Disposition and Metabolic Profiling of $[^{14}C]$Cerlapirdine Using Accelerator Mass Spectrometry

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ABSTRACT

Cerlapirdine (SAM-531, PF-05212365) is a selective, potent, full agonist of the 5-hydroxytryptamine 6 (5-HT$_6$) receptor. Cerlapirdine and other 5-HT$_6$ receptor antagonists have been in clinical development for the symptomatic treatment of Alzheimer’s disease. A human absorption, distribution, metabolism, and excretion study was conducted to gain further understanding of the metabolism and disposition of cerlapirdine. Because of the low amount of radioactivity administered, total $^{14}$C content and metabolic profiles in plasma, urine, and feces were determined using accelerator mass spectrometry (AMS). After a single, oral 5-mg dose of $[^{14}C]$cerlapirdine (177 nCi), recovery of total $^{14}$C was almost complete, with feces being the major route of elimination of the administered dose, whereas urinary excretion played a lesser role. The extent of absorption was estimated to be at least 70%..

Metabolite profiling in pooled plasma samples showed that unchanged cerlapirdine was the major drug-related component in circulation, representing 51% of total $^{14}$C exposure in plasma. One metabolite (M1, desmethylerlapirdine) was detected in plasma, and represented 9% of the total $^{14}$C exposure. In vitro cytochrome P450 reaction phenotyping studies showed that M1 was formed primarily by CYP2C8 and CYP3A4. In pooled urine samples, three major drug-related peaks were detected, corresponding to cerlapirdine-N-oxide (M3), cerlapirdine, and desmethylerlapirdine. In feces, cerlapirdine was the major $^{14}$C component excreted, followed by desmethylerlapirdine. The results of this study demonstrate that the use of the AMS technique enables comprehensive quantitative elucidation of the disposition and metabolic profiles of compounds administered at a low radioactive dose.

Introduction

Serotonin (5-hydroxytryptamine, 5-HT) is a major neurotransmitter in both the central and peripheral nervous systems. At least seven 5-HT receptor families (5-HT$_{1-7}$) have been identified (Hoyer et al., 1994). Among the 5-HT receptor families, the 5-HT$_6$ receptor is localized primarily in the central nervous system and in regions such as the cerebral cortex, nucleus accumbens, caudate-putamen, striatum, and hippocampus (Roberts et al., 2002; Hirst et al., 2003; Marazziti et al., 2012) that are associated with cognition and memory (Nyberg et al., 1996; Reinvang et al., 1998; Burgess et al., 2001). In experimental models, 5-HT$_6$ antagonists have demonstrated the ability to increase brain levels of acetylcholine and glutamate (Dawson et al., 2000; Riemer et al., 2003; Marcos et al., 2006) and to improve cognitive functions in animal models (Lindner et al., 2003; Foley et al., 2004; Lieben et al., 2005; Arnt et al., 2010; Mohler et al., 2012). Several 5-HT$_6$ antagonists have been evaluated in clinical trials for the treatment of cognitive deficits and dementia associated with Alzheimer’s disease (Upton et al., 2008; Johnson et al., 2008;提及的文献)

and other neurological diseases (Liu and Robichaud, 2009; Rosse and Schaffhauser, 2010; Codony et al., 2011). Recent clinical evidence further illustrates the potential utility of 5-HT$_6$ antagonists in the symptomatic treatment of Alzheimer’s disease (Maher-Edwards et al., 2010, 2011).

Cerlapirdine (SAM-531, PF-05212365 [N,N-dimethyl-3-[[3-(naphthalen-1-ylsulfonyl)-2H-indazol-5-yl]oxy]propan-1-amine]) is a selective, potent, full agonist at the 5-HT$_6$ receptor (Liu and Robichaud, 2010) with procognitive effects in nonclinical species (Comery et al., 2010). Clinical safety, tolerability, and preliminary efficacy studies have been conducted (Baird-Bellaire et al., 2010; Brisard et al., 2010; Mitchell, 2011). A study using radiolabeled $[^{14}C]$cerlapirdine was conducted in healthy young male subjects to gain a comprehensive understanding of the absorption, metabolism, and excretion properties of cerlapirdine. Also, in vitro metabolism and reaction phenotyping studies were conducted to elucidate the metabolic pathways and the oxidative enzymes responsible for the formation of the major metabolite that was observed in vivo.

Accelerator mass spectrometry (AMS) is an extremely sensitive method for the detection and determination of isotopic ratios (Salehpour et al., 2008). AMS has been used in pharmacoeconomic research and development since 2000 (Garner, 2000), including applications in pharmacokinetics (Barapa et al., 2005; Hah et al., 2009) and metabolism studies (Lappin and Stevens, 2008). In some radiotracer studies (Foley et al., 2004; Lieben et al., 2005; Arnt et al., 2010; Mohler et al., 2012).

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ABBREVIATIONS: AMS, accelerator mass spectrometry; ADME, absorption, distribution, metabolism, and excretion; AUC, area under the concentration-time curve; HLM, human liver microsomes; HPLC, high-performance liquid chromatography; 5-HT, 5-hydroxytryptamine, serotonin; LC/MS-MS, liquid chromatography coupled with tandem mass spectrometry; P450, cytochrome P450; tPF-05212365, SAM-531, cerlapirdine, N,N-dimethyl-3-[[3-(naphthalen-1-ylsulfonyl)-2H-indazol-5-yl]oxy]propan-1-amine; RAF, relative activity factor; WAY-211560, 2-amino-3-methyl-5-(3-phenylphenyl)-5-pyridin-4-ylimidazol-4-one.
in which only very low amounts of radioactive activity may be administered for various reasons—such as radiolytic instability or long plasma half-lives or retention in tissues—the ultra-high sensitivity of AMS enables the detection of very low levels of radioisotopes that may not be detectable by conventional techniques. In the case of cerlapidine, before the conduct of the absorption, distribution, metabolism, and excretion (ADME) study in humans using $^{14}$C-cerlapidine, dosimetry calculations were performed to evaluate the risk involved with exposure to ionizing radiation in human subjects based on in vivo distribution, pharmacokinetics, and mass balance studies in rats (data on file). Based on the absorption and distribution of radioactive activity to the whole body and major tissues, which indicated prolonged retention of radioactivity in pigmented tissues, a single, low, oral radioactive dose of $\sim 200$ nCi (actual dose 177 nCi) of $^{14}$C-cerlapidine was selected for administration in the human study. Because of the low amount of radioactivity anticipated in plasma and in excreta, which were likely to be below the levels that can be reliably measured by liquid scintillation counting, the levels of total $^{14}$C in plasma, whole blood, urine, and feces were determined using AMS. Subsequently, metabolic profiles of $^{14}$C-cerlapidine in plasma, urine, and feces were determined in pooled samples using high-performance liquid chromatography (HPLC) fractionation followed by AMS analysis of fractions (HPLC+AMS).

**Materials and Methods**

$^{14}$C-Cerlapidine (Fig. 1) was prepared under good manufacturing practices conditions by ARC Laboratories (Columbia, MO). Cerlapidine and $^{14}$C-cerlapidine were blended before the preparation of the final (capsule) dosage form. Each capsule was hand-filled, weighed, and capped. Each dose was packed in one capsule per subject, with a target dose of $5.44 \text{ mg}$ of the hydrochloride salt or $4.96 \text{ mg}$ of active moiety (free base). Each capsule was hand-filled, weighed, and capped. Each dose was packed in one capsule per subject, with a target dose of $5.44 \text{ mg}$ of the hydrochloride salt or $4.96 \text{ mg}$ of active moiety (free base). Each dose vial contained approximately $200 \text{ nCi}$ ($\sim 7.4 \text{ kBq}$; the actual mean radioactive dose was determined to be $177 \text{ nCi}$ of $^{14}$C-cerlapidine. Authentic standards of cerlapidine, desmethylcerlapidine (M1, N-desmethyl metabolite [N-desmethylerlapidine, N-methyl-3-[[3-(naphthalen-1-ylsulfonyl)-2H-indazol-5-yloxy]propan-1-amine]), N-oxide metabolite (M5) of cerlapidine, $\text{[H}_2\text{]}$cerlapidine (internal standard for cerlapidine in LC/MS-MS method), $\text{[H}_3\text{]}$desmethylcerlapidine (internal standard for desmethylcerlapidine in the liquid chromatography coupled with tandem mass spectrometry [LC/MS-MS] method), and WAY-211560 (2-amino-3-methyl-5(3-phenylenyl)-5-pyridin-4-ylmidoazol-4-one, internal standard used for reaction phenotyping study) were obtained from Pfizer Global Research & Development, Pearl River, NY or Princeton, NJ.

The following materials were used for the LC/MS-MS analyses of the plasma, urine, fecal samples and the metabolite profiling and identification. Methanol, acetonitrile, methyl-1-butyl ether, and ethyl acetate were obtained from Honeywell Burdick & Jackson (Muskegon, MI). Isopropyl alcohol and sodium borate 10-hydrate were obtained from J.T. Baker (Phillipsburg, NJ); formic acid Superpur was obtained from EMD Millipore (Billerica, MA). Materials used for the graphitization process, AMS, and HPLC+AMS analysis, and methanol (HPLC and analytic grades) was obtained from Thermo Fisher Scientific (Waltham, MA). Liquid paraffin, copper oxide wire (ACS grade), cobalt powder (100 mesh, 99%), zinc powder (100 mesh, 99%), and titanium (II) hydride (325 mesh, 98%) were obtained from Sigma-Aldrich (St. Louis, MO). ANU sugar (certified $^{13}$C/$^{12}$C ratio = 1.5061 Times Modern) used as an AMS standard was obtained from the Quaternary Dating Research Centre, Australian National University, Canberra, Australia. Synthetic graphite 200-35 mesh (99.99%) was obtained from Alfa Aesar (Ward Hill, MA) and contained aluminum powder (99.99%, obtained from Acros Organics, Morris Plains, NJ). Solid aluminum cathode (used as machine control) was obtained from National Electrostatics Corporation (Middleton, WI). Graphitization tubes, sample tubes, and borosilicate glass tubes (prebaked at 500°C for 2 to 4 hours) were obtained from York Glassware Services Ltd. (York, United Kingdom), and tin caps and Chromosorb W were obtained from Elemental Microanalysis Ltd. (Okehampton, Devon, United Kingdom). Urea standard was obtained from ThermoQuest Corporation (San Jose, CA).

The following materials were used for in vitro cytochrome P450 reaction phenotyping experiments. Sulfaphenazole, tranylcypromine, quinidine, ketoconazole, quercetin, α-naphthoflavone, and diethyldithiocarbamate were purchased from Sigma-Aldrich. HPLC-grade water, methanol, and acetonitrile were obtained from E.M. Science (Gibbstown, NJ). All other chemicals were reagent grade or better. Human liver microsomes (pool of 50 male donors) were purchased from XenoTech (Kansas City, KS). Recombinant human cytochrome P450 enzymes CYP1A2, CYP2A6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4 expressed in Escherichia coli were prepared at Pfizer (Collegeville, PA).

**Dosing of Subjects.** This was an open-label, nonrandomized, single-dose study conducted at a single clinical site (PRA International EDS, Zuidlaren, the Netherlands), in accordance with the International Conference on Harmonization (ICH) guidelines on good clinical practice and with ethical principles as per the Declaration of Helsinki. The clinical protocol and informed consent form were reviewed and approved by the institutional review board and independent ethics committee before subject enrollment. Subjects were fasted for 10 hours before dose administration, and fasted for 4 hours after dosing. On study day 1, each of six (6) healthy Caucasian male volunteers (age: 19–48 years; mean age: 27 years; weight: 66.3–89.0 kg; mean weight: 76.8 kg) was administered a single capsule containing a 5-mg (free-base) oral dose of $^{14}$C-cerlapidine (177 nCi radioactive dose) at approximately 8:00 AM, and received 240 ml of room temperature water immediately after dosing. All six study subjects were discharged on day 15 after the 14-day sample collection and study period.

**Collection of Samples.** Blood (venous) samples were collected before drug administration (predose sample) and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 16, 24, 48, 72, 96, 120, 144, 168, 192, 216, 240, 264, 288, 312, and 336 hours after drug administration. Potassium EDTA was used as an anticoagulant. Each sample was gently mixed and stored on ice until centrifugation. Aliquots of each blood sample were centrifuged at 2500 RPM for 10 minutes at 4°C to obtain plasma. The plasma was removed and stored frozen at −70°C/−80°C in polypropylene tubes before and after analysis.

Urine samples were collected before drug administration (predose specimen) and at 0–4, 4–8, 8–12, 12–24, 24–48, 48–72, 72–96, 96–120, 120–144, 144–168, 168–192, 192–216, 216–240, 240–264, 264–288, 288–312, 312–336
hours after dosing. Urine samples were collected on wet ice during each collection period, then frozen and stored at −70°C to 80°C before and after analysis. Fecal samples were collected before drug administration (predose specimen), then all bowel movements were collected after drug administration up to 336 hours after dosing. Fecal samples were quantitatively transferred into a container per subject per 24-hour interval (predose, 0–24, 24–48 hours, etc.) and the total weight for each sample at each interval was recorded. The fecal samples were homogenized with an Ultra Turax mixer (Ika Works, Wilmington, NC) after adding one to two weight equivalents of water. Aliquots of fecal homogenates were analyzed for metabolite profiling (HPLC + MS) and metabolite identification (LC/MS-MS). Separate subsamples of fecal homogenates were stored in plastic containers at −20°C, −80°C, or frozen at −30°C.

**Accelerator Mass Spectrometry (AMS) Analyses.** Whole blood, plasma, urine, and fecal samples were assayed for total 14C content using AMS at Xceleron (York, United Kingdom). Metabolite profiling of pooled plasma, urine, and fecal samples was conducted using HPLC with offline 14C detection by AMS at Xceleron. For the AMS analysis, plasma (60 μl), whole blood (20 μl), urine (diluted 10-fold with water; 100 μl), and feces (homogenized, ~40 mg; freeze-dried, ~4 mg), HPLC fractions (100 μl), or fraction pools (250 μl) were placed in tubes containing CuO2. For samples containing little carbon (i.e., urine and HPLC fractions), liquid paraffin (equivalent to ~2 mg carbon) was added as carbon carrier. Samples were sealed into quartz tubes under vacuum, heated for 2 hours at 900°C, oxidizing all carbon in the sample to CO₂. CO₂ was cryogenically transferred and sealed into a glass tube containing TiH₂ and Zn, with cobalt powder as catalyst. Samples were heated for 4 hours at 500°C, then for 6 hours at 550°C, reducing CO₂ to solid carbon. Carbon was pressed into aluminum cathodes, which were placed in the ion source of a 5 MV tandem pelletron Accelerator Mass Spectrometer (Model 15SDH-2; National Electrostatics Corporation) and ionized using a Cs⁺ ion beam, and the 14C/12C and 13C/12C ratios were determined. The 14C content of each sample was calculated, based on the 14C/12C ratio and the total carbon content. Carbon content was measured using an NA2100 Breweranlyser (CE Instruments, Wigan, United Kingdom), or generic values were used. Where appropriate, predose samples from the same subject were graphitized and analyzed to determine background 14C:12C ratios.

**LC/MS-MS Analyses of Cerlapirdine and Desmethylcerlapirdine (M1) in Human Plasma.** The concentrations of cerlapirdine and desmethylcerlapirdine (M1) in plasma and urine samples were determined at Advion BioServices (Ithaca, NY) using validated LC/MS-MS assays. Plasma or urine samples (100 μl) were extracted by a liquid-liquid extraction procedure using a 96-well format to isolate cerlapirdine and desmethylcerlapirdine. [2H₆]Cerlapirdine and [2H₆]desmethylcerlapirdine were used as internal standards for cerlapirdine and desmethylcerlapirdine, respectively. Sample extracts were injected on a 2.0 × 50 mm, 80 Å, (4 μm particle size) Synergi Hydro-RP column (Phenomenex Inc., Torrance, CA). A mobile phase gradient starting at 80% mobile phase A (10:90:0.01 methanol/water/formic acid) and 20% mobile phase B (90:10:0.01 methanol/water/formic acid) was used. Cerlapirdine and desmethylcerlapirdine concentrations in plasma extracts were determined by a Sciex API 4000 mass spectrometer (ABSciex, Framingham, MA) with turbo ion spray in the positive ionization mode. The plasma method has a lower limit of quantitation of 0.2 ng/ml and a calibration curve range of 0.2 to 100 ng/ml for both analytes. Cerlapirdine and desmethylcerlapirdine concentrations in plasma extracts were determined by a Sciex API 3000 mass spectrometer (ABSciex) with turbo ion spray in the positive ionization mode. The plasma method has a lower limit of quantitation of 10 ng/ml and a calibration curve range of 10–500 ng/ml.

**In Vitro Cytochrome P450 Reaction Phenotyping.** Experiments were conducted to determine the intrinsic clearance by substrate depletion of cerlapirdine or formation of desmethylcerlapirdine in human liver microsomes (HLM). Incubations were conducted at 37°C with shaking on Thermomixer R (Eppendorf, Hamburg, Germany) in 96-well square plates containing (1 μM) in potassium phosphate buffer (100 mM, pH 7.4), MgCl₂ (10 mM), and HLM (1 mg/ml) and were initiated with the addition of a NADPH regenerating system (glucose-6-phosphate, 3.6 mM; NADP⁺, 1.3 mM; and glucose-6-phosphate dehydrogenase, 0.4 units/ml) in the absence and presence of selective inhibitors.
TABLE 2

Pharmacokinetic parameters for total 14C in plasma and whole blood, cerlapirdine and desmethylcerlapirdine in plasma after administration of a single oral dose of 5 mg (177 nCi) of [14C]cirlapirdine to healthy male subjects

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Plasma 14C</th>
<th>Desmethylcerlapirdine</th>
<th>Cmax</th>
<th>Median</th>
<th>Mean (%CV)</th>
<th>Min</th>
<th>Max</th>
<th>Mean (%CV)</th>
<th>Min</th>
<th>Max</th>
<th>Median</th>
<th>Mean (%CV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax (ng/ml)</td>
<td>122</td>
<td>92.4</td>
<td>69.2 (22)</td>
<td>58.5</td>
<td>64.2</td>
<td>81.8 (34)</td>
<td>50.6</td>
<td></td>
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<tr>
<td>Median (ng/ml)</td>
<td>5.00 ± 3.00</td>
<td>127 ± 1.5</td>
<td>123 ± 10.5</td>
<td>128 ± 7.6</td>
<td>204 ± 7.6</td>
<td>234 ± 7.6</td>
<td>341 ± 7.6</td>
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<tr>
<td>Mean (ng/ml)</td>
<td>4.58 ± 3.00</td>
<td>78.1 ± 2.0</td>
<td>5.00 ± 3.00</td>
<td>6.00 ± 3.00</td>
<td>3.42 ± 2.00</td>
<td>7.78 ± 2.00</td>
<td>4.58 ± 2.00</td>
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<tr>
<td>Blood 14C (% of total 14C)</td>
<td>4.87 ± 2.00</td>
<td>9.27 ± 2.0</td>
<td>4.58 ± 2.00</td>
<td>5.7 ± 2.00</td>
<td>4.87 ± 2.00</td>
<td>7.78 ± 2.00</td>
<td>2.95 ± 2.00</td>
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<tr>
<td>AUCinf (ng • h/ml)</td>
<td>104 (30)</td>
<td>72.2 ± 2.0</td>
<td>48 (7)</td>
<td>4.0 (39)</td>
<td>114 (83.0)</td>
<td>83.0</td>
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<tr>
<td>t1/2 (h)</td>
<td>90 (11)</td>
<td>79.4 ± 2.0</td>
<td>7.78 ± 2.00</td>
<td>3.0 (96)</td>
<td>7.78 ± 2.00</td>
<td>9.27 ± 2.00</td>
<td>9.27 ± 2.00</td>
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<tr>
<td>CLint (l/h per kg)</td>
<td>NC</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
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<tr>
<td>F (l/kg)</td>
<td>NC</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
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<tr>
<td>P450i (nmol P450/mg protein)</td>
<td>0.327</td>
<td>0.0432</td>
<td>0.327</td>
<td>0.0432</td>
<td>0.327</td>
<td>0.0432</td>
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<tr>
<td>Km (m) of cytochrome P450</td>
<td>0.049</td>
<td>0.051</td>
<td>0.049</td>
<td>0.051</td>
<td>0.049</td>
<td>0.051</td>
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<tr>
<td>% contribution of P450</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
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CV, coefficient of variation; NA, not applicable; NC, not calculated.

Peak area ratios for the parent drug against the internal standard were used to determine the apparent intrinsic clearance (CLint) in HLM using the relative activity factor (RAF). The RAF is the ratio of the specific activity in human liver microsomes to the activity by the recombinant P450 (rP450) enzyme for the P450 enzyme of interest:

RAF = \( \frac{CL_{int\text{HLM}}}{CL_{int\text{rP450}}} \)

or

\( CL_{int\text{HLM}} = RAF \times CL_{int\text{rP450}} \)

The RAF values for CYP2C8 and CYP3A4 determined internally were 0.327 and 0.0432 nmol P450/mg protein, respectively. The % contribution of the intrinsic clearance by a particular P450 enzyme (\( CL_{int\text{P450}_{HLM}} \)) to total P450-mediated intrinsic clearance (\( CL_{int\text{P450}_{total\ HLM}} = CL_{HLM} \)) in human liver may then be calculated as follows:

\( %P450 = 100\% \times \frac{CL_{int\text{P450}_{HLM}}}{CL_{HLM}} \)

Metabolite Profiling in Plasma, Urine, and Feces. Plasma, urine, and fecal homogenate samples were pooled across all six subjects at selected time points. A single 0–72 hours plasma pooled sample was prepared from aliquots of plasma samples at each time point according to the Hamilton method (Hamilton et al., 1981). Two pooled urine samples (0–96 hours and 96–168 hours) were prepared by combining volumes of individual time point samples proportional to their respective plasma concentrations.
analyzed for 14C content by AMS as described earlier.

Fractions were analyzed either individually or pooled then 15% B). The eluent was collected as a series of fractions at 15-second intervals for 10 minutes, linear gradient to 30% B over 20 minutes, then to 95% B over 3 minutes before column re-equilibration at initial conditions for 5 minutes. Collision induced dissociation spectra were obtained for m/z 410, 396, and 426. Proposed structures were compared with available authentic standards of cerlapirdine, desmethylerlapirdine, and cerlapirdine-N-oxide.

Parent and Metabolite Excretion in Urine and Feces. Amounts of cerlapirdine and observed metabolites excreted in urine and feces were calculated by multiplying urine volume or feces weight and cerlapirdine (or metabolites) concentration in pooled urine or fecal homogenate (adjusted by homogenate volume) sample at each time interval. The total urinary or fecal excretion of cerlapirdine (or metabolites) was obtained by summation across all time intervals. Percentage dose urinary or fecal excretion of cerlapirdine (or metabolites) was calculated by dividing total amount excreted in urine or feces by the administered dose and multiplying by 100.

Pharmacokinetic and Statistical Analyses. The pharmacokinetics of total 14C, cerlapirdine, and desmethylerlapirdine in plasma were analyzed using WinNonlin (version 5.1.1; Pharsight). Pharmacokinetic parameters were calculated by noncompartmental analysis using the linear-up/log-down trapezoidal method. Based on the 336-hour plasma total 14C, cerlapirdine, and desmethylerlapirdine concentration data, peak plasma concentration (C_max), and time at peak plasma concentration (t_max) values were taken directly from the plasma concentration versus time curve for each subject. The terminal-phase disposition rate constant (λz) was determined by the log-linear regression of at least three time points (maximum of five time points) that were judged to be in the terminal phase. The apparent terminal half-life (t_(1/2)) value was estimated by dividing 0.693 by λz. Total area under the concentration-time curve to the last measurable concentration at time t (AUCt) was determined by the log-linear trapezoidal rule from time 0 to the time of last observed concentration at time t (C_t). Total area under the concentration-time curve from time zero to infinity (AUC_0→∞) was determined by: AUC_0→∞ = AUC_t + C_t/λz. CL/F was calculated as dose/total area under the concentration-time curve, and Vd/F was determined by the following: Vd/F = (dose/AUC) × (1/λz). CL/F and Vd/F were calculated only for the parent compound cerlapirdine.

Metabolite Identification. Metabolite peaks from plasma, urinary, and fecal profiling samples were collected using a fraction collector, and were analyzed by positive ion HPLC-UV-MS using a Thermo Finnigan LTQ-Orbitrap (ThermoQuest Corporation) in a full scan mode (m/z 200–650) at a resolution setting of 30,000. Capillary temperature was set at 275°C, and the source potential was 3600 V. Other potentials were optimized to get optimal ionization and fragmentation of the parent compound. UV absorption spectra were obtained by an in-line Surveyor photodiode array detector. A Polaris (Agilent) C18 column was used (4.6 × 250 mm) with a flow rate of 0.8 ml/min. Mobile phase A was comprised of 0.1% formic acid, and mobile phase B was comprised of acetonitrile. The gradient system used was as follows: initially, 10% B held for 5 minutes followed by a linear gradient to 50% B at 50 minutes, washing the column at 95% B for 10 minutes, followed by column re-equilibration to initial conditions for 5 minutes. Collision induced dissociation spectra were obtained for m/z 410, 396, and 426. Proposed structures were compared with available authentic standards of cerlapirdine, desmethylerlapirdine, and cerlapirdine-N-oxide.

Fig. 4. Plasma concentrations (mean ± S.D.) versus time profiles of cerlapirdine and desmethylerlapirdine after a single oral 5-mg (177 nCi) dose of [14C]cerlapirdine.

Fig. 5. Metabolite profiles in 0–72 hour plasma pooled sample after a single oral 5-mg (177 nCi) dose of [14C]cerlapirdine.
in plasma. Over the 96-hour sampling period, mean blood/plasma concentrations of cerlapirdine are also summarized in Table 2. The plasma concentrations related metabolites did not preferentially partition in whole blood but were found to be associated with selected erythrocyte fractions. The plasma and blood concentrations versus time profiles of total 14C are presented in Fig. 3. Pharmacokinetic parameters of total 14C contents in plasma and blood are summarized in Table 1. The excretion of 14C in urine, feces, and excreta is shown in Fig. 4. After a single oral dose of 5 mg (177 nCi) of [14C]cerlapirdine to healthy male subjects, the rate of excretion was rapid, with the majority (98.3%) recovered within 96 hours. The mean t1/2 in blood was shorter relative to plasma. This is probably due to the limited (up to 96 hours only) analysis of blood samples such that the terminal phase was not completely characterized.

### Table 3

<table>
<thead>
<tr>
<th>Excreta</th>
<th>Time Period</th>
<th>Cerlapirdine</th>
<th>Desmethylcerlapirdine</th>
<th>M3</th>
<th>Unassigned</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0–96</td>
<td>4.9</td>
<td>3.1</td>
<td>7.1</td>
<td>6.7</td>
<td>21.8</td>
</tr>
<tr>
<td>96–168</td>
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<td>1.5</td>
<td>1.1</td>
<td>0.8</td>
<td>4.0</td>
<td></td>
</tr>
<tr>
<td>0–168</td>
<td>5.5</td>
<td>4.6</td>
<td>8.2</td>
<td>7.5</td>
<td>25.8</td>
<td></td>
</tr>
<tr>
<td>96–168</td>
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<td>14.7</td>
<td>ND</td>
<td>3.3</td>
<td>12.1</td>
<td></td>
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<tr>
<td>0–168</td>
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<td>5.4</td>
<td>ND</td>
<td>20.1</td>
<td>57.9</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>33.4</td>
<td>24.7</td>
<td>8.2</td>
<td>17.4</td>
<td>83.7</td>
<td></td>
</tr>
</tbody>
</table>

ND, not detected.

*Minor peaks of 14C in urine and feces homogenate pooled samples; structures could not be identified.

*Additional 2.2% excreted in urine pooled sample and 12.5% excreted in feces homogenate pooled sample between 168 and 336 hours.

### Results

**Excretion and Mass Balance.** The study population consisted of six healthy, male, Caucasian subjects aged 19 to 48 years, with a mean age of 27 years. The subject demographic and baseline characteristics are summarized in Table 1. The excretion of 14C in urine, feces, and total excretion with time is shown in Fig. 2. After a single oral dose of 5 mg/177 nCi of [14C]cerlapirdine, excretion was essentially complete, with mean (± S.D.) total 14C recovery of 98.3% (± 4.7%) (range: 91.0–103%) up to 336 hours after the dose. Feces was the major route of excretion, accounting for 70.3% (± 5.1%) (range: 63.8–76.9%) of the administered dose, and urine was the minor excretory route, accounting for 28.0% (± 5.2%) (range: 24.4–37.8%) of the administered dose. The rate of excretion was rapid, with the majority (~68%) of the 14C dose recovered within 96 hours.

**Pharmacokinetic Profiles of Total 14C, Cerlapirdine, and Desmethylcerlapirdine (M1).** The plasma and blood concentrations versus time profiles of total 14C are presented in Fig. 3. Pharmacokinetic parameters of total 14C contents in plasma and blood are summarized in Table 2. After a single oral dose of 5 mg/177 nCi of [14C]cerlapirdine, 14C was determined in selected time points (up to 96 hours) in blood and for all time points (up to 336 hours) in plasma (Fig. 3). Mean peak concentrations (Cmax) of 14C were achieved with mean tmax values of ~3 hours and ~6 hours in blood and plasma, respectively. Mean Cmax values of 14C were 69.2 ng cerlapirdine equivalents/ml in blood and 104 ng eq/ml in plasma. Mean t1/2 of 14C was 37.0 hours in blood and 90.0 hours in plasma. It should be noted that the apparent t1/2 in blood was shorter relative to plasma. This is probably due to the limited (up to 96 hours only) analysis of blood samples such that the terminal phase was not completely characterized. AUCinf of 14C was 3216 ng eq/ml in blood and 507 ng eq/ml in plasma. Over the 96-hour sampling period, mean blood/plasma ratios for [14C] ranged between 0.59:1 (1 hour) and 0.89:1 (12 hours) after the dose for all subjects, suggesting that [14C]cerlapirdine or related metabolites did not preferentially partition in whole blood components.

The pharmacokinetic parameters of cerlapirdine and desmethylcerlapirdine are also summarized in Table 2. The plasma concentrations versus time profiles are depicted in Fig. 4. After a single oral 5-mg dose of [14C]cerlapirdine, cerlapirdine was absorbed into the systemic circulation with median time to peak plasma concentrations (tmax) of ~3 hours (range: 1.5–6 hours). Mean Cmax and AUCinf for cerlapirdine were ~82 ng/ml and 2866 ng·h/ml, respectively. Mean Vd/F and and mean CL/F were 2.1 l/kg and 0.024 l/h per kg, respectively, with a mean apparent t1/2 of ~60 hours. For desmethylcerlapirdine, median tmax value was ~13 hours (range: 4–48 hours). Mean Cmax and AUCinf values for desmethylcerlapirdine were 5.0 ng/ml and 507 ng·h/ml, respectively. Desmethylcerlapirdine was eliminated from the systemic circulation with a mean apparent t1/2 of ~85 hours. Cerlapirdine and desmethylcerlapirdine accounted for 51 and 9%, respectively, of the total 14C in plasma. The ratio of desmethylcerlapirdine to cerlapirdine concentrations in plasma, based on their respective AUCinf values, was ~0.18.

**In Vitro Cytochrome P450 Reaction Phenotyping.** In HLM, the substrate depletion or formation of desmethylcerlapirdine was inhibited minimally by the various P450 inhibitors evaluated with the exception of quercetin (48–55%) and ketoconazole (54–63%). These observations suggested that CYP2C8 and CYP3A were primarily responsible for the metabolism of cerlapirdine to desmethylcerlapirdine, apparently with similar contribution of these enzymes in HLM. Because quercetin also inhibits CYP3A (albeit to a lower extent), the contribution of CYP2C8 to desmethylcerlapirdine formation may have been somewhat overestimated.

Recombinant CYP2C8 and CYP3A4 were also shown to be primarily responsible for the metabolism of cerlapirdine to desmethylcerlapirdine, with Vmax values of 3.4 and 9.4 pmol/pmol P450/min for CYP2C8 and CYP3A4, respectively, and Km values of 3.3 and 11.2 μM for CYP2C8 and CYP3A4, respectively. When corrected by their corresponding RAF values, the intrinsic clearance (Vmax/Km) values of desmethylcerlapirdine formation were 0.34 and 0.036 ml/mg per min for CYP2C8 and CYP3A4, respectively, reflecting a corresponding contribution of ~90% for CYP2C8 by ~10% by CYP3A4. By taking into consideration results from both the recombinant P450 and chemical inhibition in HLM, it appeared that the contribution of CYP2C8 to CYP3A to desmethylcerlapirdine formation was 48–90% and 10–63%, respectively.
Cerlapidine is a 5-HT\textsubscript{6} receptor antagonist that has been evaluated as a potential treatment of cognitive deficits in mild to moderate Alzheimer’s disease (Brisard et al., 2010). As part of the clinical development program, a human ADME study with \([^{14}C]\)cerlapidine was conducted to elucidate the metabolism and disposition of cerlapidine. Because of the long retention time of cerlapidine in pigmented tissues, a low radioactive dose (\(\approx200\) nCi) was administered. Because of the low amount of radioactivity that is generally below the detection limit of conventional detection method such as liquid scintillation counting, we used accelerator mass spectrometry to determine the \(^{14}C\) drug-related material in the plasma, urine, and fecal samples obtained from this study. The overall results show that cerlapidine is well absorbed (at least 70\% based on amounts of unchanged cerlapidine in feces and amounts of metabolites excreted in urine and feces) after oral administration. Recovery of \(^{14}C\) was almost complete (mean total recovery was 98.3\%) within 14 days; feces was not available for recovery studies.
the major route of excretion, accounting for ≈70% of the total dose, and urinary excretion was a minor route, with mean excretion of ≈28% of the dose. The rate of excretion was rapid, with majority (≈68%) of the dose recovered within 96 hours.

Metabolite profiling results showed that unchanged cerlapirdine was the major drug-related compound in circulation, accounting for approximately 51% of the total 14C in plasma. One circulating metabolite, desmethylcerlapirdine (M1), was identified. The ratio of desmethylcerlapirdine relative to cerlapirdine was 0.18 (based on AUC\text{inf} values). Total desmethylcerlapirdine was approximately 9% of the total 14C in plasma. Evaluation of the partial AUC data between 0 and 72 hours also shows that cerlapirdine and M1 AUC\text{0–72 hours} values were approximately 80% of the 14C AUC\text{0–72 hours} in plasma (data not shown), indicating that the parent compound and M1 accounted for a majority of the total drug-related materials, even though the overall AUC values show that the parent and M1 metabolite accounted for ≈60% of total 14C AUC (0–336 hours). This discrepancy is primarily due to low levels of 14C in plasma samples beyond 72 hours (concentrations <15 ng eq/ml). The difference between the total 14C AUC and the measured cerlapirdine and M1 may be due to other minor metabolites (with abundance lower than desmethylcerlapirdine since no definitive peak has been observed) in plasma. The recovery of total 14C from pooled plasma was ≈72%, which was similar to the extraction recoveries of cerlapirdine and desmethylcerlapirdine from plasma when evaluated by an LC/MS-MS assay (data on file).

It is possible that some of the minor metabolites of cerlapirdine may not be quantitatively extracted and therefore not identified in this study. In vitro metabolism studies in HLM have shown that other oxidative metabolites (besides M1 and the N-oxide, M3) are formed (data on file). Other oxidative metabolites have also been observed in metabolism studies in nonclinical species in vitro and in vivo (data on file). However, in earlier metabolite profiling studies after single or multiple doses of cerlapirdine in humans, cerlapirdine and

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**Fig. 8.** HPLC/UV/HRMS-MS analysis of cerlapirdine, M1, and M3 in fractions obtained from 0–96 hours urine sample. (A) UV chromatograms of fractions containing cerlapirdine, desmethylcerlapirdine (M1), and cerlapirdine-N-oxide (M3). (B) High-resolution mass spectra for cerlapirdine, M1, and M3 metabolites.
desmethyl cerlapirdine were the major drug-related materials in circulation, whereas cerlapirdine N-oxide was observed in trace amount after multiple dosing only (data on file). It is possible that low levels of these other oxidative metabolites (observed in HLM in vitro and in nonclinical species) may be present, but the abundance of these would be relatively low compared with cerlapirdine and desmethyl-cerlapirdine because no definitive metabolite peak was observed in either the single/multiple dose metabolite profiling studies or in this study. If present, the abundance of these metabolites would be relatively low compared with cerlapirdine and desmethylcerlapirdine. It should also be noted that no other metabolites besides cerlapirdine, M1, and M3 have been observed in the pooled urine and fecal samples from later time points (96–168 hours); therefore, the presence of another metabolite at later time points (beyond 72 hours) is not likely.

The N-oxide metabolite (M3) was the major drug-related entity excreted in urine, along with desmethylcerlapirdine and unchanged cerlapirdine. Unchanged cerlapirdine was the predominant compound excreted in the feces, followed by desmethylcerlapirdine. No other metabolite was identified in the feces. CYP2C8 and CYP3A were identified as the major P450 isozymes responsible for the formation of desmethylcerlapirdine in HLM or using recombinant enzymes. Both the desmethyl and the N-oxide metabolites were also identified from in vitro systems using HLM or S9 fractions (data on file). The overall metabolism and disposition pathways of cerlapirdine are summarized and depicted in Fig. 9.

The AMS technique has been used in this metabolism study with satisfactory outcomes. However, the samples were pooled across time points and subjects because of the low amounts of radioactivity and 14C, thus time-related changes in the metabolite profiles and variability between subjects could not be assessed in this study. It should be noted that the metabolism of cerlapirdine is uncomplicated, with one major (desmethyl) and one minor (N-oxide) metabolite identified in plasma and in excreta. Other minor metabolites, if present, would likely occur at very low amounts, precluding sufficient characterization and identification.

Since its introduction, AMS has increasingly been used in pharmaceutical research and development (Lappin and Stevens, 2008; Garner, 2010; Lappin and Seymour, 2010). The ultrahigh sensitivity of AMS (by measure isotopic 14C rather than radioactivity) enables the use of low amounts of radioactivity in radiotracer pharmacokinetic and metabolism studies. This is particularly important when the compound under evaluation shows radiolytic instability, has to be administered as a very low dose, or the compound exhibits long systemic and/or tissue half-lives such that the total amount of radioactivity that can be safety administered is limited. In a conventional human ADME study using liquid scintillation counting as a quantitation method, the typical radioactivity doses are in the range of 10–100 μCi. However, in the case of ixabepilone, which shows radiolytic instability at high specific activity but is stable at 1–2 nCi/mg (Comezoglu et al., 2009), AMS provided a tool by which the mass balance and metabolism of this compound could be determined in humans. Other examples of using AMS for human mass balance and metabolism studies include vismodegib (Graham et al., 2011), which has a long half-life (over 200 hours), and pasireotide, which is a somatostatin analog administered at a low dose of 600 mg (Lin et al., 2013).

With the low amount of radioactivity administered in studies using AMS as the detection method, the radiotracer study may be exempt from regulatory review by health authorities because the radioactive exposure from the administered dose is comparable to ambient exposure. As a result, pharmacokinetics/ADME study can be conducted during early phase clinical development, and valuable insights can be gained and may result in an expedited development program (Garner et al., 2002; Garner 2010). This is particularly important in the case of identifying potential human metabolites that may require additional safety testing in animals and additional monitoring in clinical studies (Lappin and Seymour, 2010). In the case of cerlapirdine, long retention...
in pigmented tissues was observed in rodent tissue distribution studies (data on file), so the radioactivity dose was limited to <200 nCi. Despite the low amount of radioactivity, the recovery of total 14C was virtually complete, and the metabolic and elimination pathways for carbapiramide also were delineated in this study.

Authorship Contributions
Participated in research design: Tse, Leung, Raje, Seymour. Conducted experiments: Leung, Shishikura, Obach. Performed data analysis: Tse, Leung, Raje, Seymour, Shishikura, Obach. Wrote or contributed to the writing of the manuscript: Tse, Leung, Raje, Seymour, Shishikura, Obach.

References