Excretion and Metabolism of Lersivirine (5-[[3,5-Diethyl-1-(2-hydroxyethyl)(3,5-14C2)-1H-pyrazol-4-yl]oxy]benzene-1,3-dicarbonitrile), a Next-Generation Non-Nucleoside Reverse Transcriptase Inhibitor, after Administration of [14C]Lersivirine to Healthy Volunteers

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ABSTRACT:

Lersivirine [UK-453,061, 5-((3,5-diethyl-1-(2-hydroxyethyl)(3,5-14C2)-1H-pyrazol-4-yl)oxy)benzene-1,3-dicarbonitrile] is a next-generation non-nucleoside reverse transcriptase inhibitor, with a unique binding interaction within the reverse transcriptase binding pocket. Lersivirine has shown antiviral activity and is well tolerated in HIV-infected and healthy subjects. This open-label, Phase I study investigated the absorption, metabolism, and excretion of a single oral 500-mg dose of [14C]lersivirine (parent drug) and characterized the plasma, fecal, and urinary radioactivity of lersivirine and its metabolites in four healthy male volunteers. Plasma Cmax for total radioactivity and unchanged lersivirine typically occurred between 0.5 and 3 h postdose. The majority of radioactivity was excreted in urine (~80%) with the remainder excreted in the feces (~20%). The blood/plasma ratio of total drug-derived radioactivity [area under the plasma concentration-time profile from time zero extrapolated to infinite time (AUCinf)] was 0.48, indicating that radioactive material was distributed predominantly into plasma. Lersivirine was extensively metabolized, primarily by UDP glucuronosyltransferase and cytochrome P450-dependent pathways, with 22 metabolites being identified in this study. Analysis of precipitated plasma revealed that the lersivirine-glucuronide conjugate was the major circulating component (45% of total radioactivity), whereas unchanged lersivirine represented 13% of total plasma radioactivity. In vitro studies showed that UGT2B7 and CYP3A4 are responsible for the majority of lersivirine metabolism in humans.
Lersivirine (formerly UK-453,061), an NNRTI with a unique binding interaction within the reverse transcriptase binding pocket (Phillips et al., 2007), is currently in clinical development for the treatment of HIV-1 infection. Lersivirine has shown potent in vitro activity against both wild-type and clinically relevant drug-resistant viruses (Mori et al., 2008). Furthermore, lersivirine has shown synergy in vitro with other classes of compounds, particularly those in the nucleoside reverse transcriptase inhibitor class. In vitro studies suggest that lersivirine has good membrane permeability (Allan et al., 2008) and is predominantly cleared by metabolism, via glucuronidation and cytochrome P450 (P450)-mediated oxidation (Vourvahis et al., 2009). Furthermore, the clinical safety, tolerability, and pharmacokinetics of lersivirine have been studied in both HIV-infected and healthy subjects (Davis et al., 2007; Fatkenheuer et al., 2009). In a 7-day monotherapy study, lersivirine was found to be well tolerated at all the doses used, achieving significant mean viral load reductions of $\geq 1.62 \log_{10}$ for dosing regimens of 500 mg once daily, 750 mg once daily, and 500 mg b.i.d. in HIV-infected patients (Fatkenheuer et al., 2009).

This open-label Phase I study investigated the absorption, distribution, metabolism, and excretion (ADME) of a single oral 500-mg dose of [$^{14}$C]lersivirine (parent drug) to characterize the plasma, fecal, and urinary radioactivity of lersivirine and its metabolites in healthy male subjects. In addition, in vitro data identifying the major drug metabolism enzymes responsible for the metabolism of lersivirine are described.

Materials and Methods

Chemicals and Reagents. [$^{14}$C]Lersivirine was synthesized by GE Healthcare (Little Chalfont, Buckinghamshire, UK) at a specific activity of 3.7

![MS/MS spectrum of lersivirine (A) and proposed fragmentation of lersivirine (B).](image1)

![The mean blood and plasma concentration-time profiles for drug-derived total radioactivity and lersivirine (parent drug) after a single oral administration of 500 mg of [$^{14}$C]lersivirine.](image2)
Kilobecquerels per milligram and radiochemical purity of 97.6% as assessed by high-performance liquid chromatography (HPLC) (Alliance 2695 Separations Module with 2487 Dual Absorbance Detector; Waters, Milford, MA). Authentic, nonradiolabeled lersivirine was supplied by Pfizer Global Research and Development; purity was 99.3% as assessed by HPLC. Authentic standards of known metabolites of lersivirine [PF-04580552 (M15), PF-03139905 (M17), and UK-533,713 (M19)] were synthesized at Pfizer Global Research and Development (Sandwich, UK). Aquasafe 500 Plus liquid scintillation fluid was obtained from Zinsser Analytic (Maidenhead, UK). Carbo-Sorb CO$_2$ absorbing solution and Permafluor E$_3$ scintillation fluid, used in conjunction with the Packard Tri-Carb 307 Automatic Sample Oxidizer, were supplied by PerkinElmer Life and Analytical Sciences (Waltham, MA). Spec-Check-14C, used to estimate combustion efficiency, was also from PerkinElmer Life and Analytical Sciences. Luna C18 (for radiopurity; Phenomenex, Torrance, CA) and HIRPB (for urine, feces, and plasma profiling; Hichrom Ltd., Theale, UK) columns were used for HPLC analyses. All the other commercially available chemicals and solvents were of analytical grade where available.

**Clinical ADME Study Design.** Study design. This study, conducted at Charles River Clinical Services Ltd. (Edinburgh, UK), was an 8-day, open-label, Phase I study of lersivirine in four healthy male subjects. All the subjects gave written informed consent; the study was conducted in accordance with the Declaration of Helsinki and was approved by an accredited institutional ethics committee. After an overnight fast, all the subjects received a single oral 500-mg dose of [14C]lersivirine as a 5-ml suspension in aqueous Avicel RC 591, containing a maximum of 62 μCi of [14C]; this amount of radioactivity was chosen to comply with the International Commission on Radiological Protection category IIa guidelines (radioactive exposure not exceeding 1 mSv), followed by 240 ml of water. Participants remained in an upright position and refrained from eating or drinking for 4 h postdose.

**Sample collection.** Blood samples (12 ml) were collected into nonbeaded lithium-heparinized tubes immediately predose and at 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 18, 24, 36, 48, 72, 96, 120, 144, and 168 h postdose. One milliliter of whole blood per sample was transferred into a nonheparinized tube and stored at 4°C for subsequent liquid scintillation counting. The remaining samples were processed for plasma via centrifugation at 1500g for 10 min at 4°C; samples were then divided into three aliquots for HPLC tandem mass spectrometry (HPLC/MS/MS) analysis of lersivirine (1 ml), liquid scintillation counting (1 ml), and metabolite profiling (approximately 3 ml).

Urine samples were collected at 12-h intervals postdose until day 4 and then at 24-h intervals until 168 h postdose. Urine was stored at 4°C until the end of the collection period. A 10-ml aliquot of each sample was withdrawn for scintillation counting, and 2 × 50-ml aliquots were frozen at −20°C for subsequent metabolite profiling.

Fecal samples were collected in polypropylene containers at 24-h intervals until 168 h postdose, and stored at −20°C. After each collection, samples were homogenized in water. Duplicate portions of the homogenate (0.4 g each) were taken for combustion in oxygen, and a separate 100-g homogenate aliquot was used for metabolite profiling. Sample collection was stopped when ≥90% of administered radioactivity had been recovered in the combined urine and fecal samples or when <1% of radioactivity was recovered in 24 h.

**Determination of total radioactivity.** Whole blood, plasma, urine, and fecal samples were assayed for drug-derived total radioactivity. Liquid scintillation counting of [14C]lersivirine in urine (A), feces (B), and in total (C).

**TABLE 1** Lersivirine pharmacokinetic parameter values (geometric means and intersubject variability)

<table>
<thead>
<tr>
<th>PK Parameter (Units)</th>
<th>Lersivirine (Plasma)</th>
<th>Total Drug-Related Radioactivity (Plasma)</th>
<th>Total Drug-Related Radioactivity (Whole Blood)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$n$</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>$AUC_{0-t}$ (ng·h/ml)$^a$</td>
<td>4100 (19)</td>
<td>39,130 (4)</td>
<td>18,650 (7)</td>
</tr>
<tr>
<td>$C_{max}$ (ng/ml)$^a$</td>
<td>1010 (40)</td>
<td>6042 (21)</td>
<td>2767 (27)</td>
</tr>
<tr>
<td>$T_{max}$ (h)$^b$</td>
<td>1.50 (0.50–3.00)</td>
<td>1.50 (1.00–3.00)</td>
<td>1.50 (1.00–3.00)</td>
</tr>
<tr>
<td>$t_{1/2}$ (h)$^b$</td>
<td>5.83 (26)</td>
<td>9.41 (30)</td>
<td>9.91 (19)</td>
</tr>
<tr>
<td>$CL/F$ (l/h)$^b$</td>
<td>122 (18)</td>
<td>12.78 (4)</td>
<td>26.81 (7)</td>
</tr>
<tr>
<td>$V/F$ (liter)$^b$</td>
<td>994 (24)</td>
<td>169.4 (26)</td>
<td>37,804 (15)</td>
</tr>
</tbody>
</table>

$^a$AUC$_{0-t}$, area under the plasma concentration-time profile from time zero extrapolated to infinite time; $C_{max}$, maximum plasma concentration; $T_{max}$, time to reach maximum concentration; $V/F$, apparent volume of distribution.

$^b$Units for radioactive compound are ng-Eq·h/ml for AUC$_{0-t}$ and ng-Eq/g for $C_{max}$.

$^c$Geometric mean (percentage coefficient of variation).

$^d$Median (range).

$^e$Arithmetic mean (percentage coefficient of variation).
counting was used for both urine (2300 TR; Canberra Industries, Meriden, CT) and plasma (Guardian; PerkinElmer Life and Analytical Sciences) samples after mixing with scintillation mixture. Whole-blood samples were first mixed with SOLVABLE (PerkinElmer Life and Analytical Sciences), EDTA, and H₂O₂ before mixing with scintillation mixture and subjected to liquid scintillation counting (Guardian; PerkinElmer Life and Analytical Sciences). Fecal samples were homogenized in water using a Waring (Stamford, CT) industrial blender, combusted in a biological oxidizer (model 307 MK2 Tri-Carb; PerkinElmer Life and Analytical Sciences), and the evolved ¹⁴CO₂ was trapped and measured by liquid scintillation counting in Permafluor E+ scintillation mixture (PerkinElmer Life and Analytical Sciences). A 1-ml aliquot of each plasma sample was analyzed by scintillation counting. The detection limit was 2.5 dpm above background. Samples of plasma (0.2 g) were mixed with scintillation mixture (16 ml, Starscint; PerkinElmer Life and Analytical Sciences) before liquid scintillation counting (Guardian; PerkinElmer Life and Analytical Sciences). Total radioactivity (percentage of dose) was determined for urine, feces, and their combination.

**Plasma assay.** Plasma samples were analyzed for lersivirine concentrations using a validated HPLC/MS/MS method (Allan et al., 2008). The method was linear over the range of 1 to 2000 ng/ml. The lower limit of quantification was 1 ng/ml.

**Metabolite profiling.** Urine samples (0–36 h) were prepared for metabolite profiling by centrifugation (3500 rpm, 15 min, 4°C); resultant pools (2 ml/subject) were profiled by HPLC using PU-2080 Plus pumps coupled to an HIRPB column (250 × 7.75 mm; Hichrom Ltd.). Separation was achieved with a binary solvent gradient at 2 ml/min, comprising methanol and 0.1 M ammonium acetate, pH 5. The gradient consisted of 30% methanol held for 10 min, increased to 50% over 1 min, held at 50% for 14 min, increased to 80% methanol over 20 min and held for 5 min, before returning to 30% methanol over 1 min, which was maintained for a further 4 min. Fractions (6.88 s) were collected into 96-well Scintiplates (PerkinElmer Life and Analytical Sciences), which were then dried and counted on a Microbeta scintillation counter (PerkinElmer Life and Analytical Sciences). Composite plasma samples (0–24 h time-normalized pools) (Hamilton et al., 1981; Hop et al., 1998) were treated 1:4 with methanol, centrifuged, and resultant supernatants were collected, dried, and profiled by HPLC in a manner similar to that of the urine samples. Drug-related material was extracted from composite fecal homogenate samples (0–96 h) by mixing with methanol, followed by sonication. The mixture

![Graph A: Plasma (0 to 24 hours)](image)

![Graph B: Urine (0 to 36 hours)](image)

![Graph C: Feces (24 to 96 hours)](image)

**FIG. 4.** Representative radiochromatograms showing lersivirine metabolites in the plasma (A), urine (B), and feces (C) of one healthy male subject after a single oral (500 mg) administration of [¹⁴C]lersivirine.
was centrifuged at 3500 rpm for 15 min, and the supernatant was collected. This extraction procedure was repeated with a mixture of methanol (1 ml) and Tris buffer (0.1 M, pH 6, 24 ml) and a mixture of methanol (23 ml) and Tris buffer (0.1 M, pH 9, 2 ml). The extracts were combined and reduced to dryness under nitrogen at 37°C in a Turbovap (Zymark, Hopkinton, MA), and the residue was suspended in mobile phase for HPLC analyses. The fecal extracts were profiled using the same HPLC system as urine and plasma; radiochromatographic peaks were isolated manually via on-line radiochemical detection (β-RAM: IN/US Systems, Tampa, FL) and reduced to dryness under nitrogen at 37°C in a Turbovap (Zymark).

Metabolite identification. Initial identification of drug-related metabolites isolated from human plasma and excreta was determined by direct-infusion MS on a Sciex API 4000 Q Trap mass spectrometer (Applied Biosystems, Foster City, CA) using appropriate precursor and neutral loss experiments based on the MS/MS fragmentation of lersivirine (Fig. 1, A and B). The mass spectrometer was operated with a Turbo Spray source and controlled by Analyst 1.4.1 software (Applied Biosystems). For MS/MS experiments, the collision energy was typically 40 V; declustering potential was set to 50 V; and the ion spray voltage was set to 5000 eV. Additional confidence in structural assignments was obtained by HPLC/MS/MS and MS/MS experiments using appropriate precursor and neutral loss experiments based on the MS/MS fragmentation of lersivirine (Thermo Fisher Scientific, Waltham, MA). The mass spectrometer was operated in MS/MS mode with an activation time of 30 ms. The HPLC/MS system consisted of Agilent Technologies (Santa Clara, CA) 1100 binary pumps coupled to a Sunfire C18 column (3.5 μm, 100 × 2.1 mm; Waters), with a binary solvent gradient (200 μl/min) of formic acid (0.1% aqueous) and acetonitrile (plus 0.1% formic acid) held at 5% acetonitrile for 1 min, increased to 98% over 7 min, held for 1 min, then returned to 5% over 0.1 min and held for 3.9 min.

Glucuronide conjugates isolated from human urine were subjected to deconjugation using Helix pomatia extract (Sigma-Aldrich, St. Louis, MO) to provide additional structural information on these metabolites. Dried isolates were reconstituted in water and incubated with an equal volume of H. pomatia extract for up to 24 h. Incubations were terminated by addition of acetonitrile, diluted with 0.1 M ammonium acetate, pH 5, and centrifuged (3500 rpm, 15 min, 4°C) to enable analysis of each sample on the same HPLC system used for urine profiling. Regions of interest were then analyzed by HPLC/MS/MS using a Thermo LTQ Orbitrap MS (Thermo Fisher Scientific) as described previously.

Safes and tolerability. Blood pressure, pulse rate, ECG, and physical exams, urine drug screening, and monitoring of adverse events (AEs) were reviewed on an ongoing basis throughout the study. The investigator obtained and recorded all the observed or volunteered AEs, the severity (mild, moderate, or severe) of the events, and the investigator’s opinion of the relationship to lersivirine.

Statistics. Pharmacokinetic and radioactivity parameters were calculated for each subject by using noncompartmental analysis of lersivirine and total radioactivity plasma and blood concentration versus time profiles. Values were summarized using descriptive statistics.

In Vitro Preclinical Metabolism Studies. Pooled human liver microsomes and microsomes prepared from baculovirus-infected insect cells engineered to individually express recombinant human UDP-glucuronosyl-

![Fig. 5. Proposed metabolic pathways of lersivirine in human excreta (A), with percentage of total excreted dose, and in human plasma (B), with percentage of AUC data for composite (0–24 h) samples after oral (500-mg single dose) administration of lersivirine.](image-url)
transferase (rUGT) isoforms UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A7, UGT1A8, UGT1A9, UGT1A10, UGT2B4, UGT2B7, UGT2B15, and UGT2B17 were obtained from BD Gentest (Woburn, MA). Human recombinant P450s (CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A4, and CYP3A5) were obtained from PanVera Corp. (Madison, WI), whereas isozymes CYP2C8 and CYP2B6 were obtained from BD Gentest.

For glucuronidation studies, lersivirine (50 μM) was incubated with human liver microsomes (0.5 mg/ml) or each of the rUGTs (0.5 mg/ml) for up to 120 min at 37°C. Incubations comprised 50 mM Tris-HCl (pH 7.4 at 25°C), 5 mM saccharocharine, alamethicin at 50 μg/mg protein, 10 mM MgCl₂, and 5 mM UDP-glucuronic acid. Microsomes and rUGTs were activated and incubated as described previously (Hyland et al., 2009). The reaction was terminated by adding 3X volume ice-cold acetone and centrifuged at specific times throughout the duration of the incubation, centrifuged at 3000 rpm for 45 min at 4°C, and analyzed for lersivirine and lersivirine glucuronide on a Sciex API 3000 mass spectrometer (Applied Biosystems).

For glucuronidation studies, lersivirine (50 μM) was incubated with human liver microsomes (0.5 mg/ml) or each of the rUGTs (0.5 mg/ml) for up to 120 min at 37°C. Incubations comprised 50 mM Tris-HCl (pH 7.4 at 37°C), 5 mM saccharocharine, alamethicin at 50 μg/mg protein, 10 mM MgCl₂, and 5 mM UDP-glucuronic acid. Microsomes and rUGTs were activated and incubated as described previously (Hyland et al., 2009). The reaction was terminated by adding 3X volume ice-cold acetone acid at specific times throughout the duration of the incubation, centrifuged at 3000 rpm for 45 min at 4°C, and analyzed for lersivirine and lersivirine glucuronide on a Sciex API 3000 mass spectrometer (Applied Biosystems). For UGT, enzyme kinetic study incubations were initially conducted to optimize incubation time and protein concentration before the kinetic study, which was conducted over a lersivirine range of 10 to 1500 μM. Rates of glucuronide formation were quantified against an authentic standard and used to obtain values for Kₘ and Vₘₐₓ.

For P450 studies, lersivirine (1 μM) was incubated with each of the recombinant P450 isoforms (150 pmol/ml) for 60 min. Incubations comprised 50 mM phosphate buffer, pH 7.4, 5 mM MgCl₂, and 1 mM NADPH, regenerated in situ from an isocitric acid/isocitric acid dehydrogenase regenerating system (Youdim et al., 2008). Reactions were terminated by the addition of acetonitrile containing midazolam (as internal standard), followed by centrifugation at 3000 rpm for 45 min at 4°C. Samples were then analyzed for lersivirine and two of the major oxidized products identified from the human ADME study (M19 and M17) on a Sciex API 4000 mass spectrometer (Applied Biosystems).

**Results**

**Clinical ADME Study. Study subjects.** Four healthy white male subjects completed the study; mean age (±S.D.) was 37.3 years (±7.4; range, 31–48 years), and mean body weight was 81.3 kg (±12.8).

**Table 2**

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>m/z</th>
<th>%Circulating Radioactivity</th>
<th>Urine</th>
<th>Feces</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>mean (range)</td>
<td>mean (range)</td>
<td>mean (range)</td>
</tr>
<tr>
<td>M1</td>
<td>345</td>
<td>1 (N.D.–3)</td>
<td>&lt;1 (N.D.–&lt;1)</td>
<td>N.D. (N.A.)</td>
<td>&lt;1 (N.D.–&lt;1)</td>
</tr>
<tr>
<td>M2</td>
<td>459</td>
<td>2 (2–3)</td>
<td>2 (1–2)</td>
<td>1 (N.D.–1)</td>
<td>2 (2–3)</td>
</tr>
<tr>
<td>M3</td>
<td>503</td>
<td>&lt;2 (&lt;1–&lt;2)</td>
<td>&lt;1 (N.A.)</td>
<td>N.D. (N.A.)</td>
<td>&lt;1 (N.A.)</td>
</tr>
<tr>
<td>M4</td>
<td>521</td>
<td>&lt;2 (&lt;1–&lt;2)</td>
<td>&lt;1 (N.A.)</td>
<td>N.D. (N.A.)</td>
<td>&lt;1 (N.A.)</td>
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<tr>
<td>M5</td>
<td>343</td>
<td>N.D. (N.A.)</td>
<td>N.D. (N.A.)</td>
<td>1 (N.D.–2)</td>
<td>1 (N.D.–2)</td>
</tr>
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<td>503</td>
<td>3 (2–3)</td>
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<td>N.D. (N.A.)</td>
<td>2 (N.A.)</td>
</tr>
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<td>M7</td>
<td>459</td>
<td>1 (N.A.)</td>
<td>1 (N.A.)</td>
<td>N.D. (N.A.)</td>
<td>1 (N.A.)</td>
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<td>M8</td>
<td>503</td>
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<td>3 (3–4)</td>
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<td>3 (3–4)</td>
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<td>M9</td>
<td>443</td>
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<td>N.D. (N.A.)</td>
<td>5 (3–7)</td>
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<td>501</td>
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<td>2 (N.A.)</td>
<td>N.D. (N.A.)</td>
<td>2 (N.A.)</td>
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<td>M11</td>
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<td>N.D. (N.A.)</td>
<td>2 (1–2)</td>
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<tr>
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<td>341</td>
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<td>N.D. (N.A.)</td>
<td>1 (&lt;1–1)</td>
<td>1 (&lt;1–1)</td>
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<tr>
<td>M14</td>
<td>501</td>
<td>1 (1–2)</td>
<td>1 (1–2)</td>
<td>N.D. (N.A.)</td>
<td>1 (1–2)</td>
</tr>
<tr>
<td>M15</td>
<td>487</td>
<td>45 (41–49)</td>
<td>54 (49–66)</td>
<td>N.D. (N.A.)</td>
<td>54 (49–66)</td>
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<td>M16</td>
<td>286</td>
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<td>N.D. (N.A.)</td>
<td>1 (N.D.–2)</td>
<td>1 (N.D.–2)</td>
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<tr>
<td>M17</td>
<td>327</td>
<td>8 (6–10)</td>
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<td>1 (N.D.–1)</td>
<td>2 (1–2)</td>
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<tr>
<td>M18</td>
<td>791</td>
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<td>1 (N.A.)</td>
<td>1 (1–2)</td>
<td>2 (3–3)</td>
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<tr>
<td>M19</td>
<td>325</td>
<td>3 (2–3)</td>
<td>3 (2–3)</td>
<td>N.D. (N.A.)</td>
<td>3 (2–3)</td>
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<tr>
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<td>325</td>
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<td>N.D. (N.A.)</td>
<td>N.D. (N.A.)</td>
</tr>
<tr>
<td>M21</td>
<td>267</td>
<td>&lt;1 (N.D.–1)</td>
<td>N.D. (N.A.)</td>
<td>1 (1–2)</td>
<td>1 (1–2)</td>
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<tr>
<td>M22</td>
<td>283</td>
<td>N.D. (N.A.)</td>
<td>N.D. (N.A.)</td>
<td>1 (N.D.–2)</td>
<td>1 (N.D.–2)</td>
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<tr>
<td>Lersivirine</td>
<td>311</td>
<td>13 (10–16)</td>
<td>&lt;1 (N.A.)</td>
<td>&lt;1 (N.D.–1)</td>
<td>&lt;1 (N.D.–1)</td>
</tr>
<tr>
<td>Total</td>
<td>92</td>
<td>90–92)</td>
<td>77 (69–89)</td>
<td>22 (14–27)</td>
<td>99 (96–103)</td>
</tr>
</tbody>
</table>

N.A., not available; N.D., not detected. Data from all the subjects were included in the calculation of the mean, N.D. being equal to zero for the calculation. Where there is no range stated it is because there was no variability between subjects.

**Pharmacokinetics.** The mean plasma concentration-time profiles for total drug-derived radioactivity and lersivirine are shown in Fig. 2, and the geometric means and intersubject variability for pharmacokinetic parameters for lersivirine and total drug-derived radioactivity are shown in Table 1. There were appreciable differences in the Cₘₐₓ and AUCₘₜ for total drug-derived radioactivity in plasma and blood and lersivirine in plasma occurred between 0.5 and 3 h postdose, and the mean blood/plasma ratio of total drug-derived radioactive material AUCₘᵢᵣ was 0.48.

**Mass balance.** The majority of radioactivity was excreted in urine, accounting for 80% of the dose, whereas 23% was recovered in feces (Fig. 3C). Mean total recovery of radioactivity (urine plus feces) was 103.7%. By 120 h postdose, all the radioactivity had been excreted, with the largest recovery occurring within the first 12 h for urine and first 48 h for fecal samples (Fig. 3, A and B).

**Metabolite profiling.** HPLC profiling of precipitated plasma (0–24 h) after time-normalized pooling, pooled urine (0–36 h), and pooled fecal homogenate (0–96 h) using radiochemical detection showed extensive metabolism for all the matrices in all the subjects (Fig. 4). Based on cochromatography, unchanged lersivirine was a major component of human plasma but only a minor component in excreta.

**Metabolite identification.** MS analysis of components isolated from human plasma and excreta identified 22 metabolites of lersivirine (Fig. 5, A and B). The MS/MS spectrum and proposed fragmentation of lersivirine are shown in Fig. 1, A and B, with major ions at m/z 293 and 267 representing loss of water and loss of C₃H₄O, respectively. Analysis of precipitated plasma (0–24 h; Table 2) revealed that the glucuronide conjugate (M15) was the major circulating component (45% of total radioactivity). The MS/MS spectrum and proposed fragmentation of M15 (m/z 487) is shown in Fig. 6, A and B, showing the major fragment ion at m/z 311, representing the characteristic loss of the glucuronic acid moiety (176 atomic mass units). As expected,
the MS³ spectrum of m/z 311 is essentially identical to the MS/MS spectrum of lersivirine. A product of mono-oxidation of one of the ethyl side chains (M17) accounted for 8% of the total radioactivity, whereas unchanged lersivirine accounted for 13%. The MS/MS spectrum and proposed fragmentation of M17 (Fig. 7, A and B) showed a major ion at m/z 309, consistent with loss of water. The MS³ spectrum indicated a further loss of water to yield m/z 291 and a loss of C₂H₄O to give m/z 265. Nine additional products of glucuronidation were detected; metabolites M3, M6, M8, and M11 were derived by glucuronidation in conjunction with monohydroxylation, and each accounted for between 1 and 4% of the total radioactivity; metabolites M2 and M7 were produced by glucuronidation in conjunction with N-dealkylation and mono-oxidation, both accounting for 3% of circulating radioactivity; M9 was a product of N-dealkylation and glucuronidation (6%); M4 was a product of oxidation, glucuronidation, and hydrolysis of one of the nitrile moieties to form an amide (<2%); whereas M10 formation was postulated to involve oxidation to form a ketone on one of the ethyl side chains and glucuronidation (2%).

Additional characterization of four of the glucuronic acid conjugates was achieved by enzymatic hydrolysis with β-glucuronidase followed by HPLC/MS/MS analysis of the deconjugated analog. Figure 8, A and B, shows the MS/MS spectrum and proposed fragmentation of M3, whereas Fig. 8C shows the radiochromatogram for M3 before and after hydrolysis together with chromatographic comparison with the mono-oxidized authentic standard UK-508,550. The approach resulted in identification of M3 as a product of oxidation on the terminal carbon of one of the ethyl side chains followed by glucuronidation. The same approach was used to provide further structural information for M6, M8, and M11, which shows that M6 was a glucuronic acid conjugate of the hydroxylated metabolite PF-03230716, whereas M8 and M11 were both glucuronides of the hydroxy metabolite M17, involving conjugation on both alcohol moieties. The MS/MS data for the glucuronic acid metabolite M9 showed the characteristic loss of 176 mass units to yield a major fragment ion at m/z 267 (Fig. 9, A and B). The specific site of glucuronidation for M9 was not determined but is postulated as a product of glucuronida-
tion of the pyrazole ring. Further low-level drug-related products of 
N-dealkylation, hydroxethyl side chain oxidation, glucuronidation, 
nitrile group hydrolysis, and ethyl side chain oxidation were also 
detected in the plasma (Fig. 4A).

Profiling of human urine and extracted fecal homogenates also 
revealed similar and extensive metabolism in all four subjects (Fig. 4, 
A and B). The major components were the glucuronide of lersivirine, 
M15, accounting for 54% of the dosed radioactivity; a metabolite 
involving N-dealkylation and glucuronidation (M9, 10%); and M19, a 
product of oxidation of the hydroxyethyl moiety to a carboxylic acid 
(8%). The MS/MS data for the carboxylic acid metabolite (M19) are 
shown in Fig. 10, A and B, with the major ion at 
\( m/z = 279 \) representing 

\[ \text{loss of CH}_2\text{O}_2. \]

Sulfation of the parent compound and nitrile group 
hydrolysis were also detected in excreta, whereas unchanged lersi-

virine accounted for <1% of the dose in both urine and feces.

Safety. There were no serious AEs and no discontinuations as a 
result of AEs after administration of radioactive lersivirine (ADME 
study). The only AE reported during the ADME study was mild 
headache, which was not considered treatment-related and resolved 
during the course of the study.

In Vitro Preclinical Metabolism Studies. Of the UGTs investi-
gated only rUGT2B7 was able to metabolize lersivirine. Further 
investigations through enzyme kinetic experiments performed in hu-
man liver microsomes and rUGT2B7 showed that formation of glucu-
ronide was linear with time up to 60 min and protein up to 1 mg/ml. 
Kinetic studies were performed at 0.5 mg/ml for 20 min in both 
rUGT2B7 and human liver microsomes over a substrate concentration 
range of 10 to 1500 \( \mu \text{M} \) (Fig. 11).

Data analysis indicated that in human liver microsomes glucu-
ronidation exhibited typical Michaelis-Menten kinetics, characterized 
by a \( K_m \) of 224.7 ± 56.7 \( \mu \text{M} \) and a \( V_{\text{max}} \) of 1583.3 ± 468.0 

\( \text{pmol/min/mg protein} \). Enzyme kinetics in rUGT2B7 also exhibited 
Michaelis-Menten kinetics with a \( K_m \) of 120.2 ± 31.8 \( \mu \text{M} \) and a \( V_{\text{max}} \) 
of 725.1 ± 64.8 \( \text{pmol/min/mg protein} \).

In incubations with recombinant P450 enzymes, CYP3A4 metabo-
lized lersivirine most rapidly with an intrinsic clearance of 0.9 
\( \mu \text{l}/\text{pmol P450/min} \). The only other enzyme shown to metabolize 
lersivirine based on a substrate depletion approach was CYP3A5, with 
a greater than 10-fold lower rate of metabolism (<0.08 \( \mu \text{l}/\text{pmol} 
P450/min \). The in vitro clearance in rCYP3A4 was scaled to activity
FIG. 8. MS/MS and MS3 spectrum of M3 (A), proposed fragmentation of M3 (B), and radiochromatogram for M3 before and after hydrolysis and comparison with UK-508,550 (C).

FIG. 9. MS/MS and MS3 spectrum of M9 (A) and proposed fragmentation of M9 (B).
in human liver microsomes using a relative activity factor of 0.28 (determined in-house) and a CYP3A4 abundance of 120 pmol P450/mg microsomal protein. The scaled recombinant P450 clearance of 8.55 \(\mu\)l/min/mg liver microsomal protein compared well with the human liver microsomal clearance of 8.23 \(\mu\)l/min/mg. In addition, CYP3A4 was shown to be capable of forming the oxidative metabolites seen in vivo (M19 and M17).

**Discussion**

In this human ADME study, recovery of the oral \([^{14}C]\)lersivirine dose was complete (103.7%) and occurred within 5 days after ingestion in healthy volunteers. The high urinary excretion of radioactivity (80.4%) is evidence that lersivirine is well absorbed after oral dosing in humans. After absorption lersivirine was extensively metabolized, with unchanged lersivirine constituting 13% of total plasma radioactivity (AUC\(_{\text{tot}}\)) and \(\leq 1\%\) of excreted radioactivity.

The blood/plasma ratio of total drug-derived radioactivity AUC\(_{\text{tot}}\) was 0.48, indicating limited distribution of radiolabeled material to red blood cells. This result likely reflects limited penetration of the polar lersivirine glucuronide (the major circulating metabolite in plasma) into red blood cells because in vitro studies with \([^{14}C]\)lersivirine show a higher blood/plasma ratio of 0.77 (Pfizer Inc., data on file).

Characterization of the radioactivity in plasma and excreta of humans dosed with \([^{14}C]\)lersivirine showed that at least 22 different metabolites were present and indicated that glucuronidation is the primary metabolic pathway of lersivirine. In both plasma and excreta the major metabolite was a glucuronide conjugate of lersivirine (M15). In vitro studies showed that of the isozymes studied only UGT2B7 was capable of forming lersivirine glucuronide. Furthermore, 9 of the 22 metabolites identified in both plasma and excreta were products of glucuronidation.

The renal clearance of M15 was 2 ml/min/kg. Although the plasma protein binding of M15 has not been determined, given the greater polarity of M15 compared with lersivirine and that the plasma protein binding of lersivirine is itself low to moderate (fraction unbound = 0.424), M15 would be expected to have minimal plasma protein binding. In this case, the unbound renal clearance of M15 would be essentially equivalent to glomerular filtration rate.
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Fig. 11. Kinetics of in vitro glucuronidation of lersivirine in human liver microsomes (A) and rUGT2B7 (B) with Eadie-Hofstee plots as inset enzymes. Data points represent the mean of three determinations.

Although M15 is the major circulating metabolite in humans, it does not circulate in the plasma of rat, mouse, or dog, but it is formed in the rat and excreted into bile. This glucuronide is significantly less potent than the parent compound (see later in Discussion) and does not contribute to the activity of lersivirine in vivo. In vitro, M15 is resistant to hydrolysis with β-glucuronidase, as was observed for the major pyrazole glucuronide of JNJ-10198409 (Yan et al., 2006).

The formation of a pyrazole N-glucuronide (M9) has previously been identified for the investigative anticancer agent JNJ-10198409 in human, rat, and monkey liver microsomes (Yan et al., 2006). M9 was resistant to hydrolysis with β-glucuronidase, as was observed for the major pyrazole N-glucuronide of JNJ-10198409 (Yan et al., 2006).

A number of oxidative metabolites of lersivirine were identified, of which metabolites with oxidation of the alcohol to a carboxylic acid group (M19) and a hydroxylated metabolite (M17) were the most abundant. In vitro, CYP3A4 was found to be the predominant P450 isoform tested that metabolized lersivirine. In addition, CYP3A4 was shown to be capable of forming the major oxidative metabolites formed in vivo (M19 and M17). Although these data are consistent with previous observations that CYP3A4 is the primary contributor to oxidative metabolism of lersivirine, in a previous study, using slightly different buffers and reagents, other P450 enzymes were able to metabolize lersivirine (Allan et al., 2008). This result most likely reflects the different source of the P450 isoforms used in the two studies and the inherent metabolic capabilities of the two systems.

CYP3A5*1, a polymorphic-expressed enzyme in which the allelic frequency of wild-type is only 10 to 15% in the white population (increasing to approximately 50% in blacks) (Daly, 2006), was also assessed in this study. Although it is not currently possible to make a quantitative extrapolation from recombinant CYP3A5 data because of the lack of an appropriate CYP3A5 probe substrate for the generation of relative activity factors, the low rate of metabolism of lersivirine by CYP3A5 compared with CYP3A4 suggests that no dosage adjustment will be needed in populations in which the expression of this polymorphic enzyme is high.

Compared with the parent compound (IC50, 2 nM), the metabolites M17 (138 nM), M15 (5 μM), and M19 (15.8 μM) are significantly less potent against the laboratory-adapted HIV-1 strain NL 4-3. In fact, the potency of either glucuronide could be accounted for by 0.1% parent in the glucuronide standard or by a small amount of degradation of M15 to parent in the culture. When the average for each of the metabolites is compared with the IC50 of these four components, the ratio is 325, 3, 0.5, 0.01 for lersivirine, M17, M15, and M19, respectively, suggesting that the antiviral activity is mainly associated with the lersivirine parent molecule.

In addition to glucuronidation and oxidative metabolism, metabolic pathways of lersivirine also involve sulfation and nitrile hydrolysis. It is well recognized that the extent of a drug-drug interaction as a result of enzyme inhibition will depend on the fraction of the substrate metabolized by that enzyme (Brown et al., 2005; Ito et al., 2005). The balanced clearance of lersivirine is expected to reduce the interindividual variability and drug-drug interaction liability of the compound by reducing the reliance on a single enzyme to clear the compound. Furthermore, the predominant clearance pathway in humans in vivo is UGT2B7-mediated glucuronidation. In general, glucuronidation is a high-capacity low-affinity metabolic pathway, and drug-drug interactions mediated through UGTs are known to be small in magnitude (Williams et al., 2004; Kiang et al., 2005). Coadministration with valproic acid, a potent inhibitor of UGT2B7, results in increased exposure of a number of compounds that undergo glucuronidation via UGT2B7, including zidovudine and lamotrigine; however, the magnitude of the changes in exposure is low [1.8-fold (Lertora et al., 1994) and 2.6-fold (Morris et al., 2000), respectively]. It is not expected that the plasma levels of lersivirine will increase dramatically on coadministration with medications that inhibit UGT metabolism because only a 1.3-fold increase in lersivirine AUC was observed after coadministration with valproic acid (Langdon et al., 2008).

Conclusion

In conclusion, this open-label Phase I study has shown that lersivirine is almost completely absorbed, extensively metabolized by both UGT- and P450-dependent pathways, and radiolabeled material is eliminated entirely by renal and fecal routes. The major human circulating metabolites examined thus far do not appear to contribute to the antiviral activity of lersivirine.

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References


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