Bicifadine: A New Non-Narcotic Analgesic in Humans

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ABSTRACT:
Bicifadine [DOV 220,075; (±)-1-(4-methylphenyl)-3-azabicyclo[3.1.0]hexane HCl] is a non-narcotic analgesic that has proven to be effective for the treatment of acute pain in clinical studies. The pharmacokinetics, disposition, and metabolism of bicifadine were determined in eight healthy adult male subjects following a single oral dose of 200 mg of [14C]bicifadine in solution. The maximum concentration of total drug equivalents and bicifadine in plasma was at approximately 1 h; the elimination half-life was 2.6 and 1.6 h for radioactivity and bicifadine, respectively. Unchanged bicifadine represented 15% of the area under the concentration-time curve for total drug equivalents; the rest was due mainly to the lactam (M12), the acid (M3), and the lactam acid (M9). Total recovery of the dose was 92%, with most of the radioactivity recovered in the urine in the first 24 h; fecal excretion accounted for only 3.5% of the dose. Approximately 64% of the dose was metabolized to M9 and its acyl glucuronide; another 23% was recovered as M3 and its acyl glucuronide. Neither bicifadine nor M12 were detected in urine or feces. There were no reported serious or severe adverse events during the study.

Chronic peripheral neuropathic pain is difficult to treat, and side effects may limit the usefulness of a drug. Although opiates have been used for neuropathic pain management, tolerance develops to their analgesic efficacy, and they cause drowsiness and constipation. Empirical observations have demonstrated the efficacy of tricyclic antidepressants in treating peripheral neuropathy (Sindrup et al., 2005). Research has shown that this effect is produced by inhibition of the neuronal reuptake of NE and 5-HT and blocks the postsynaptic α-adrenergic, H1-histaminergic, and muscarinic cholinergic receptors. Duloxetine is a selective 5-HT and NE reuptake inhibitor that has been approved for treatment of diabetic neuropathy. Bupropion is a weak blocker of NE and DA, but not 5-HT, reuptake (Ascher et al., 1995) but was effective in reducing neuropathic pain in patients with human immunodeficiency virus (Semenchuck et al., 2001). The involvement of DA in analgesic mechanisms suggests that a broadened range of neurotransmitters may result from the ability to enhance dopaminergic neurotransmission, in addition to activating serotonergic and noradrenergic pathways. This, agents that simultaneously inhibit DA, NE, and 5-HT transporters may possess a unique analgesic profile.

Bicifadine (Fig. 1) is being developed for the treatment of neuropathic pain. It inhibits NE (IC50 55 nM) and 5-HT (117 nM) reuptake, with lesser potency in blocking DA reuptake (910 nM), as determined using recombinant human transporter systems (Basile et al., 2007). In vivo microdialysis studies in the brains of rats indicate that bicifadine increases the extracellular levels of all three neurotransmitters following oral administration of analgesic doses (Basile et al., 2007). Bicifadine was an effective antinociceptive agent in both the early (acute) and late (tonic) phases of paw-licking in the formalin test using rats and mice, unlike duloxetine or COX inhibitors, which are active only on the late stage (Iyengar et al., 2004). It is not an inhibitor of either COX-1 or COX-2 (Basile et al., 2007) and does not induce dependence in either rodents or primates after as much as 48 days of administration (A. Basile and J. Tizzano, unpublished observations). Clinically, it has been shown to be effective in the treatment of acute dental (Stern et al., 2005) and bunionectomy pain (Riff et al., 2006); studies in humans with diabetic neuropathy are ongoing.

A human metabolism study using [14C]bicifadine was conducted to support its clinical trials and registration. In addition to assaying the plasma concentrations of total drug equivalents and unchanged bicifadine, the mass balance of drug-derived radioactivity in the urine and feces was determined. Metabolites in the plasma, urine, and feces were identified and compared with those formed in vitro (Erickson et al., 2007) and in the species used for toxicology studies (Musick et al., 2008).

Materials and Methods
Radiolabeled Bicifadine and Dosage Form. [14C]Bicifadine was synthesized by ViTrax (Placentia, CA) and supplied as a bulk powder with a specific activity of 95 μCi/mg. The radiolabeled compound was incorporated into a 200 mg tablet formulation. 252
activity of 0.52 µCi/mg. The radiochemical purity was 99.9% as determined by HPLC. The compound was formulated by Covance Clinical Research on the morning of dosing. [14C]Bicifadine powder was dissolved in sterile water for irrigation to produce a solution with a concentration of 91.9 mg/g. The appropriate volume was divided into three 00 capsules, which were then double-encapsulated in 000 capsules. The capsules were administered within 20 min of preparation. The mean dose and amount of radioactivity administered was 201 mg (range, 201–202) and 105 Ci (range, 104–105 µCi), respectively.

Subjects and Dose Administration. The study was reviewed and approved by the Covance Clinical Research Unit Institutional Review Board. Informed consent was obtained from each volunteer prior to study initiation. The study was conducted in accordance with Good Clinical Practice and the Declaration of Helsinki.

Eight healthy male subjects (seven white and one African-American) participated in the study. The subjects had a mean age of 26.4 years (range, 19 – 43 years), a mean weight of 79.4 kg (range, 61.3–93.2 kg), a mean body mass index of 25.5 kg/m² (range, 20.2–28.6 kg/m²), and met all of the eligibility criteria. Subjects were in good health based on their medical history, a physical examination, a 12-lead electrocardiogram, and clinical laboratory test results. Subjects were in good health based on their medical history, a physical examination, a 12-lead electrocardiogram, and clinical laboratory test results. Eight healthy adult male subjects (n = 8) ingested the [14C]bicifadine in three capsules with 240 ml of water. They were fed a standard lunch approximately 4 h after dosing. The subjects were confined to the study site until at least 168 h postdose, at least 90% of the administered radioactivity was recovered, or until their excreta contained nondetectable amounts of radioactivity for at least 48 h. Samples were collected for 168 h for five subjects and 192 h for the other three subjects.

Sample Collection. Blood samples (10 ml) were collected into chilled, heparinized tubes at 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 48, 72, 96, 120, 144, 168, and 192 h postdose. Additional blood samples were collected at 0.5, 2, 4, and 6 h postdose for plasma metabolite profiling and identification. Before centrifugation, a 2-ml sample was saved for analysis of radioactivity in whole blood. The rest was centrifuged within 45 min of collection in a refrigerated centrifuge, and the plasma was divided into two tubes. One sample was used for the determination of the plasma radioactivity concentration; the other was used for analysis of concentrations of bicifadine and the lactam metabolite M12 (DOV 255,828). Plasma and blood samples were stored at −70°C.

Urine was collected predose and at intervals of 0 to 4 h, 4 to 8 h, 8 to 24 h, and at 24-h intervals thereafter up to 192 h postdose. Samples were refrigerated until the end of the block collection periods when the total volume of each urine collection was recorded. Samples were stored at −70°C. All bowel movements and fecal samples were collected before administration of [14C]bicifadine and at 24-h intervals up to 192 h postdose. The fecal samples were refrigerated until assay for total radioactivity; the wipes were not assayed. After homogenization and sampling, the remainder of the sample was stored at approximately −20°C until extracted for metabolite profiling.

Radioactivity Assay. All samples were assayed on a daily basis for total radioactivity by liquid scintillation counting using a Packard model 2900TR Liquid Scintillation Counter (PerkinElmer Life and Analytical Sciences, Boston, MA). Plasma and urine samples (0.2 ml) were added to Ultima Gold XR scintillation cocktail and counted. Blood and fecal homogenates (0.2 g) were oxidized in a Packard model 307 Sample Oxidizer; the resulting 14CO2 was trapped in a mixture of Perma Fluor and Carbo-Sorb (PerkinElmer Life and Analytical Sciences, Boston, MA). Plasma and urine samples (0.2 ml) were added to Ultima Gold XR scintillation cocktail and counted. Blood and fecal homogenates (0.2 g) were oxidized in a Packard model 307 Sample Oxidizer; the resulting 14CO2 was trapped in a mixture of Perma Fluor and Carbo-Sorb (PerkinElmer Life and Analytical Sciences, Boston, MA).

Mean pharmacokinetics of radioactivity, bicifadine, and M12 in male subjects (n = 8) administered a single oral dose of [14C]bicifadine in solution

<table>
<thead>
<tr>
<th>Parameter (Units)</th>
<th>Plasma 14C</th>
<th>Blood 14C</th>
<th>Bicifadine</th>
<th>M12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax (ng/ml)*</td>
<td>5154 ± 683</td>
<td>2716 ± 392</td>
<td>1780 ± 695</td>
<td>1205 ± 362</td>
</tr>
<tr>
<td>T1/2 (h)</td>
<td>1.19 ± 0.26</td>
<td>1.25 ± 0.38</td>
<td>1.06 ± 0.42</td>
<td>1.50 ± 0.46</td>
</tr>
<tr>
<td>AUC0-1822 (ng · h/ml)*</td>
<td>21,413 ± 3311</td>
<td>11,577 ± 1822</td>
<td>3373 ± 1851</td>
<td>23.7 ± 13.5</td>
</tr>
<tr>
<td>AUC0-1822 (ng · h/ml)</td>
<td>N.C.</td>
<td>N.C.</td>
<td>3427 ± 1934</td>
<td>1851 4641 ± 1803</td>
</tr>
<tr>
<td>t (h)</td>
<td>2.57 ± 0.28</td>
<td>2.62 ± 0.17</td>
<td>1.63 ± 0.56</td>
<td>1.83 ± 0.42</td>
</tr>
<tr>
<td>tf (h)</td>
<td>12.0 ± 0.0</td>
<td>12.0 ± 0.0</td>
<td>10.0 ± 2.1</td>
<td>13.5 ± 4.2</td>
</tr>
<tr>
<td>CL/F (l/h)</td>
<td>N.C.</td>
<td>N.C.</td>
<td>59.1 ± 23.7</td>
<td>N.C.</td>
</tr>
<tr>
<td>Vz/F (l)</td>
<td>N.C.</td>
<td>N.C.</td>
<td>125 ± 31.5</td>
<td>N.C.</td>
</tr>
</tbody>
</table>

N.C., not calculated; tf, time of last measurable concentration.

*Units for Cmax and AUC0-1822 of radioactivity are ng Eq/g and ng Eq · h/g.
Samples with less than 2 × the background dpm were recorded as zero.

**Bioanalytical Assay.** The analysis of plasma samples for bicifadine and M12 (DOV 255,828) were performed using a validated liquid chromatography (LC)/tandem mass spectrometry assay as detailed in the companion article on page 241 of this issue (Musick et al., 2008). The only changes were that 0.5 ml of plasma was assayed and a Symbiosis Pharma System (Spark Holland Inc., Plainsboro, NJ) was used. The latter consisted of a Symbiosis Pharma LC system, Reliance Autosampler, and an Applied Biosystems API 4000 tandem quadrupole mass spectrometer with a heated nebulizer probe (Applied Biosystems, Foster City, CA).

**Pharmacokinetic Analysis.** Blood and plasma concentrations of radioactivity, bicifadine, and M12 above their respective lower limit of quantitation were used for pharmacokinetic analyses using noncompartmental methods (Gibaldi and Perrier, 1982). WinNonlin, version 4.1 (Pharsight, Mountain View, CA), was used for the calculations. The values of $C_{\text{max}}$ and $T_{\text{max}}$ were the observed values for each subject. AUC(0–t) was determined using the linear trapezoidal rule whenever the concentration data were increasing and the logarithmic trapezoidal rule any time the concentration data were decreasing. The apparent $\lambda_r$ was calculated as the negative slope of the log-linear terminal portion of the blood concentration-time curve using linear regression. A minimum of three observations was used to calculate $\lambda_r$. The $t_{1/2}$ was calculated as in $2/\lambda_r$. AUC(0–t) was extrapolated to infinity (AUC$_{\text{inf}}$) as AUC$_{\text{0–t}} = \text{AUC}_{\text{0–t}} + C_{\text{t last}}/\lambda_r$, where $C_{\text{t last}}$ was the last quantifiable concentration. CL/F of bicifadine was calculated as Dose/AUC$_{\text{0–t}}$. $V_z/F$ was calculated as Dose/(\lambda_r \times \text{AUC}_{\text{0–t}}). Variation around the mean is expressed as the standard deviation.

**Metabolite Profiling and Identification.** Plasma samples (approximately 0.6–1.3 ml) collected from each subject at 0.5, 2, 4, and 6 h postdose were
combined to provide a single pooled plasma sample at each time point. Each pool was extracted twice with 3 volumes of acetonitrile and centrifuged. Extraction recoveries ranged from 82.6 to 131%. Each supernatant was evaporated to dryness under a stream of nitrogen and reconstituted in water/acetonitrile (v/v 1:1). The supernatant was dried again and reconstituted in water. Urine samples collected from 0 to 24 h postdose were pooled such that approximately 0.2% of each urine sample was included to prepare a single 0- to 24-h pooled urine sample for each subject. A percentage of each individual’s pooled urine was combined for a single overall pooled urine sample for metabolite profiling. After clarification by centrifugation through a centrifugal filtration device, recovery of radioactivity was 102%. Approximately 3% of each fecal homogenate was included in the pools for each individual such that they represented greater than 80% of the amount excreted into feces. Due to differences in the rate of fecal excretion, pooled samples from individuals were profiled separately. Samples were extracted twice with 3 volumes of acetonitrile and centrifuged. The extracts were combined and analyzed by liquid scintillation chromatography; extraction efficiencies were 74 to 83%. Each supernatant was evaporated to dryness under a stream of nitrogen and reconstituted in water/acetonitrile (v/v 1:1). For all samples, extraction and recovery efficiencies were determined by liquid scintillation counting, and corrections were made to subsequent data.

**HPLC and Radiometric Detection.** The HPLC system used for profiling of metabolites consisted of an HP 1100 series pump, autoinjector, column heater, and ultraviolet detector set to 254 nm (Hewlett Packard, Palo Alto, CA). The column was a Zorbax SB-Phenyl column (250 × 4.6 mm, 5-μm particle size; Agilent Technologies, Santa Clara, CA) with a Phenomenex propyphenyl guard column (4 × 3 mm; Phenomenex, Torrance, CA). The initial mobile phase was 95% 0.05% formic acid in reverse osmosis water/5% acetonitrile. The percentage of acetonitrile increased linearly to 25% over 45 min and then increased linearly to 95% over the next 5 min, where it was held for 6 min. The column was re-equilibrated to the initial conditions over the next 11 min. The flow rate was 1.0 ml/min. Column fractions were collected every 10 s for 62 min using an ISCO Foxy 200 fraction collector (Cole-Parmer Instrument Co., Vernon Hills, IL). The fractions were analyzed using a TopCount Microplate Scintillation and Luminescence counter (Packard Instrument). To determine the percentage of the total dose in each radioactive metabolite from the pooled urine and fecal extracts, the percentage of radioactivity that eluted in each peak was multiplied by the percentage of administered radioactivity excreted in the pooled sample and corrected for extraction and reconstitution recoveries.

Full-scan LC/MS analyses were conducted using an Applied Biosystems 4000 Q Trap with a turbospray source. The mass spectrometer software was Analyst 1.4.1 from Applied Biosystems. The HPLC system was a Shimadzu model SIL-HTC autoinjector and system controller and Shimadzu model LC-10AD VP pumps (Shimadzu, Columbia, MD). A Radiomatic Series 500 with Flo-One software, version 3.65 (PerkinElmer Life and Analytical Sciences), was used for radiochemical detection. The HPLC column, solvents, and gradient were the same as used for metabolite profiling. After passing through the column switcher, the column effluent was split with approximately 25% diverted to the mass spectrometer and 75% to the radiometric detector. To minimize contamination of the mass spectrometer source, the first 4.5 min of each run were diverted to waste. Samples were scanned in the positive-ion full-scan mode from 80 to 700 amu with a Q1 scan time of 0.8 s for 52 min. The ion spray voltage was 5000 V, the source temperature was 500°C, and the exit potential was 10 V. Nitrogen was used as the curtain, nebulizer (GS1), and turbo (GS2) gas. Product-ion analyses used the same instrumentation and conditions as LC/MS with the following exceptions: enhanced product ion was used for the scan function; the ionization mode was positive turbo spray; nitrogen was used as the collision gas; and the collision energy, mass range, and scan time were variable. The contribution of the 14C-tracer to the mass of the metabolites was low enough (∼0.15%) so that it did not contribute to the m/z ratios as determined by mass spectrometry. For consistency across studies, the metabolite numbering system is the same as that used in the in vitro and nonclinical metabolism studies (Erickson et al., 2007; Musick et al., 2008).

The high-resolution mass spectrum of the ion at the nominal m/z 135 in M9 was obtained using a Thermo LTQ/Orbitrap Fourier transform mass spectrometer (Thermo Fisher Scientific Inc., Waltham, MA). A solution of M9 was infused using an electrospray ionization source. The product ion spectrum was obtained at a resolution of 60,000 and using a lock mass of 218.08117 amu (protonated molecular ion of M9).

### Results

**Pharmacokinetics of Radioactivity, Bicifadine, and M12.** The mean concentration of radioactivity in blood and plasma concentrations of radioactivity, bicifadine, and M12 (DOV 255,828) following a single oral dose of [14C]bicifadine in solution are presented in Fig. 2. The C<sub>max</sub> of radioactivity in plasma was 5154 ng Eq · h/g at 1.19 h (Table 1). Its t<sub>1/2</sub> was approximately 2.6 h; the last measurable concentration of plasma radioactivity was at 12.0 h postdose. C<sub>max</sub> and AUC<sub>0-t</sub> values of radioactivity in blood were 53 to 54% of the values in plasma, indicating that bicifadine and its metabolites did not concentrate in red blood cells. The subjects’ mean hematocrit of 44% is in agreement with these results.

The C<sub>max</sub> of bicifadine, 1780 ng/ml, was observed at 1.06 h postdose (Table 1). Its AUC<sub>0-t</sub> was 3373 ng · h/ml, approximately 15% of the total drug equivalents in plasma. Both the t<sub>1/2</sub> and the time to the last measurable concentration of plasma bicifadine were slightly shorter than for plasma radioactivity. CL/F was 59.1 l/h, and V<sub>F</sub>/F was 125 l; based on the weight of the subjects, CL/F was 0.74 ± 0.28 l/h/kg, and V<sub>F</sub>/F was 1.58 ± 0.32 l/kg. The lactam metabolite, M12, had a C<sub>max</sub> that was slightly lower than that of bicifadine and a T<sub>max</sub> that occurred later. Although exposure to M12 was approximately 50% higher compared with that of bicifadine, their t<sub>1/2</sub> values were similar.

**Excretion of Radioactivity.** Almost the entire orally ingested radioactivity was excreted into urine (Fig. 3). By 24 h postdose, 87.3 ± 5.3% of the dose was recovered in the urine; another 1.5% was excreted over the next 72 h. Only 3.52% of the dose was excreted into the feces over the collection period. Overall recovery of radioactivity was 95.3 ± 2.8%.

### Table 2

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Hour Postdose</th>
<th>Retention Time (min)</th>
<th>Percentage of Dose in Urine (%)</th>
<th>Mean Percentage of Dose in Feces (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bicifadine</td>
<td>0.5‡</td>
<td>12.8</td>
<td>5.11</td>
<td>0.85</td>
</tr>
<tr>
<td>M3</td>
<td>2</td>
<td>148</td>
<td>37.7</td>
<td>—</td>
</tr>
<tr>
<td>M9</td>
<td>4</td>
<td>464</td>
<td>614</td>
<td>342</td>
</tr>
<tr>
<td>M12</td>
<td>6</td>
<td>922</td>
<td>914</td>
<td>496</td>
</tr>
<tr>
<td>M12F</td>
<td>8</td>
<td>1210</td>
<td>537</td>
<td>186</td>
</tr>
</tbody>
</table>

—, metabolite not detected at this time period.

### Table 3

The concentrations of radioactivity in plasma followed a single oral dose of [14C]bicifadine. The mean values are shown in Table 3. The percentage of dose excreted in each peak was multiplied by the percentage of administered radioactivity in plasma and corrected for extraction and reconstitution recoveries.

<table>
<thead>
<tr>
<th>Proposed Identification</th>
<th>Retention Time (min)</th>
<th>Percentage of Dose in Urine (%)</th>
<th>Mean Percentage of Dose in Feces (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M3</td>
<td>12.8</td>
<td>5.11</td>
<td>0.85</td>
</tr>
<tr>
<td>M14A-F</td>
<td>4.7–8.0</td>
<td>17.3</td>
<td>—</td>
</tr>
<tr>
<td>M9</td>
<td>34.2</td>
<td>55.6</td>
<td>1.34</td>
</tr>
<tr>
<td>M31B-F</td>
<td>18.8–23.3</td>
<td>7.33</td>
<td>—</td>
</tr>
<tr>
<td>M32</td>
<td>31.7</td>
<td>0.57</td>
<td>—</td>
</tr>
<tr>
<td>M33</td>
<td>46.0</td>
<td>0.11</td>
<td>—</td>
</tr>
<tr>
<td>M34</td>
<td>49.7</td>
<td>1.75</td>
<td>—</td>
</tr>
<tr>
<td>Unknown</td>
<td>50.6</td>
<td>—</td>
<td>0.23</td>
</tr>
</tbody>
</table>

—, metabolite not detected at this time period.

“‡” Percentage of dose in urine determined from radiochemical profile of pooled urine from all eight subjects.
FIG. 5. HPLC chromatogram of radioactivity in pooled urine (0–24 h) (top) and feces (subject 6, 24–120 h) (bottom) from male subjects following a single oral dose of 200 mg of [14C]bicifadine. The urine was a pooled sample from all eight subjects. Feces from individual subjects were profiled due to differing times for excretion of radioactivity.

FIG. 6. Proposed metabolic scheme of bicifadine in adult male subjects.
was 92.3 ± 5.2% (range, 88.1–104%). Two subjects had recoveries that were slightly below 90% (88.1 and 89.4%); neither had detectable amounts of radioactivity in their feces and urine from 96 to 192 h postdose.

**Metabolite Profiling. Plasma.** The pooled plasma contained four main radioactive peaks (Fig. 4). The highest concentration of bicifadine occurred at 0.5 h and then declined to nondetectable levels by 6 h postdose (Table 2). The main metabolites identified were the acid M3, the lactam acid M9, and the lactam M12. With the exception of M3 at 0.5 h, all of the metabolites had concentrations that were higher than bicifadine. The highest concentration of the metabolites was at 2 or 4 h and declined by 6 h. These data are consistent with the results using the validated assay that indicated that most of the plasma radioactivity was due to metabolites rather than bicifadine. A number of smaller radioactive peaks were detected in the chromatograms with concentrations that were too low to obtain reliable mass spectra. All had concentrations that were ≤31 ng Eq/g plasma with the exception of a peak at an Rt of 51.5 min, which had a concentration of 63.5 ng Eq/g plasma at 0.5 h.

**Urine.** The major urinary metabolite was the lactam acid M9; it constituted 55.6% of the administered radioactivity (Table 3; Fig. 5). A series of smaller peaks that corresponded to the acyl glucuronide of M9 and its rearrangement products eluted with Rt values ranging from 18.8 to 23.2 min. These peaks represented 7.3% of the dose and, together with M9, accounted for 62.9% of the dose. The other carboxylic acid-containing metabolite, M3, and its acyl glucuronide(s), M14A-F, were also detected in urine. Combined, M3 and its conjugate accounted for another 22.4% of the dose. A smaller unidentified peak, M32, eluted at an Rt of 31.7 min, but it represented only 0.57% of the dose. Unchanged bicifadine and the lactam M12 were not detectable in the pooled urine.

**Feces.** M3 and M9 represented most of the radioactivity in feces (0.85 and 1.34% of the dose, respectively) (Table 3; Fig. 5). Two other unidentified metabolites, with Rt values of 46.0 and 50.6 min, accounted for <0.4% of the dose.

**Metabolite Structure Identification.** The proposed metabolic scheme for bicifadine in humans is displayed in Fig. 6.

### Bicifadine, M9, and M12.

The product-ion mass spectra of bicifadine, M9, and M12 in the pooled plasma collected 4 h after the oral dose of [14C]bicifadine were essentially identical to the mass spectra of the corresponding standards (Table 4). The HPLC retention times of the plasma peaks were also the same as the standards.

The high-resolution mass spectrum of the ion at m/z 135 in the product-ion mass spectrum of M9 was also determined. The fragment had an observed amu of 135.04405; the calculated amu for C2H5O+ is 135.04406. The difference between the calculated and observed amu was 0.07 ppm. This empirical formula corresponds to an ion with the structure H2O-C-phenyl-CH2-. Two other possible formulas with a nominal amu of 135 were C6H7O2- and C8H5NO3-, but their calculated molecular weights were 269 and 176 ppm higher, respectively, than the observed. A similar fragmentation mechanism was observed for bicifadine (m/z 105), M3 (m/z 135) (Fig. 7), and M2 (m/z 121) (Musick et al., 2008).

**M3 (Bicifadine Carboxylic Acid).** The full-scan LC/MS mass spectrum of M3 had an [M+H]+ at m/z 204, 30 amu (+2 oxygen = 2 hydrogen) higher than bicifadine (Fig. 7). The presence of ions at m/z 135 and 163 in the product-ion mass spectrum of M3 indicated that the methyl group had been oxidized to a carboxylic acid.

**M14A-F (M3 Acyl Glucuronides).** A series of peaks eluted between 5.24 and 8.42 min in the extract of the 0- to 24-h pooled urine sample. The [M+H]+ of all peaks was at m/z 204, 176 amu (glucuronic acid) higher than M3. The ion at m/z 204 was due to the loss of an empirical formula corresponding to an ion with a nominal amu of 135 and 163 in the product-ion mass spectrum of M3 indicated that the methyl group had been oxidized to a carboxylic acid.
the glucuronic acid moiety. It could not be determined which of the six peaks was the initial 1-O-β-acyl glucuronide.

**M31B-F (M9 Acyl Glucuronides).** The metabolites M31B-F (Rt, 18.8–23.2 min) all had an [M + H]+ at 394 amu, 176 amu (glucuronic acid) higher than that of M9 (Fig. 7). They all had product ions at m/z 218 (−glucuronic acid), 200 (m/z 218 − water), and 171, which were also detected in the spectrum of M9.

**M32, M33, and M34.** The urine and feces contained three minor metabolites for which mass spectra could be obtained, but possible structures were not identified. M32 had an [M + H]+ at 366, 192 amu higher than that of bicifadine. The ions at m/z 190 and 105 indicated the loss of glucuronic acid and that the methyl group had not been oxidized, respectively. M33 was detected in fecal samples and had an [M + H]+ of 232, 58 amu higher than that of bicifadine. Its enhanced product-ion spectrum was similar to M3. M34 (Rt, 49.7 min) had an [M + H]+ at m/z 174 and enhanced product-ion MS that was virtually identical to the bicifadine standard, but its Rt of 49.7 min indicated that it had a different structure. Two other metabolites, constituting <0.4% of the dose, with Rt values of approximately 30 and 50 min were detected in fecal extracts, but reliable mass spectra could not be obtained.

**Safety.** There were 28 adverse events that were either possibly or probably related to the study drug; they were all mild or moderate in severity. There were no clinically significant changes or abnormalities in the clinical laboratory evaluations, vital sign measurements, physical examinations, or 12-lead electrocardiograms in the study. Overall, the compound was well tolerated.

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**Discussion**

When [14C]bicifadine was administered as an oral solution, the Cmax of both bicifadine and total drug equivalents was approximately 1 h. The elimination half-life of unchanged parent drug was 1.6 h. The material used in clinical trials is a sustained release formulation with a significantly longer half-life (N. Huang, unpublished observations).

The oral clearance of bicifadine in this study was 59 l/h. The plasma flow of the human liver is approximately 48 l/h (Davies and Morris, 1993), slightly less than the oral clearance of bicifadine. Extrahepatic clearance of the compound is likely since the main route of metabolism is formation of the lactam metabolite by monoamine oxidase B (Erickson et al., 2007), which is widely distributed in the body (Cesura and Pletscher, 1992).

Exposure to unchanged bicifadine, based on values of AUC0-τ, accounted for approximately 15% of the total drug-derived radioactivity. The rest of the plasma radioactivity was due predominantly to M12 and the two carboxylic acids, M3 and M9. At 4 h postdose, these two acids were the predominant peaks in plasma. The concentration of M12 had declined by 4 h, whereas the concentration of bicifadine was minor. No acyl glucuronide conjugate of M3 was detected in human plasma as it was in mouse (Musick et al., 2008).

Bicifadine was well absorbed, with approximately 89% of the dose recovered in the urine. The small amount in the feces was composed only of metabolites. Neither unchanged bicifadine nor M12 were detected in urine or feces.

M9 and its glucuronide accounted for 64.3% of the radioactive dose in the excreta over the 192-h study period, whereas M3 and its
glucuronide represented another 23.3% of the dose. It was not determined which of the M14 peaks was the initial 1-O-acyl-beta-glucuronide, which is known to rearrange at neutral to slightly basic pH (Compernolle et al., 1978; Hasegawa et al., 1982; Janssen et al., 1982). Monkeys and humans produced the glucuronide conjugate of M9 (M31B-F), but rats and mice did not (Musick et al., 2008).

In conclusion, bicifadine was well absorbed by humans when administered as an oral solution. The $T_{\text{max}}$ of radioactivity and bicifadine was approximately 1 h. Most of the drug-derived radioactivity in plasma was due to metabolites, especially the lactam M12, the acid M3, and the lactam acid M9. Most of the radioactivity was recovered in urine in the first 24 h. M9 and its acyl glucuronide conjugate accounted for almost two-thirds of the dose; M3 and its glucuronide represented another 23% of the dose. No human-specific metabolites were identified, and the compound was well tolerated by the subjects with no reported serious or severe adverse events.

References


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