Disposition and Metabolism of $[^{14}C]$Brasofensine in Rats, Monkeys, and Humans

Mingshe Zhu, Daisy B. Whigan, Shu Y. Chang, and Randy C. Dockens

Departments of Pharmaceutical Candidate Optimization (M.Z., D.B.W., S.Y.C.) and Clinical Discovery (R.C.D.), Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, New Jersey

Received April 4, 2007; accepted September 26, 2007

ABSTRACT:

Brasofensine (BMS-204756) is a novel 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 $[^{14}C]$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 190 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 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 $[^{14}C]$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 190 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.

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 $[^{14}C]$brasofensine (Fig. 1) as the maleate salt had a radiochemical purity of 97.6% and specific activity of 50 $\mu$Ci/mg free base. $[^{14}C]$Brasofensine, unlabeled brasofensine, unlabeled BMS-205912, and metabolite standards [O-desmethyl brasofensine (ODME), O-desmethyl BMS...

ABBREVIATIONS: brasofensine, BMS-204756, (+)-(E)-(1R,2R,3S)-3-(3,4-dichlorophenyl)-8-methyl-8-azabicyclo[3.2.1]octane-2-carbaldehyde O-methylxime; BMS-205912, (+)-(Z)-(1R,2R,3S)-3-(3,4-dichlorophenyl)-8-methyl-8-azabicyclo[3.2.1]octane-2-carbaldehyde O-methylxime; BMS-180448, (3S-trans)-N-(4-chlorophenyl)-N'-cyano-N'-[6-cyano-3,4-dihydro-3-hydroxy-2,2-dimethyl-2H-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]-1H-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$_{0\text{inf}}$, 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).
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 [14C]brasofensine. The rats were fasted overnight and for 4 h after dosing, chaired for 12 h after 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 [14C]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 ± 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 (K3EDTA) and placed in chipped 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 butyl ether) and shaken for 40 min. 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 × 100-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 [M+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 electro spray LC/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 [M+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 internal standard for human plasma) and urine) were analyzed using an electrospray ionization source.
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, liver, lung, muscle, plasma, spleen, and testes.

![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.](image-url)
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 NH₄OH) 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%...
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 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 ([H]9252-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-[H]9262-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).
DISPOSITION OF BRASOFENSINE

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 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 AUCINF 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...
a terminal plasma half-life of 1.7 to 3.5 h. MRT was 2.1 to 2.5 h. CLr of brasofensine in rats was 199 ml/min/kg, and Vdso was 24 l/kg.

In the monkey, mean brasofensine AUCINF values were 507 ng \cdot h/ml after i.v. dosing (12 mg), with a mean absolute bioavailability of 0.8%. The corresponding exposure to BMS-205912 was approximately 20 ng \cdot h/ml. The decrease in plasma concentrations yielded a mean terminal plasma half-life of 3.9 h. MRT was 5.4 h. Mean CLr of brasofensine in monkeys was 32 ml/min/kg, and mean Vdso was 46 l/kg.

In humans, mean AUCINF values were 39.0 and 35.5 ng \cdot h/ml for brasofensine and BMS-205912, respectively, after p.o. dosing (50 mg) of brasofensine. 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 CLr of brasofensine in monkeys was 32 ml/min/kg, and mean Vdso was 46 l/kg.

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 (Z; BMS-205912), the ODMZ metabolite, the ODMEZ metabolite, and the ODME 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 ODMZ (20%), ODMEZ (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 ODMZ, 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 [14C]brasofensine in rat urine is shown in Fig. 7A, which was similar to that in rat plasma. M1, M2, ODMZ, and ODMEZ were observed as major urinary metabolites. The parent drug and its Z-isomer were not observed in rat urine. The metabolite profile...
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, MS^n 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. MS^3 (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). Most likely, the glucuronic acid was attached to the oxime oxygen (Fig. 9A). M2 had a protonated molecule at m/z 475 (Fig. 10B). 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
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. MS3 spectrum of M2 (475 → 299) 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). MS2 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 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). MS2 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 demethylation (−14). MS3 spectra of (505 → 329) M13 and M17 were identical (Fig. 11B; Table 5). The observed loss of the OCH3 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 O,N-didesmethyl brasofensine (Table 5).
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 (Tmax 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). CLT 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-
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 [14C]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 metabolites 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 μM) 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 (1 h to reach Cmax), whereas it took ~7 h to reach Cmax following p.o. administration in humans. [14C]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.

Acknowledgments. We thank the personnel of the Technical Support Unit of the Department of Metabolism and Pharmacokinetics for assisting in the conduct of the monkey study; Covance (Madison, WI) for the conduct of the rat studies; Pharma BioResearch (The Netherlands) for the conduct of the human study; Xiaohui Jiang for her analytical work; James Mitroka for his support in drug metabolism studies; and the reviewers for their insightful comments.

References


Address correspondence to: Randy C. Dockens, Bristol-Myers Squibb, Route 206 & Province Line Road, Princeton, NJ 08543-4000. E-mail: randy.dockens@bms.com