.o. dosing (12 mg), with a mean absolute bioavailability of 0.8%. The corresponding exposure to BMS-205912 was approximately 20 ng · h/ml following i.v. brasofensine administration but was undetectable following p.o. brasofensine administration. After p.o. dosing, brasofensine was absorbed rapidly, with a peak concentration of 3.3 ng/ml achieved within 1 h of dosing. The decrease in plasma concentrations yielded a mean terminal plasma half-life of 3.9 h. MRT was 5.4 h. Mean CL_T of brasofensine in monkeys was 32 ml/min/kg, and mean Vd_{ss} was 46 l/kg.

In humans, mean AUC_{INF} values were 39.0 and 35.5 ng · h/ml for brasofensine and BMS-205912, respectively, after p.o. dosing (50 mg) of brasofensine. After p.o. dosing, brasofensine was absorbed relatively slowly with a peak concentration of 1.2 ng/ml achieved within 7 h of dosing. The peak concentration for BMS-205912 following brasofensine administration was approximately one third the brasofensine concentration and occurred much later (~24 h). The decrease in plasma concentrations yielded a mean terminal plasma half-life of 24 and 55 h for brasofensine and BMS-205912, respectively. MRT were 39 and 96 h, respectively.

Distribution of Radioactivity in Tissues and Fluids. Figure 5 depicts the concentrations of total radioactivity in selected tissues in LE rats after i.v. and p.o. administrations of brasofensine. The tissues selected represent organs associated with absorption (stomach and small intestine), organs associated with elimination (kidneys, liver, and large intestine), organ associated with site of action (brain), and another organs that showed significant concentrations of radioactivity at the last time of measurement (eye, lung, adrenal gland, spleen, and blood).

Highest concentrations of radioactivity after either p.o. or i.v. dosing were observed at 1 h postdose in most tissues except for the eyes and small intestine following i.v. administration where peak concentrations occurred at 3 h postdose, and except for the eyes, small intestine, and large intestine following p.o. dosing where peak concentrations occurred at 12, 3, and 12 h postdose, respectively. Except for CSF, all the other tissues had higher concentrations of total radioactivity than plasma for at least the first 12 h. All the tissues, except CSF, had detectable concentrations at 168 h postdose. The

decrease of radioactivity in plasma and blood was almost superimposable until around 48 h, when the radioactivity in blood started to decrease less than the decrease in plasma. The decrease in concentration in most tissues seemed to parallel the decrease in blood, whereas some tissues, like the organs of excretion, tended to parallel the decrease in plasma.

Metabolite Profiles. HPLC radiochromatograms of rat, monkey, and human plasma are shown in Fig. 6. There were seven radioactivity peaks in the human plasma profile. Based on their retention times, five of these peaks corresponded to the parent drug (E), its Z-isomer (Z; BMS-205912), the ODME metabolite, the ODMZ metabolite, and the NDME metabolite (Fig. 1). Together, these five radioactive peaks accounted for 85% of the total radioactivity in plasma (Table 4). Parent drug in human plasma was the predominant component (45%), followed by ODME (20%), ODMZ (11%), Z-isomer of the parent drug (6%), and NDME (3%). The remainder of the radioactivity (15%) was present as two minor metabolite peaks (M1 and M2), identified as glucuronide conjugates of ODME and *N,O*-didesmethyl brasofensine, respectively (Fig. 6). All these metabolites in human plasma had the same HPLC retentions as those of the metabolite standards [ODMZ, ODME, Z-isomer (BMS-205912), and NDME] and metabolites identified in rat urine (M1 and M2) by LC/MS/MS.

The plasma metabolite profiles in rats and monkeys were more complex in terms of the number of radioactivity peaks compared with humans. The rat plasma contained all of the metabolites observed in human plasma (Table 4; Fig. 6). The Z-isomer (BMS-205912) in the rat plasma was detected by LC/MS/MS but not by HPLC/radioactivity analysis because of its low abundance (<2% of the total radioactivity). All the human plasma metabolites were also observed in monkey plasma (Table 4; Fig. 6). The parent drug and its Z-isomer (BMS-205912) were detected by LC/MS/MS (data not shown) but not by radiochromatographic analysis because each had less than 2% of the total plasma radioactivity. However, the radioactivity distribution in monkey plasma was different from that in human plasma. Human metabolites only accounted for 9% of the total radioactivity in monkey plasma, and none of the prominent radioactivity peaks were observed in human plasma.

The metabolite profile of [^{14}C]brasofensine in rat urine is shown in Fig. 7A, which was similar to that in rat plasma. M1, M2, ODME, and ODMZ were observed as major urinary metabolites. The parent drug and its Z-isomer were not observed in rat urine. The metabolite profile

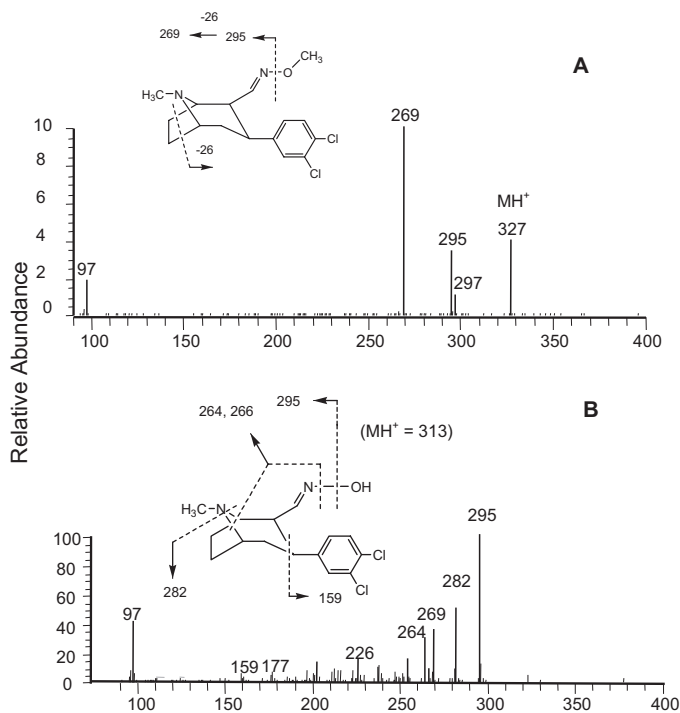


FIG. 8. Product spectra and structures of brasofensine (A) and ODME (B).

of [¹⁴C]brasofensine in monkey is shown in Fig. 7B, which was consistent with that in monkey plasma (Fig. 6B). The major drug-related components in monkeys were glucuronides of oxidative metabolites, such as *O*- and *N*-desmethyl metabolite (Table 5). No brasofensine or *Z*-isomer was detected in monkey urine (Fig. 7B), suggesting that brasofensine was extensively metabolized in monkeys because more than 60% of dose was recovered in the urine (Table 1).

Identification of Urine Metabolites by LC/MS/MS. Brasofensine metabolites in rat and monkey urine were structurally characterized using ion trap LC/MS. Protonated molecules, MSⁿ spectra ($n = 2$ and 3), and proposed structures of brasofensine metabolites in monkey urine are summarized in Table 5. The product ion spectrum of brasofensine (E) is shown in Fig. 8A. ODME and its geometric isomer ODMZ had the same protonated molecule (MH⁺) at m/z 313, 14 atomic mass units (amu) less than that of the parent drug, indicating these two metabolites were formed via demethylation. The product ion spectrum and its interpretation of ODME are shown in Fig. 8B, which were identical to those of ODMZ (data not shown). The product ion at m/z 282 from the loss of a CH₃N group (-31) suggests that the metabolite was the *O*-demethylation product. The product ions at m/z 295 and 269 were produced from both ODME and the parent drug (Fig. 8), indicating that the *N*-methyl group remains in ODME. The identity of ODME and ODMZ was further confirmed by chromatographic and mass spectral comparison with those of the synthetic standards.

M1 had an MH⁺ at m/z 489 and the most intensive product ion at m/z 313 from the loss of 176 amu (Fig. 9A), indicating M1 was a glucuronide of a demethylated product. MS³ (489 → 313 →) spectrum of M1 showed several major fragment ions at m/z 295, 282, 266, and 264 (Fig. 9B) identical to product ions of ODME (Fig. 8B). Therefore, M1 was identified as a glucuronide conjugate of ODME (or its *Z*-isomer). M2 had a protonated molecule at m/z 475 (Fig. 10A). The product ion at m/z 299, resulting from a loss of 176 amu, clearly indicates M2 is a glucuronide (Fig. 10A). The protonated ion

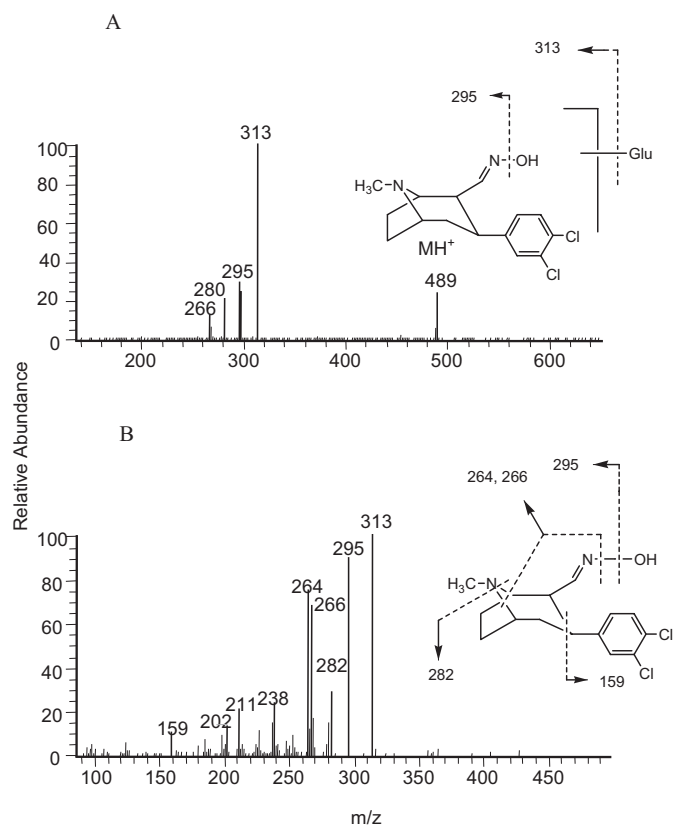


FIG. 9. Mass spectra and proposed structure of M1. A, product ion spectrum of M1 ($M + H^+ = 489$). B, MS³ spectrum (489 → 313 →) of M1.

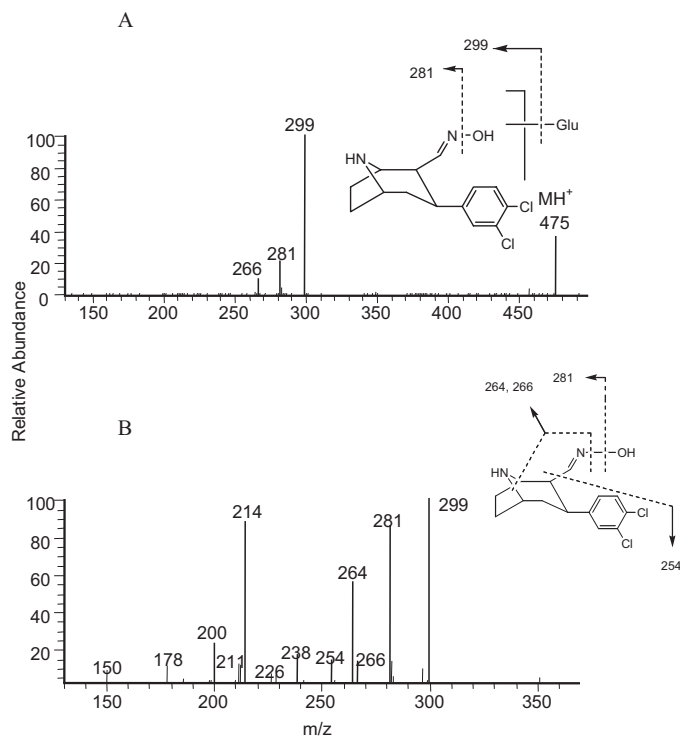


FIG. 10. Mass spectra and proposed structure of M2. A, product ion spectrum of M2 ($M + H^+ = 475$). B, MS³ spectrum (474 → 299) of M2.

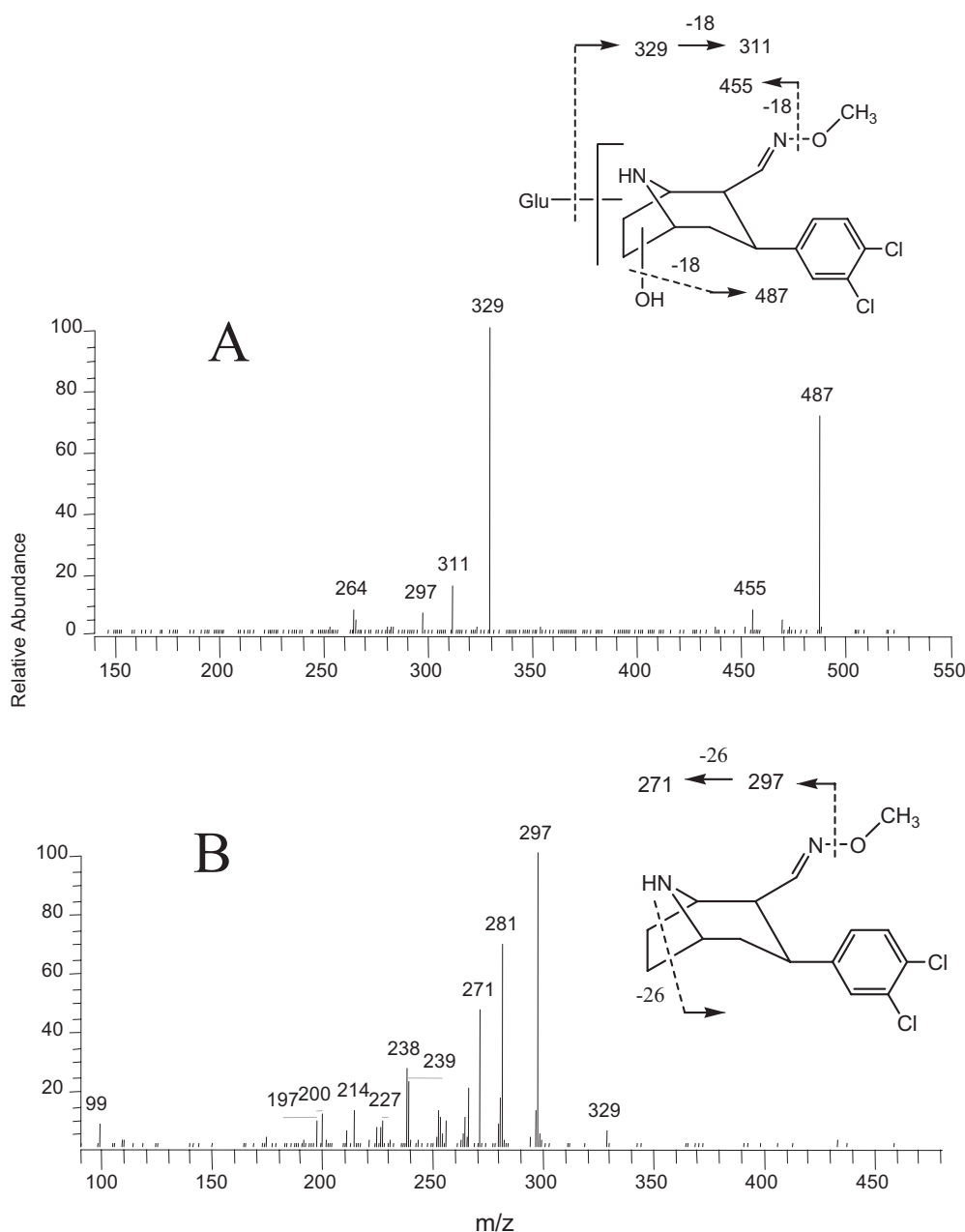


FIG. 11. Mass spectra and proposed structure of M13. A, product ion spectrum of M13 ($M + H^+ = 505$). B, MS^3 spectrum ($505 \rightarrow 329$) of M13.

(m/z 299) of the aglycone was 28 amu less than that of the parent drug ($MH^+ = 327$), suggesting the loss of two methyl groups from brasofensine or its *Z*-isomer. MS^3 spectrum of M2 ($475 \rightarrow 299 \rightarrow$) displayed product ions at m/z 281, 266, 264, 254, and 214 (Fig. 10B), consistent with the structure of *O,N*-desmethyl brasofensine (Fig. 10). Therefore, M2 is assigned as a glucuronide of *O,N*-didesmethyl brasofensine. Most likely, the glucuronic acid was attached to the oxime group (Fig. 10A).

M13, M15, and M17 were major metabolites in monkey plasma (Fig. 6B) and urine (Fig. 7B), but these metabolites were not presented significantly in human plasma (Fig. 6A), rat plasma (Fig. 6C), and rat urine (Fig. 7A). M13 and M17 had the same protonated molecule at m/z 505 (Table 5). MS^2 spectra of these metabolites showed the formation of an ion at m/z 329, resulting from a loss of 176 Da (Fig. 11A and Table 5), indicating M13 and M17 were glucuronide conjugates of brasofensine derivatives. The ion at m/z 329 corresponded to the molecular ion of a metabolite formed by the combination of

monohydroxylation (+16) and demethylation (-14). MS^3 spectra of ($505 \rightarrow 329 \rightarrow$) M13 and M17 were identical (Fig. 11B; Table 5). The observed loss of the OCH_3 group to yield an ion at m/z 297 suggests that a methyl group was attached on the oxygen atom. Therefore, M13 and M17 were identified as glucuronide conjugates of monohydroxylated NDME.

M11, M14, and M15 had the same molecular ion at m/z 491 but different HPLC retention times (Fig. 6B). MS^2 analysis of the three metabolites showed the formation of an ion at m/z 315 by the loss of 176 Da, indicating that M11, M14, and M15 were glucuronide conjugates of brasofensine derivatives. The ion at m/z 315 ($MH^+ - 176$) corresponded to the protonated aglycone that was formed by the combination of monohydroxylation (+16) and didemethylation (-28). MS^3 spectra of M15 and the interpretation of the fragmentation are displayed in Fig. 12. Based on MS^n spectral data, M11, M14, and M15 were identified as glucuronide conjugates of monohydroxylated *O,N*-didesmethyl brasofensine (Table 5).

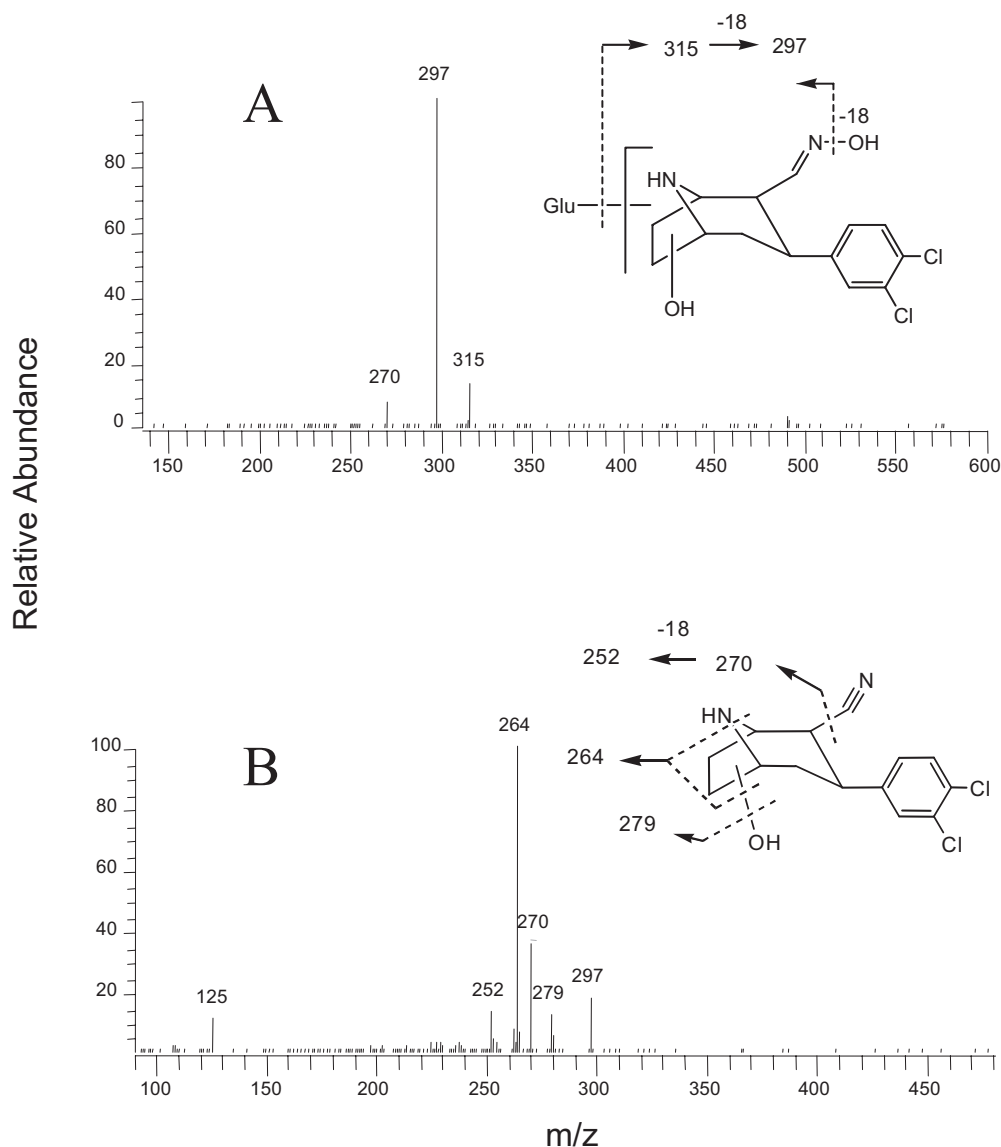


FIG. 12. Mass spectra and proposed structure of M15. A, product ion spectrum of M15 ($M + H^+ = 491$). B, MS³ spectrum ($491 \rightarrow 297$) of M15.

Discussion

From the BDC rats dosed with [¹⁴C]brasofensine, 64% of the administered radioactivity was recovered in bile, indicating that at least this amount plus that recovered in the urine was absorbed from the gastrointestinal tract in rats (i.e., at least 85%). Although similar data are not available from the monkey, mean recovery of total radioactivity in urine after p.o. administration of [¹⁴C]brasofensine was comparable with that after i.v. administration, suggesting almost complete absorption of brasofensine. Also, at least 86% of the dose was absorbed in humans because this percent of dose was recovered in urine following p.o. administration of brasofensine. Despite this high percentage of administered dose being absorbed, the absolute bioavailability of brasofensine was 7% in the rat and 0.8% in the monkey. Also, the AUC ratio of unchanged drug to total radioactivity was highest in humans, with a value of 15%. This suggests a significant portion of the p.o. administered dose is subjected to first-pass metabolism.

Absorption of brasofensine in rat and monkey after p.o. administration was rapid compared with that in humans (T_{max} of 1 versus 7 h). Plasma levels of brasofensine and BMS-205912 in the rats decreased more quickly than in the monkeys and in a comparable fashion following both i.v. and p.o. administration. Plasma concentrations of

brasofensine and BMS-205912 in the monkey decreased more quickly following p.o. administration compared with i.v. administration, probably reflecting the degree of first-pass metabolism. The plasma levels of BMS-205912 following brasofensine administration were one to two orders of magnitude lower than parent drug. However, in humans, there were significant concentrations of BMS-205912 following p.o. brasofensine administration yielding a 1:1 AUC ratio.

Brasofensine appears to be extensively distributed into tissues, as indicated by its high volume of distribution in rats (24 l/kg) and monkeys (46 l/kg), compared with that of total body water of 0.7 l/kg in these species (Davies and Morris, 1993). CL_T of brasofensine was approximately 7-fold higher than the liver plasma flow of 30 ml/min/kg in the rat and 120% of the liver plasma flow of 26 ml/min/kg in the monkey (Davies and Morris, 1993), probably reflecting the high degree of extraction into the liver and subsequent metabolism.

After single 4-mg/kg p.o. and 1.5-mg/kg i.v. doses of [¹⁴C]brasofensine to male LE rats, radioactivity was distributed throughout the body, and there was no evidence that radioactivity tended to accumulate in any tissue. The increase in the concentration of radioactivity in the intestinal tract up to 6 h after i.v. administration indicated the biliary secretion of the drug. After p.o. administration, the concentra-

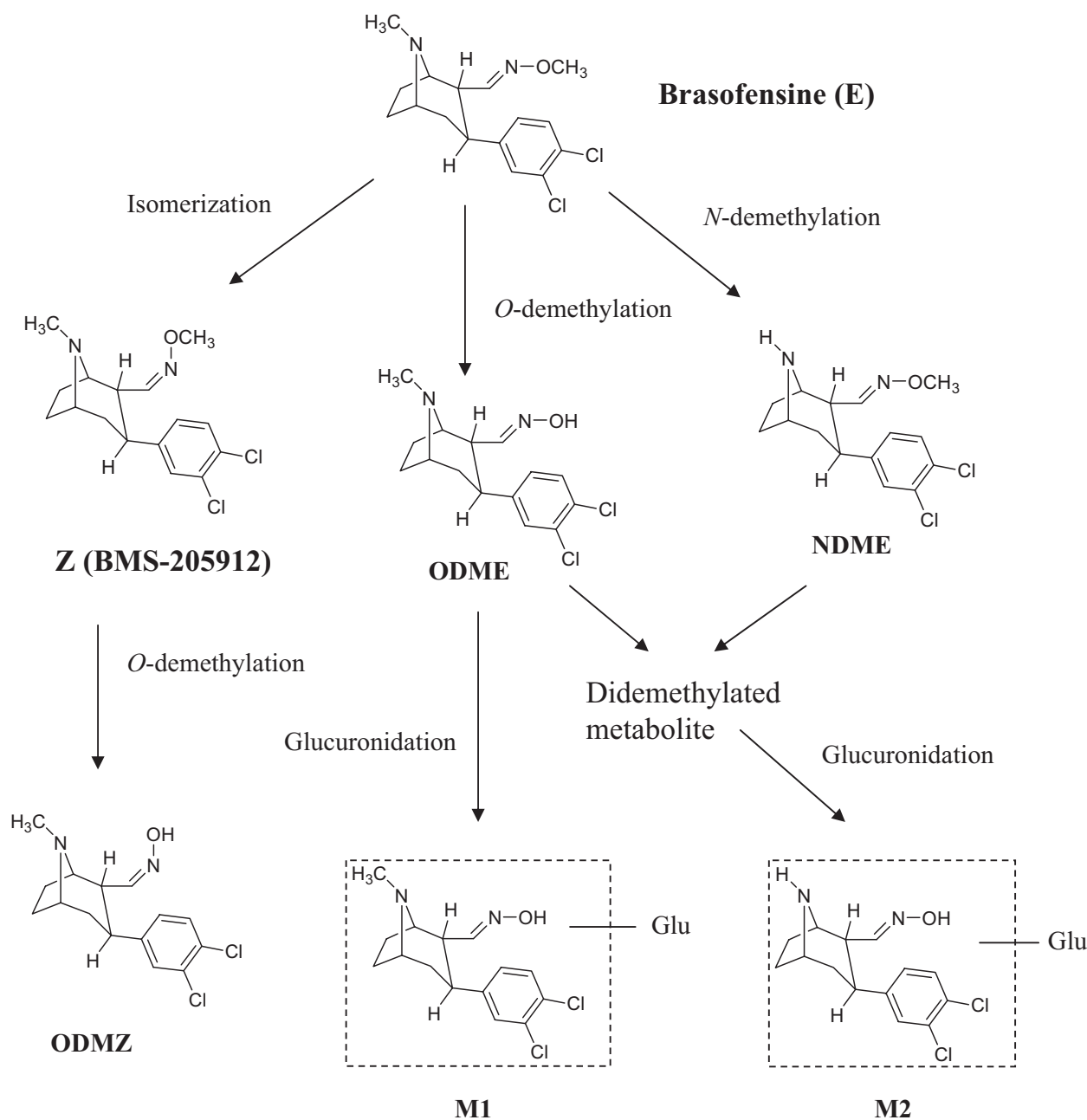


FIG. 13. Proposed primary biotransformation pathways of brasofensine in humans, monkeys, and rats.

tions in the intestinal tract probably represented any unabsorbed drug and the drug secreted in bile. After p.o. and i.v. doses to LE rats, the maximum concentrations of total radioactivity were generally observed at the 1- or 3-h time for all the tissues, with the exception of the large and small intestines. Concentrations of total radioactivity were detectable in all the tissues examined after p.o. and i.v. doses at 168 h after dosing, indicating extensive distribution of [^{14}C]brasofensine, its metabolites, or both into most tissues. Radioactivity was observed in brain, suggesting that brasofensine and/or its metabolites cross the blood-brain barrier to reach its proposed site of action.

In human plasma, brasofensine was the most abundant drug-related component, accounting for 45% of the total plasma radioactivity (Table 4). The metabolites observed in human plasma were ODME, ODMZ, M1, M2, Z, and NDME (Fig. 6), which accounted for the rest of the human plasma radioactivity (55%). These human plasma me-

tabolites were also detected in both rat and monkey plasma, although their relative abundance was different across species. Urinary excretion was the major elimination pathways of brasofensine in humans and monkeys, accounting for 86.4 and 64.7% of a p.o. dose, respectively (Table 1). Brasofensine and its Z-isomer were not detected in human (data not shown) and monkey urine (Fig. 7) by HPLC/radioflow detection, suggesting metabolism played a dominant role in drug clearance in both humans and monkeys. Metabolite profiling of rat urine (21.2% of a p.o. dose) showed the major drug-related components in rats were metabolites rather than the parent drug and Z-isomer (Fig. 6A), indicating that metabolism might significantly contribute to brasofensine clearance in rats.

The metabolite profiles in monkey and rat urine (Fig. 7) suggest that brasofensine underwent three primary biotransformation pathways in monkeys and rats (Fig. 13): 1) demethylation to ODME from

brasofensine and to ODMZ from Z-isomer; 2) *N*-demethylation of brasofensine to NDME; and 3) isomerization of the drug to form Z-isomer (BMS-205912). ODME, NDME, and the didemethylated product further formed glucuronides. Although metabolite profile in human urine was not determined in the present study, human plasma metabolite profile (Fig. 6A) suggests the similar biotransformation reactions occurred in humans. Additionally, incubations of radiolabeled brasofensine (50 μ M) in liver microsomes from humans and liver S9 preparations from humans, rats, and monkeys in the presence of NADPH only generated two metabolites, ODME and NDME (data not shown), indicating that the *O*- and *N*-demethylation reactions of brasofensine were primary cytochrome P450-mediated metabolic pathways in these species. However, the biochemical mechanism of the brasofensine isomerization remains to be determined.

In summary, a number of differences were observed in the disposition of brasofensine in rats, monkeys, and humans. Brasofensine was rapidly absorbed after p.o. administration in rats and monkeys (\sim 1 h to reach C_{\max}), whereas it took \sim 7 h to reach C_{\max} following p.o. administration in humans. [14 C]Brasofensine shows extensive distribution into rat tissues. The levels of BMS-205912 following brasofensine administration are one to two orders of magnitude lower than parent compound in the rat and monkey but are comparable in the human, yielding a 1:1 AUC ratio. The terminal plasma half-life of brasofensine following p.o. administration is 2 to 4 h in rats and monkeys but approximately 24 h in humans. Biliary excretion was the predominant route of elimination in the rat, and urine was the predominant route of elimination in the monkey and in humans. Brasofensine is subjected to significant first-pass metabolism in all three species, which plays a significant role in drug clearance. In humans, monkeys, and rats, brasofensine primarily undergoes isomerization

and demethylation biotransformation. Some of the desmethyl metabolites are further converted to glucuronides.

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