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ABSTRACT

Cerlapirdine (SAM-531, PF-05212365) is a selective, potent, full antagonist 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, desmethylcerlapirdine) 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 desmethylcerlapirdine. In feces, cerlapirdine was the major $[^14]C$ component excreted, followed by desmethylcerlapirdine. 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; 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 (Lipp et al., 2005; Hah et al., 2009) and metabolism studies (Lippin and Stevens, 2008). In some radiotracer studies 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) is a selective, potent, full antagonist 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.
in which only very low amounts of radioactivity 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 cerlapirdine, before the conduct of the absorption, distribution, metabolism, and excretion (ADME) study in humans using [14C]cerlapirdine, 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 radioactivity to the whole body and major tissues, which indicated prolonged retention of radioactivity in pigmented tissues, a single, low, oral radioactive dose of ~200 nCi (actual dose 177 nCi) of [14C]cerlapirdine 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 14C in plasma, whole blood, urine, and feces were determined using AMS. Subsequently, metabolic profiles of [14C]cerlapirdine 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

[14C]Cerlapirdine (Fig. 1) was prepared under good manufacturing practices conditions by ABC Laboratories (Columbia, MO). Cerlapirdine and [14C]cerlapirdine 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 mg of the hydrochloride salt or approximately 4.96 mg of active moiety (free base). Each dose vial contained approximately 200 nCi (~7.4 kBq; the actual mean radioactive dose was determined to be 177 nCi) of [14C]cerlapirdine. Authentic standards of cerlapirdine, desmethylcerlapirdine (M1, N-desmethyl metabolite [N-desmethylerlapirdine, N-methyl-3-((3-naphthalen-1-ylsulfonil)-2H-indazol-5-yl)oxy]-propan-1-amine]), N-oxide metabolite (M3) of cerlapirdine, [H3]cerlapirdine (internal standard for cerlapirdine in LC/MS-MS method), [H3]desmethylcerlapirdine (internal standard for desmethylcerlapirdine in the liquid chromatography coupled with tandem mass spectrometry [LC/MS-MS] method), and WAY-211560 (2-amino-3-methyl-5(3-phenylphenyl)-5-pyridin-4-ylimidazol-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 Supersol 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, 99.9%), zinc powder (100 mesh, 99%), and titanium (II) hydride (325 mesh, 98%) were obtained from Sigma-Aldrich (St. Louis, MO). ANU sugar (certified 13C/12C 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) was purchased from XenoTech (Kansas City, KS). Recombinant human cytochrome P450 enzymes CYP1A2, CYP2A6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4 as Excherichia col·f 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 [14C]cerlapirdine (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
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 + LC/MS-MS) after adding one to two weight equivalents of water. Separate subsamples of fecal homogenates were stored in plastic containers 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.

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 were 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), 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:13C ratio and the total carbon content. Carbon content was measured using an NA2100 Breweranalyser (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.

Mass Balance and Blood:Erythrocyte Distribution. Mass balance was determined based on the recovery of total 14C in urine and feces. Distribution of blood radioactivity to erythrocytes at selected time points (up to 96 hours after the dose) was calculated from 14C contents in blood (Cblood), plasma (Cplasma), and hematocrit (Hct) using the formula: (Cblood − Cplasma)(1 − Hct)/Hct. Ratios of plasma cerlapirdine and M1 area under the concentration-time curve (AUC) to total plasma 14C AUC were also determined as follows:

Cerlapirdine or M1 AUC ratio = AUCcerlapirdine or AUCM1/AUC (total 14C)

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. [3H]Cerlapirdine and [3H]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 urine extracts were determined by a Sciex API 3000 mass spectrometer (ABSciex) with turbo ion spray in the positive ionization mode. The urinary 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

<table>
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<tr>
<th>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</th>
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<tr>
<td><strong>Plasma 14C</strong> (n = 6)</td>
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<td>Mean (%CV)</td>
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<td>Plasma 14C</td>
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<td>Blood 14C</td>
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<td>Cerlapirdine</td>
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<td>Desmethylcerlapirdine</td>
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**CV, coefficient of variation; NA, not applicable; NC, not calculated.**

The apparent P450-mediated intrinsic clearance (CLint,P450sum HLM) in human liver may be calculated as follows:

$$\text{RAF} = \frac{\text{CLint,HLM}}{\text{CLint,rP450}}$$

or

$$\text{CLint,HLM} = \text{RAF} \times \text{CLint,rP450}$$

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 (CLint,P450) to total P450-mediated intrinsic clearance (CLint, sum HLM) in human liver may then be calculated as follows:

$$\% \text{P450 Contribution} = 100 \% \frac{\text{CLint, P450}_i}{\text{CLint, sum HLM}}$$

**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 (Hamiltion 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 of cytochrome P450 enzymes including ketoconazole (1 μM) for CYP3A, quinine (10 μM) for CYP2D6, sulfaphenazole (10 μM) for CYP2C9, tranylcypromine (50 μM) for CYP2C19, α-naphthoflavone (5 μM) for CYP1A2, tranylcypromine (10 μM) for CYP2A6, quercetin (20 μM) for CYP2C8, or diethylthiocarbamate (50 μM) for CYP2E1. Reactions were quenched by the addition of 0.5 ml acetonitrile containing the internal standard (WAY-211560). The plates were then centrifuged in a Therma Forma (Marietta, OH) centrifuge at room temperature for 10 minutes at 3400 rpm. Supernatants (200 μl) were transferred to 1-ml polystyrene 96-well plates for the determination of the parent drug or M1 concentrations by LC/MS-MS. Similar incubations were conducted in recombinant human cytochrome P450 enzymes CYP1A2, CYP2A6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, or CYP3A4 (100 pmol/ml) expressed in E. coli. The kinetics of desmethylcerlapirdine formation were determined in recombinant CYP2C8 and CYP3A4 based on results from an initial study, and in HLM at parent drug concentrations of 1, 5, 10, 25, and 50 μM similarly for an incubation duration of 10 minutes.

HPLC analysis was performed on an Agilent model 1100 HPLC equipped with a binary pump (Agilent Technologies, Santa Clara, CA) and using an HTS PAL autosampler (LEAP Technologies, Raleigh, NC) maintained at 10°C. Aliquots (10 μl) of the cytochrome P450 metabolite supernatants (containing cerlapidine, desmethylcerlapidine, and the internal standard) were injected into the HPLC. The column was a 5-μm Thermo Aquasil C18 column, 2.1 mm ID × 50 mm (Bellefonte, PA). Mobile phase A was 0.1% formic acid in water, and mobile phase B was 0.1% formic acid in acetonitrile. Mass spectrometry analyses were conducted using an Applied Biosystems PE Sciex API 4000 triple quadrupole mass spectrometer (Applied Biosystems, Foster City, CA), with a turbo spray interface using the positive electrospray ionization mode with electrospray ionization source using a multiple reaction monitoring technique.

Data analysis was performed using Analyst, v.1.4.1 (Applied Biosystems). Peak area ratios for the parent drug against the internal standard were used to express the amount of parent remaining compared with that at time 0 in both HLM with or without chemical inhibitors and in recombinant cytochrome P450 (P450) enzymes. Intrinsic clearance by substrate depletion was determined by linear regression of the log % substrate remaining versus time plots using Microsoft Excel, v.7.0 (Microsoft, Redmond, WA).

Concentrations of desmethylcerlapirdine formed were determined by extrapolating the response calculated from the peak-area ratios (peak areas of desmethylcerlapirdine versus the internal standard) to that of the standard curve. Vmax and Km values were determined by nonlinear regression in plots generated from the simple Emax model (model 101) using WinNonlin Professional, version 4.1 (Pharsight, Mountain View, CA). The intrinsic clearance (Vmax/Km) of desmethylcerlapirdine formation determined in a recombinant human P450 enzyme was converted to the intrinsic clearance 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 enzyme (Nakajima et al., 2002). The specific activity is measured as intrinsic clearance (Vmax/Km) using a specific probe substrate in HLM and in the recombinant P450 (rP450) enzyme for the P450 enzyme of interest:

$$\text{RAF} = \frac{\text{CLint,HLM}}{\text{CLint,rP450}}$$

or

$$\text{CLint,HLM} = \text{RAF} \times \text{CLint,rP450}$$

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 (CLint,P450) to total P450-mediated intrinsic clearance (CLint, sum HLM) in human liver may then be calculated as follows:

$$\% \text{P450 Contribution} = 100 \% \frac{\text{CLint, P450}_i}{\text{CLint, sum HLM}}$$
to the volume collected over each time period for each subject. Two pooled feces samples (0–96 hours and 96–168 hours) were prepared for each subject by combining a mass of each individual sample proportional to the total mass excreted over each time period by that subject. An equal proportion of each individual subject feces pool was then pooled to provide two cross-subject pooled samples (0–96 hours and 96–168 hours) for analysis.

Plasma extracts for metabolite profiling were prepared by adding 1 ml of pooled plasma sample to 3 ml of acetonitrile, vortex mixing, then centrifuging (4500 rpm; 10 minutes; ambient temperature). The supernatant was transferred and evaporated under a stream of nitrogen to approximately 0.6 ml, followed by addition of 0.4 ml of 5% v/v acetonitrile in 0.2% v/v formic acid. Aliquots of pooled urine samples were centrifuged, and metabolite profiles were determined in the supernatants.

Fecal homogenate samples for metabolite profiling were prepared by mixing approximately 200 mg of each overall pooled homogenate sample with 1 ml of water. Acetonitrile (4 ml) was added to 0.8 ml of the mixture, vortex mixed, and centrifuged. The supernatant was transferred to a clean tube and evaporated under a stream of nitrogen to approximately 0.6 ml, followed by addition of 0.4 ml of 5% v/v acetonitrile in 0.2% v/v formic acid. Aliquots of pooled urine samples were centrifuged, and metabolite profiles were determined in the supernatants.

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

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 desmethylcerlapirdine 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 desmethylcerlapirdine concentration data, peak plasma concentration (Cmax), and time to peak plasma concentration (tmax) 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 (t1/2) value was estimated by dividing 0.693 by λz. Total area under the concentration-time curve to the last measurable concentration at time t (AUC) was determined by the log-linear trapezoidal rule from time 0 to the time of last observed concentration at time t (C). Total area under the concentration-time curve from time zero to infinity (AUC∞) was determined by: AUC ∞ = AUC + C/λz. CL/F was calculated as dose/total area under the concentration-time curve, and V/F was determined by the following: V/F = (dose/AUC) × (1/λz). CL/F and V/F were calculated only for the parent compound cerlapirdine.

Fig. 5. Metabolite profiles in 0–72 hour plasma pooled sample after a single oral 5-mg (177 nCi) dose of [14C]cerlapirdine.
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 $^{14}$C in urine, feces, and total excretion with time is shown in Fig. 2. After a single oral dose of $[^{14}$C$]$cerlapirdine, excretion was essentially complete, with mean total excretion with time being 98.3% (range: 91.0–103%) up to 336 hours after the dose. Feces was the major route of excretion, accounting for 70.3% (range: 24.4–28.0%) of the administered dose. The rate of excretion was rapid, with the majority (~68%) of the $^{14}$C dose recovered within 96 hours.

Pharmacokinetic Profiles of Total $^{14}$C, Cerlapirdine, and Desmethylcerlapirdine (M1). The plasma and blood concentrations versus time profiles of total $^{14}$C are presented in Fig. 3. Pharmacokinetic parameters of total $^{14}$C contents in plasma and blood are summarized in Table 2. After a single oral dose of 5 mg/177 nCi of $[^{14}$C$]$cerlapirdine, $^{14}$C 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 ($C_{\text{max}}$) of $^{14}$C were achieved with mean $t_{\text{max}}$ values of ~3 hours and ~6 hours in blood and plasma, respectively. Mean $C_{\text{max}}$ values of $^{14}$C were 69.2 ng cerlapirdine equivalents/ml in blood and 104 ng eq/ml in plasma. Mean $t_{1/2}$ of $^{14}$C was 37.0 hours in blood and 90.0 hours in plasma. It should be noted that the apparent $t_{1/2}$ 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. $\text{AUC}_{\text{inf}}$ 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 $^{14}$C ranged between 0.59:1 (1 hour) and 0.89:1 (12 hours) after the dose for all subjects, suggesting that $[^{14}$C$]$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 $[^{14}$C$]$cerlapirdine, cerlapirdine was absorbed into the systemic circulation with median time to peak plasma concentrations ($t_{\text{max}}$) of ~3 hours (range: 1.5–6 hours). Mean $C_{\text{max}}$ and $\text{AUC}_{\text{inf}}$ for cerlapirdine were ~82 ng/ml and 2866 ng eq/ml, respectively. Mean $V_{\text{z/F}}$ and mean $CL/F$ were 2.1 l/kg and 0.024 l/h per kg, respectively, with a mean apparent $t_{1/2}$ of ~60 hours. For desmethylcerlapirdine, median $t_{\text{max}}$ value was ~13 hours (range: 4–48 hours). Mean $C_{\text{max}}$ and $\text{AUC}_{\text{inf}}$ values for desmethylcerlapirdine were 5.0 ng/ml and 507 ng eq/ml, respectively. Desmethylcerlapirdine was eliminated from the systemic circulation with a mean apparent $t_{1/2}$ of ~85 hours. Cerlapirdine and desmethylcerlapirdine accounted for 51 and 9%, respectively, of the total $^{14}$C in plasma. The ratio of desmethylcerlapirdine to cerlapirdine concentrations in plasma, based on their respective $\text{AUC}_{\text{inf}}$ 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 $V_{\text{max}}$ values of 3.4 and 9.4 pmol/pmol P450/min for CYP2C8 and CYP3A4, respectively, and $K_M$ values of 3.3 and 11.2 μM for CYP2C8 and CYP3A4, respectively. When corrected by their corresponding RAF values, the intrinsic clearance ($V_{\text{max}}/K_M$) 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.

![Fig. 6. Metabolite profiles in 0–96 hour urine pooled sample after a single oral 5-mg (177 nCi) dose of $[^{14}$C$]$cerlapirdine.](image-url)
The reason behind the apparent discrepancy between the chemical inhibition method and the recombinant P450 method on the contribution assessed for CYP2C8 and CYP3A4 is not clear; therefore, a range for their respective contributions was reported. The interindividual variability in the systemic exposure of cerlapirdine in the present study is relatively low (25–30% coefficient of variation), which suggests that CYP3A4 may not play a major role in the clearance of cerlapirdine. CYP2C8 genetic polymorphisms have been reported where the reduced function alleles CYP2C8*2 (found only in African Americans with an allele frequency of 0.18) and CYP2C8*3 (occurred primarily in Caucasians with an allele frequency of 0.13) may potentially lead to variability in pharmacokinetics, clinical response, and toxicity (Dai et al., 2001). However, the size of the present study (n = 6 subjects) is likely insufficient to detect such occurrences if they indeed exist.

Metabolite Profiling and Identification in Plasma, Urine, and Feces. The radiochromatogram from the 0–72 hours AUC plasma pool is depicted in Fig. 5. The major peak (~92% of total sample radioactivity) coeluted with cerlapirdine. One other peak was observed, coeluting with desmethylcerlapirdine. No other metabolite was identified in the plasma pooled sample.

The amounts of 14C associated with peaks observed in the radiochromatograms obtained for pooled urine and feces samples are summarized in Table 3, and the metabolite profiles are depicted in Figs. 6 and 7. In the pooled 0–96 hours urine sample (Fig. 6), the major peak present (7.1% of the administered dose) coeluted with cerlapirdine-N-oxide (M3). A second peak coeluted with cerlapirdine (4.9% of the administered dose), and another with desmethylcerlapirdine (M1; 3.1% of the administered dose). A fourth peak, representing 2.6% of the administered dose, did not align with any of the available reference standards.

In the 96–168 hours pooled urine sample (data not shown), desmethylcerlapirdine, cerlapirdine-N-oxide, the unknown peak, and cerlapirdine were observed, representing 1.5, 1.1, 0.6, and 0.8% of the administered dose, respectively. In the 0–96 hours pooled feces homogenate radiochromatograms (Fig. 7), cerlapirdine and desmethylcerlapirdine peaks were observed, with the parent compound showing higher abundance (24.5% of the administered dose) compared with the metabolite (14.7%) in the earlier time point sample and the metabolite showing higher abundance (5.4% compared with 3.4%) in the later time point (96–168 fecal pooled sample, data not shown). Cerlapirdine-N-oxide or any other metabolite peak besides cerlapirdine and M1 was not observed in either the 0–96 hours or the 96–168 hours feces homogenate sample pools.

Metabolite structures in the collected metabolite peaks from plasma, urine, and feces homogenate sample pools were further confirmed by comparing the collision-induced mass spectra and with high resolution mass spectra of the metabolites to the authentic reference standards. Representative exact mass spectra for cerlapirdine, desmethylcerlapirdine, and cerlapirdine-N-oxide observed in fractions collected from the 0–96 hours urine sample pool, and the corresponding authentic standards are shown in Fig. 8. Similar results were observed in fractions from plasma, fecal homogenates, and the 96–168 hours urine sample pools. For cerlapirdine, the parent ion at m/z 365.15413 (2.1 ppm) possesses the proper exact mass protonated molecular ion. The main fragment ions at m/z 365.09621 (2.1 ppm) represents the loss of dimethyamine, whereas fragments at 219.02271 (C9H7N2O2S; 2.0 ppm) and 173.07123 (C9H8N2O; 1.68 ppm) represent fragmentation around both the dimethyamino and sulfone moieties (Fig. 8A). The desmethylcerlapirdine (M1) peak, which elutes on the liquid chromatography column slightly ahead of the parent compound, shows a parent ion at m/z 396.13779 and is consistent with an empirical formula of C21H22N3O3S (0.38 ppm). The fragment ions at m/z 365.09582, 219.02249, and 173.07098 are the same as for the parent drug (Fig. 8B).

Cerlapirdine-N-oxide, M3, was observed only in urine. The mass spectrum of metabolite in urine fraction collected at a retention time aligned with the N-oxide shows a protonated molecular ion of m/z 426.14812, which is consistent with an empirical formula of C22H34N2O,S (0.20 ppm). The later retention time than parent is consistent with an N-oxide metabolite on the dimethylamino nitrogen; however, the daughter spectrum was not informative.

The pooled urine radiochromatogram shows a small peak not associated with the parent drug, desmethyl, or N-oxide metabolites at a retention time of approximately 37.5 minutes, which may suggest the presence of another metabolite. This peak accounted for ~10 and 15% of the total 14C recovered for the 0–96 hours and the 96–168 hours sample pools, respectively. However, the mass spectral data did not offer enough information to propose a structure for this material.

Discussion

Cerlapirdine is a 5-HT6 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 [14C]cerlapirdine was conducted to elucidate the metabolism and disposition of cerlapirdine. Because of the long retention time of cerlapirdine in pigmented tissues, a low radioactive dose (≤200 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 14C drug-related material in the plasma, urine, and fecal samples obtained from this study. The overall results show that cerlapirdine is well absorbed (at least 70% based on amounts of unchanged cerlapirdine in feces and amounts of metabolites excreted in urine and feces) after oral administration. Recovery of 14C was almost complete (mean total recovery was 98.3%) within 14 days; feces was...
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<sub>inf</sub> 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<sub>0-72</sub> values were approximately 80% of the 14C AUC<sub>0-72</sub> 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

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.
desmethylcerlapirdine 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-
ceralpirdine 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
ceralpirdine. 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
\(^{14}C\), 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 characteriza-
tion and identification.

Since its introduction, AMS has increasingly been used in phar-
maceutic research and development (Lappin and Stevens, 2008;
Garner, 2010; Lappin and Seymour, 2010). The ultrahigh sensitivity
of AMS (by measure isotopic \(^{14}C\) 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 mCi. However, in the
case of ixabepilone, which shows radiolytic instability at high specific
activity but is stable at 1–2 nCi/mg (Comezolglu 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 ex-
posure. 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 $^{14}$C was virtually complete, and the metabolic and elimination pathways for caripapride 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.

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