In Vitro Hepatic Metabolism of Cediranib, a Potent Vascular Endothelial Growth Factor Tyrosine Kinase Inhibitor: Interspecies Comparison and Human Enzymology

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ABSTRACT:
The in vitro metabolism of cediranib (4-[(4-fluoro-2-methyl-1H-indol-5-yl)oxy]-6-methoxy-7-[3-(1-pyrrolidinyl)propoxy]quinazoline), a vascular endothelial growth factor (VEGF) tyrosine kinase inhibitor (TKI) of all three VEGF receptors in late-stage development for the treatment of colorectal cancer and recurrent glioblastoma was investigated in hepatic proteins from preclinical species and humans using radiolabeled material. In human hepatocyte cultures, oxidative and conjugative metabolic pathways were identified, with pyrrolidine N'-glucuronidation being the major route. The primary oxidative pathways were di-and trihydroxidations and pyrrolidine N-oxidation. All metabolites with the exception of the N'-glucuronide metabolite were observed in rat and cynomolgus monkey hepatocyte preparations. Additional metabolism studies in liver microsomes from these or other preclinical species (CD-1 mouse, Han Wistar rat, Dunkin Hartley guinea pig, Goettingen mini-pig, New Zealand White rabbit, beagle dog, and cynomolgus and rhesus monkey) indicated that the N'-glucuronide metabolite was not formed in these additional species. Incubations with recombinant flavin-containing mono-oxygenase (FMO) and UDP-glucuronosyltransferase (UGT) enzymes and inhibition studies using the nonselective cytochrome P450 (P450) chemical inhibitor 1-aminobenzotriazole in human hepatocytes indicated that FMO1 and FMO3 contributed to cediranib N'-glucuronidation. P450 enzymes had only a minor role in the metabolism of cediranib. In conclusion, species differences in the formation of the N'-glucuronide metabolite of cediranib were observed. All other metabolites of cediranib found in humans were also detected in rat and cynomolgus monkey. Non-P450 enzymes are predominantly involved in the metabolism of cediranib, and this suggests that clinical drug interactions involving other coadministered drugs are unlikely.

Introduction

Cediranib (4-[(4-fluoro-2-methyl-1H-indol-5-yl)oxy]-6-methoxy-7-[3-(1-pyrrolidinyl)propoxy]quinazoline; Recentin; AZD2171) (Fig. 1) is a highly potent vascular endothelial growth factor (VEGF) inhibitor, with activity against all three VEGF receptor tyrosine kinases and stem cell factor receptor. It is currently in phase III clinical trials for the treatment of colorectal cancer and recurrent glioblastoma. VEGF is involved in the regulation of key processes throughout the angiogenic cascade, and overproduction of VEGF in tumors facilitates tumor progression by stimulating angiogenesis and increasing vascular permeability. Inhibition of VEGF receptor tyrosine kinases stabilizes the progression of tumors by disrupting tumor-induced angiogenesis (Kim et al., 1993). Preclinical studies have shown that cediranib prevents VEGF-induced angiogenesis in vivo and inhibits the growth of established human tumor xenografts in athymic mice in a dose-dependent manner (Wedge et al., 2005). In the clinic, cediranib has exhibited activity in patients with advanced colorectal cancer and recurrent glioblastoma (Batchelor et al., 2007; Drevs et al., 2007; Chen et al., 2009).

To gain an understanding of the species differences in the disposition of cediranib and preclinical coverage of human metabolites, the hepatic metabolism of [14C]cediranib was investigated in liver preparations from preclinical species and humans. The in vitro metabolic profiles and identity of cediranib metabolites were assessed, and a proposed metabolic pathway was suggested. The identity of the hepatic human phase I and II enzymes involved in the oxidation and conjugation of cediranib were confirmed and are reported here.

Materials and Methods

Chemicals and Materials. Dexamethasone, insulin, penicillin-streptomycin, Williams’ E medium, β-NADPH, alamethicin, t-(+)-glucuronic acid, acetaminophen, 1-aminobenzotriazole (1-ABT), dimethyl

ABBREVIATIONS: AZD2171, 4-[(4-fluoro-2-methyl-1H-indol-5-yl)oxy]-6-methoxy-7-[3-(1-pyrrolidinyl)propoxy]quinazoline; VEGF, vascular endothelial growth factor; 1-ABT, 1-aminobenzotriazole; UGT, UDP glucuronosyltransferase; FMO, flavin monoxygenase; HPLC, high-performance liquid chromatography; RAD, radiochemical detection; MS, mass spectrometry; UDPGA, uridine diphosphate glucuronic acid; P450, cytochrome P450; amu, atomic mass units.
sulfoxide-d$_4$, [14C]7-ethoxyquinazoline (specific activity, 56 mCi/mmol), and [14C]acetaminophen (specific activity, 5.4 mCi/mmol) were purchased from Sigma-Aldrich (Poole, UK). Fetal bovine serum (10%), gentamicin, Leibovitz L-15 media, and HEPES buffer were purchased from Invitrogen (Paisley, UK). Matrigel was obtained from BD Biosciences (San Jose, CA). [14C]acetaminophen (specific activity, 5.4 mCi/mmol) was purchased from Novacta Biosystems (Welwyn Garden City, UK). Thermo Fisher Scientific (Waltham, MA) supplied acetonitrile and methanol. All other chemicals or solvents were purchased from commercial suppliers and were of analytical grade or the best equivalent.

Hepatocytes. Viable fresh hepatocytes were isolated from male Han-Wistar rats (AstraZeneca UK Ltd.) using the standard collagenase perfusion technique. Freshly isolated human hepatocytes from two male (donor 1, unknown race, aged 39 years; donor 3, Asian, aged 38 years) and three female donors (donor 2, unknown race, aged 68 years; donor 4, white, aged 60 years; and donor 5, unknown race, aged 45 years) were purchased from the United Kingdom Human Tissue Bank (Leicester, UK). All human liver-derived samples were obtained with permission from the relevant local ethics committees. Freshly isolated cynomolgus monkey hepatocytes were purchased from Covance (Harrogate, UK). Cell yield and viability were assessed using trypan blue.

Liver Microsomes. Pooled human liver microsomes (28 male donors and 5 female donors; lot 63103) were obtained from BD Biosciences. Pooled liver microsomes from cynomolgus monkey (8 male donors; lot 3), Dunkin-Hartley guinea pig (8 male donors; lot 12635), and rhesus monkey (3 male donors; lot 71911), rats (4 male donors; lot 1), New Zealand White rabbit (3 male donors; lot 98358), CD-1 mouse (150 male donors; lot 80662), beagle dog (20 male donors; lot 11969), and Han-Wistar rat (30 male donors; lot 37975) were purchased from BD Biosciences. These samples were stored at −80°C in a controlled tissue collection until use.

Recombinant Enzymes. Samples of microsomal fractions prepared from insect cells transfected with plasmid vectors expressing human UGT1A1 (lot 13), UGT1A3 (lot 13), UGT1A4 (lots 9, 10, 11, 34582, and 59840), UGT1A6 (lot 10), UGT1A7 (lot 41931), UGT1A8 (lot 36867), UGT1A9 (lot 8), UGT1A10 (lot 51067), UGT2B4 (lot 4), UGT2B7 (lot 55297), UGT2B15 (lot 8), and UGT2B17 (lot 4) were purchased from BD Biosciences. Microsomes prepared from insect cells that expressed human FMO1 (lot 5251), FMO3 (lot 64186), and FMO5 (lot 56381) and microsomes from cells that contained the empty transfection vector were also obtained from BD Biosciences.

Characterization of [14C]Cediranib. Stock solutions of [14C]cediranib were diluted in either 0.2% (v/v) formic acid in water-0.2% formic acid in acetonitrile (95:5; v/v) or water-acetonitrile (80:20, v/v) to concentrations in the range of 74 to 100 μM (dependent on specific activity of the batch used for each experiment), before the radiochemical purity was assessed by HPLC with radiochemical detection (RAD).

Hepatocyte Suspension Incubations. Human hepatocytes (donors 1, 2, and 3; approximately 1 × 10^6 cells/ml) were preincubated at 37°C in Leibovitz L-15 media in the absence and presence of 1-ABT (1 mM final) for 15 min before incubation with [14C]cediranib. [14C]Cediranib (prepared as a 3 mM solution in methanol) was diluted 1:300 with the microsomal suspension to achieve a final concentration of 10 μM (arbitrary concentration). Incubations with [14C]cediranib were maintained at 37°C for up to 4 h in a shaking water bath and were terminated with an equal volume of ethanol. Appropriate control incubations were also performed. The positive control compound, [14C]7-ethoxyquinazoline (20 μM), was incubated in parallel to [14C]cediranib incubations. All samples were vortex-mixed and centrifuged at 14,171 g for up to 10 min, and the resulting supernatants were stored at −20°C until analysis by HPLC with RAD and mass spectrometry (MS*) detection. Assays were performed in duplicate.

Hepatocyte Culture Incubations. Incubations of hepatocytes (donors 4 and 5) with [14C]cediranib were based on the method described previously (LeCluyse et al., 2000) with minor modifications. Hepatocytes (132,000 viable cells/cm²) were plated onto 12-well plates (growth areas of 3.8 cm²/well) precoated with collagen I (BD Biosciences) and sandwiched with a top layer of Matrigel (250 μg/ml). After a 12- to 24-h stabilization period, hepatocytes were incubated with [14C]cediranib [10 μM final (arbitrary concentration); initially dissolved in methanol as a 3 mM stock solution] in Williams’ E medium containing dexamethasone (30 nM), gentamicin (50 μg/ml), HEPES buffer (10 mM), insulin (170 nM), and penicillin-streptomycin (102 U/ml; 100 μg/ml). The total incubation volume was 1 ml. Hepatocytes were incubated for up to 72 h (24 h for donor 4) at 37°C in an atmosphere of 5% CO₂ in air. Reactions were terminated with 1/2 volume of ethanol. Appropriate control incubations were also performed. [14C]7-ethoxyquinazoline (20 μM), was incubated in parallel to [14C]cediranib incubations. Samples were vortexed and centrifuged at 14,171 g for 10 min to remove precipitated protein/cell pellets, and the resulting supernatants were stored at −20°C until analysis by HPLC with RAD and MS*. Assays were performed in duplicate.

Hepatic Subcellular Fraction Incubations. [14C]Cediranib [10 μM final (arbitrary concentration); initially dissolved in methanol as a 2 mM stock solution] was incubated with human liver microsomes, cytosol, and S9. The incubations were performed at a protein concentration of 1 mg/ml in phosphate buffer (100 mM; pH 7.4), with 1 mM NADPH or NAD. Incubations were maintained at 37°C for up to 120 min in a shaking water bath and terminated with an equal volume of ethanol. All samples were centrifuged and stored in the dark at −20°C until analysis by HPLC with radiometric detection.

Phase II Hepatic Microsomal and Recombinant UGT Enzyme Incubations. Incubations (1 ml) of liver microsomes from humans, cynomolgus monkey, Dunkin-Hartley guinea pig, Göttingen mini-pig, rhesus monkey, New Zealand White rabbit, CD-1 mouse, beagle dog and Han-Wistar rat (all at 1 mg of protein/ml) with [14C]cediranib [10 μM final (arbitrary concentration); initially dissolved in methanol as a 3 mM stock solution] were conducted in phosphate buffer (100 mM; pH 7.4) containing 3.3 mM magnesium chloride (MgCl₂) and 50 μg of alamethicin/mg protein. The solvent attributable to the alamethicin was evaporated to dryness under a stream of nitrogen at 35°C in the incubation tubes before the addition of the remaining preincubation components. After a 5-min preincubation period, the reactions were initiated by the addition of UDPGA (4 mM final). After a 60-min incubation at 37°C, aliquots were removed into an equal volume of ethanol. Appropriate control incubations were also performed. The positive control compound, [14C]acetaminophen (10 μM), was incubated in parallel to [14C]cediranib incubations. Samples were centrifuged (14,171 g) for 10 min to remove precipitated protein, and the resulting supernatant was stored at −80°C until subjected to analysis by HPLC-RAD and HPLC-MS*. Assays were performed in duplicate.

For incubations with individual recombinant UGT enzymes, the incubation mixtures contained the same components, except that a final protein concentration of 0.25 mg of UGT protein/ml was used. In addition, after a 60-min incubation at 37°C, aliquots were removed into an equal volume of 0.05% formic acid in ethanol.

Recombinant FMO Enzyme Incubations. Microsomes prepared from insect cell lines that heterologously expressed human FMO1, FMO3, and FMO5 (all at 0.2 mg/ml) were incubated with [14C]cediranib [5 μM final (arbitrary concentration); initially dissolved in methanol as a 1 mM stock

![Chemical structure of [14C]cediranib](https://example.com/structure.png)

**Fig. 1.** Chemical structure of [14C]cediranib.
solution] in phosphate buffer (100 mM; pH 7.4), with 1 mM NADPH. Incubations were maintained at 37°C for 120 min and terminated with an equal volume of ethanol. In addition, control incubations were performed with microsomes from cells that contained the empty transfection vector. All samples were centrifuged and stored in the dark at −20°C until analysis by HPLC with radiometric detection.

**Enzyme Kinetics of [14C]Cediranib N-Oxidation in Recombinant FMO1 and FMO3 Enzymes.** N-Oxidation of [14C]cediranib was conducted under linear conditions with respect to incubation time and protein concentration. A range of concentrations (0.2–50 μM) of [14C]cediranib were incubated with either 0.02 mg/ml FMO1 or 0.2 mg/ml FMO3 protein in phosphate buffer (100 mM; pH 7.4) for 10 (FMO1) or 20 min (FMO3) at 37°C in the presence of NADPH (1 mM). Incubations were terminated by the addition of an equal volume of ethanol followed by centrifugation at 14,171 g for 10 min. The resultant supernatants were stored at −20°C until analysis by HPLC with radiochemical detection.

**Metabolite Profiling by HPLC-RAD.** [14C]Cediranib and its metabolites were separated on an ACE AQ column (4.6 × 150 mm, 5 μm; Advanced Chromatography Technologies, Aberdeen, UK) using 200 Series pumps (PerkinElmer Life and Analytical Sciences, Beaconsfield, UK) at a flow rate of 1 ml/min. The aqueous mobile phase (solvent A) consisted of 0.2% (v/v) formic acid in deionized water, whereas the organic mobile phase (solvent B) consisted of 0.2% (v/v) formic acid in acetonitrile. The initial mobile phase consisted of 5% solvent B, which was increased to 20% over a period of 15 min and then further increased to 25% in 20 min, maintained for 25 min, and finally increased to 50% over 5 min. This value was held for 10 min before a return to 5% solvent B for column equilibration for 10 min before the next injection. Radiochemical detection was performed using a Radiomatic Flo-One Beta 500 TR series detector (PerkinElmer Life and Analytical Sciences) with a 500-μl liquid flow cell and Ultima Flo-M scintillation cocktail running at 3 ml/min. The data were collected and analyzed using either FLO-ONE (version 3.65; FIG. 2. Representative HPLC-RAD metabolite profiles after incubation of [14C]cediranib (10 μM) with human hepatocyte suspensions (donor 2; 1 × 10⁶ cells/ml, at 37°C for 240 min) in the absence (A) and presence (B) of 1-ABT (1 mM) and with human hepatocyte cultures (C) (donor 5, 132,000 viable cells/cm², at 37°C for 72 h; 5% CO₂). The proportion of radioactivity attributed to each metabolite is shown in Tables 1 and 2.

FIG. 3. Representative HPLC-RAD metabolite profiles after incubation (at 37°C for 72 h; 5% CO₂) of [14C]cediranib (10 μM) with rat (A) and cynomolgus monkey (B) hepatocyte cultures (132,000 viable cells/cm²). The proportion of radioactivity attributed to each metabolite is shown in Table 1.
Metabolite Identification/Characterization by 1H NMR Spectroscopy.

The metabolism of [14C]cediranib was assessed by both qualitative and quantitative analysis of the chromatographic patterns. Radio-labeled components were assumed to have the same specific activity as parent molecule. MS² data were obtained for the metabolites identified in the analysis by HPLC-MS² with radiometric detection. Components were identified as being derived from [14C]cediranib if they demonstrated elements of the characteristic isotopic and fragmentation patterns observed with the parent molecule. Enzyme kinetic parameters (Km and Vmax) were determined by nonlinear regression using GraFit (version 5.0.10; Erithacus Software, Horley, Surrey, UK).

Results

Metabolite Profile of [14C]Cediranib in Hepatocytes. The metabolic profiles of [14C]cediranib in human hepatocyte suspension and culture are as highlighted in Figs. 2 and 3, respectively. In human hepatocyte suspension incubations, only one major metabolite, M3, was detected, which accounted for up to 10.8% of total chromatogram radioactivity (Fig. 2A; Table 1). Minor components included metabolites M1 and M2. The formation of M3 as well as that of M1 and M4 was not inhibited in the presence of the nonselective P450 chemical inhibitor, 1-ABT (Fig. 2B).

Consistent with the findings in human hepatocyte suspensions, [14C]cediranib was metabolized to these four components as well as to two further minor metabolites M5 and M7 in human hepatocyte cultures (Fig. 2C; Table 1). With the exception of metabolite M3, all other metabolites detected in human hepatocytes were also observed in either rat and/or cynomolgus monkey hepatocyte cultures (Fig. 3).

Unlike in humans, M4 was the major metabolite in both rat and cynomolgus hepatocyte incubations and accounted for 30 and 9%, respectively, of total chromatogram radioactivity. Metabolites M8, M9, M10, M11, and M12 could not be separated chromatographically; the value stated for M1 is the combined proportion of total chromatogram radioactivity for metabolites M1 and M5.

| TABLE 1 | Metabolite profiles after incubations of 10 μM [14C]cediranib with human hepatocyte suspensions (1 × 10⁶ cells/ml, 240 min) in the absence and presence of 1 mM 1-ABT and with hepatocyte cultures (132,000 viable cells/cm², 72 h; 24 h for human donor 4) of rat, cynomolgus monkey, and human

<table>
<thead>
<tr>
<th>Compound</th>
<th>In Vitro Hepatic Metabolism of Cediranib</th>
<th>Incubations with Human Donor 1</th>
<th>Incubations with Human Donor 2</th>
<th>Incubations with Human Donor 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Without 1-ABT</td>
<td>With 1-ABT</td>
<td>Without 1-ABT</td>
</tr>
<tr>
<td>M1</td>
<td></td>
<td>1.6</td>
<td>1.2</td>
<td>1.3</td>
</tr>
<tr>
<td>M2</td>
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<td>9.6</td>
<td>9.6</td>
<td>9.6</td>
</tr>
<tr>
<td>M3</td>
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<td>1.2</td>
</tr>
<tr>
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<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td>M5</td>
<td></td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
</tr>
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<td>M6</td>
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<td>1.3</td>
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</tr>
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<td>M8</td>
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<td>M9</td>
<td></td>
<td>2.5</td>
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<tr>
<td>M10</td>
<td></td>
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<td>1.3</td>
<td>1.3</td>
</tr>
<tr>
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<td></td>
<td>2.5</td>
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</tr>
<tr>
<td>M12</td>
<td></td>
<td>1.3</td>
<td>1.3</td>
<td>1.3</td>
</tr>
<tr>
<td>Cediranib</td>
<td></td>
<td>30.1</td>
<td>30.1</td>
<td>30.1</td>
</tr>
</tbody>
</table>

### Notes

- a Data with 1-ABT are not available.
- b, c, d, and e refer to peaks for M1 and M5 that could not be separated chromatographically; the value stated for M1 is the combined proportion of total chromatogram radioactivity for metabolites M1 and M5.

### References

- [14C]Acetaminophen and its metabolites were separated on an Eclipse XDB C18 column (4.6 × 150 mm, 5 μm; Agilent Technologies UK Ltd.) using 1200 Series HPLC pumps with a flow rate of 1 ml/min. The aqueous mobile phase (solvent A) consisted of ammonium acetate in deionized water (10 mM; pH 5.5), and the organic mobile phase (solvent B) consisted of methanol.

- The initial mobile phase consisted of 10% solvent B, which was maintained for 3 min before the next injection.

- This value was held for 4.5 min before a return to 5% solvent B for column equilibration for 4.9 min before the next injection.

- The metabolism of [14C]cediranib was assessed by both qualitative and quantitative analysis of the chromatographic patterns. Radio-labeled components were assumed to have the same specific activity as parent molecule. MS² data were obtained for the metabolites identified in the analysis by HPLC-MS² with radiometric detection. Components were identified as being derived from [14C]cediranib if they demonstrated elements of the characteristic isotopic and fragmentation patterns observed with the parent molecule. Enzyme kinetic parameters (Km and Vmax) were determined by nonlinear regression using GraFit (version 5.0.10; Erithacus Software, Horley, Surrey, UK).
TABLE 2

Summary of mass spectrometric data for [14C]cediranib and its metabolites

<table>
<thead>
<tr>
<th>Assignment</th>
<th>[M + H]+ (m/z)*</th>
<th>Proposed Structure</th>
<th>Diagnostic MS² Fragment Ions (m/z)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cediranib</td>
<td>453 (P)</td>
<td><img src="image" alt="Cediranib" /></td>
<td>342, 326, 112</td>
</tr>
<tr>
<td>M1</td>
<td>485 (P + 32)</td>
<td><img src="image" alt="Di-oxygenated" /></td>
<td>467 (loss of H₂O), 358, 342</td>
</tr>
<tr>
<td>M5</td>
<td>501 (P + 48)</td>
<td><img src="image" alt="Tri-oxygenated" /></td>
<td>483 (loss of H₂O), 389, 374</td>
</tr>
<tr>
<td>M6</td>
<td>Unassigned</td>
<td><img src="image" alt="Fluoro-indole, carboxylic acid" /></td>
<td>No data available</td>
</tr>
<tr>
<td>M7</td>
<td>483 (P + 30)</td>
<td><img src="image" alt="Fluoro-indole, carboxylic acid" /></td>
<td>465 (loss of H₂O), 439, 372, 356</td>
</tr>
<tr>
<td>M2</td>
<td>485 (P + 32)</td>
<td><img src="image" alt="Di-oxygenated" /></td>
<td>342</td>
</tr>
<tr>
<td>M8</td>
<td>248 (P - 285 + 80)</td>
<td><img src="image" alt="Fluoro-indole monooxygenated sulfate" /></td>
<td>168</td>
</tr>
<tr>
<td>M3</td>
<td>629 (P + 176)</td>
<td><img src="image" alt="N-glucuronide" /></td>
<td>453 (aglycone), 342, 326</td>
</tr>
</tbody>
</table>
M10, and M11 were detected in rat and cynomolgus monkey hepatocyte samples but not in human hepatocytes, whereas metabolites M6 and M10 were detected only in cynomolgus monkey hepatocytes (Table 1). The mass spectral data for cediranib and its metabolites are summarized in Table 2.

The recovery of sample radioactivity from the HPLC column for human hepatocyte suspension (donor 3) and culture (donor 5) incubations was 102 and 99.3%, respectively. For rat and cynomolgus monkey hepatocyte cultures, these values were 98.5 and 94.3%, respectively.

**Metabolite Identification in Hepatocytes.** Based on mass spectrometric data, metabolite M3 showed a [M + H]$^+$ ion of m/z 629 corresponding to a gain of 176 atomic mass units (amu) from $[^{14}C]$cediranib (m/z 453) (Table 2), consistent with the addition of a glucuronic acid moiety to the molecule. The exact site of conjugation in metabolite M3 was confirmed by HPLC-MS$^n$ and $[^1H]$ NMR, which indicated conjugation on the pyrrolidine nitrogen (Lenz et al., 2010). The proton NMR chemical shifts and coupling constants of a biosynthesized standard of metabolite M3 are included in Table 3.

For metabolite M4, an [M + H]$^+$ ion at m/z 469 was characteristic of monooxygenation. In addition, a major fragment at m/z 128, corresponding to a gain of 16 amu on the propyl pyrrolidinyl moiety (m/z 112) suggested that oxidation occurred on this group (Table 2). The synthetic standard for cediranib pyrrolidine N-oxide showed a similar retention time and mass fragmentation profile, thus confirming that the structural identities of metabolite M4 and cediranib N-oxide were identical. The proton NMR chemical shifts and coupling constants of the synthetic standard for cediranib pyrrolidine N-oxide (M4) are included in Table 3.

The mass spectrum of metabolite M7 showed an [M + H]$^+$ ion at m/z 483, 30 amu higher than that of protonated $[^{14}C]$cediranib. In addition, the presence of two major fragments at m/z 356 and 372, corresponding to increments of +30 amu on the fluoro-indole quinazoline moiety (m/z 326 and 342, respectively), suggested that oxidations had occurred on this group, resulting in the formation of the carboxylic acid M7 (Table 2).

Metabolites M1, M2, and M5 had mass spectral profile characteristics of di- and trioxygenations. However, precise positional assignment of these oxidations was not possible on the basis of the MS$^n$ fragmentation data.

The mass spectrum of metabolite M8 showed an [M + H]$^+$ ion at m/z 248, 205 amu less than that of protonated $[^{14}C]$cediranib. Tandem mass spectrometry of protonated M8 produced one major fragment at m/z 168, corresponding to the loss of a sulfate moiety. The results suggest that metabolite M8 is a fluoro-indole monoxygenated sulfate.

Metabolite M11 displayed an [M + H]$^+$ ion of m/z 414, corresponding to a loss of 39 amu from $[^{14}C]$cediranib (m/z 453) (Table 2) and a predominant MS/MS fragment at m/z 342. This finding is consistent with the loss of the pyrrolidine ring and oxidation to form the propanoic acid. No molecular ion assignments were possible for metabolites M6, M9, M10, and M12.

**Oxidative Metabolic Profiles of $[^{14}C]$Cediranib in Human Hepatic Microsomal Fractions.** Minor metabolism (<12% depletion of $[^{14}C]$cediranib total chromatogram radioactivity) was observed after incubation of $[^{14}C]$cediranib with human liver microsomes and S9 in the presence of NADPH (data not shown). Up to five components were observed; however, the structure of only one could be elucidated. This component identified in S9 accounted for 6.7% of the total radiochromatogram radioactivity, and the mass fragmentation profile was consistent with that for metabolite M4.

The oxidative metabolites M1, M2, and M5 that were detected in human hepatocytes were not formed in the presence of NADPH in either human liver microsomes or S9. No metabolism was observed in the human cytosolic incubate samples. Likewise, no metabolites were detected in the incubation of $[^{14}C]$cediranib with human liver microsomes or S9 in the presence of the cofactor, NAD.

**Involvement of FMO Enzymes in the Metabolism of $[^{14}C]$Cediranib.** A role for FMO enzymes in the metabolism of $[^{14}C]$cediranib was anticipated on the basis of the role of these enzymes in N-oxidation reactions (Cashman, 1995) and on the inability of the nonselective P450 inhibitor, 1-ABT, to inhibit the formation of the N-oxide metabolite of $[^{14}C]$cediranib (metabolite M4) in human hepatocytes (Fig. 2).

Incubation of $[^{14}C]$cediranib (5 μM) with microsomes prepared from cell lines expressing a single FMO isoform indicated that in the
presence of FMO1, extensive metabolism of [14C]cediranib was observed, with only metabolite M4 (accounting for 85.0% of the total chromatogram radioactivity) being generated (data not shown). Likewise, for FMO3 only metabolite M4 was detected, which accounted for 60.6% of the total chromatogram radioactivity. FMO5 and insect control enzymes displayed no catalytic activity for the formation of metabolite M4.

Enzyme kinetic experiments for [14C]cediranib N-oxide (metabolite M4) in recombinant FMO1 showed that the rate of formation conformed to Michaelis-Menten kinetics characterized by values for an apparent $K_m$ of 11.9 M and $V_{max}$ of 8.2 nmol Eq/(min mg) protein (Fig. 4). The kinetic parameters for metabolite M4 in recombinant FMO3 could not be calculated, because the rate of formation did not reach $V_{max}$ over the concentration range tested (Fig. 4). Solubility limitations prevented the use of higher [14C]cediranib concentrations.

**Phase II Metabolic Profiles of [14C]Cediranib in Liver Microsomes.** Human liver microsomal incubations containing [14C]cediranib and UDPGA resulted in the formation of a single metabolite (M3; 15.2% of total chromatogram radioactivity), as observed by HPLC with radiochemical detection (results not shown). A small component representing approximately 1% of the total chromatogram radioactivity with a retention time of 27.5 min was also detected in the human liver microsome sample; however, because this component was also present in the control incubates, it was considered not of metabolic origin. No metabolites were detected after incubation of [14C]cediranib in microsomes from cynomolgus monkey, Dunkin-
Hartley guinea pig, Göttingen mini-pig, rhesus monkey, New Zealand White rabbit, CD-1 mouse, beagle dog, or Han Wistar rat in the presence of UDPGA (data not shown).

Involvement of UGT Enzymes in the Metabolism of [14C]Cediranib. The UGT enzymes responsible for the N-glucuronidation of [14C]cediranib were identified using recombinant UGT enzymes. UGT enzyme identification studies in human liver microsomes were not performed because of limited availability of a complete set of UGT selective chemical inhibitors (Miners et al., 2004; Uchaipichat et al., 2006).

Incubation of [14C]cediranib (10 μM) with microsomes prepared from cell lines expressing a single UGT isoform indicated that the metabolism of [14C]cediranib was limited to UGT1A4 (Fig. 5), with a reduction observed in the amount of chromatogram radioactivity represented by [14C]cediranib and the formation of one major component (M3; 16.2% of total chromatogram radioactivity). Incubations with UGTs 1A1, 1A3, 1A6, 1A7, 1A8, 1A9, 1A10, 2B4, 2B7, 2B15, and 2B17 showed no evidence of metabolism of [14C]cediranib (Fig. 5).

Discussion

The present study was undertaken to elucidate the biotransformation pathways and identify the enzymes involved in the in vitro metabolism of cediranib. It has been demonstrated that the in vitro metabolism of cediranib proceeds predominantly via non-P450 enzymes, with the UGT and FMO enzymes playing a major role.

In vitro metabolism studies have shown that cediranib was metabolized to at least 11 components in hepatic proteins from human, rat, and cynomolgus monkey. The proposed metabolic pathway of [14C]cediranib is summarized in Fig. 6.

Incubations with hepatic in vitro systems have highlighted the fact that marked species differences in the N-glucuronidation of cediranib were observed. The pyrrolidine N-glucuronide metabolite (M3) was only produced in humans and was not detected in the other species tested. This finding was supported by previous studies in which quaternary N-glucuronidation of cyclic tertiary amines was shown to exhibit distinct species differences, with a general trend that only human and higher primates have the capability of catalyzing such reactions (Chaudhuri et al., 1976; Fischer et al., 1980; Delbressine et al., 1992; Chiu and Huskey, 1998). These studies have shown that several cyclic tertiary amine-containing drugs, the antihistamines cyproheptadine and tripelennamine and the antidepressant mianserin, also undergo human-specific N-glucuronidation. Of all the species studied, the N-glucuronide metabolites of these drugs were only detected in the urine of higher primates and/or humans. For cediranib, the N-glucuronide metabolite (M3) has been identified in the
plasma and feces of humans after a single oral dose of 45 mg of [14C]cediranib; however, it has not been detected in vivo in plasma, urine, or feces after a single oral dose of 0.5 mg/kg [14C]cediranib to beagle dogs, in vivo or urine or feces after a single oral dose of 1.0 mg/kg [14C]cediranib to cynomolgus monkeys, or in vivo in urine, plasma, or bile after a single oral dose of 30 mg/kg [14C]cediranib to Sprague-Dawley rats (unpublished data). Additional data to be published at a later date will support the conclusion that M3 is a major metabolite in humans and should be considered in this context.

The enzyme catalyzing the N\textsuperscript{-}glucuronidation of cediranib was shown using recombinant proteins to be human UGT1A4. This observation is consistent with previous studies confirming that N\textsuperscript{-}glucuronidation of cyclic tertiary amines usually proceeds via UGT1A4 (Green et al., 1995; Green and Tephly, 1996, 1998; Kubota et al., 2007). Information on the interspecies variation in the expression of this enzyme is limited, although it is well established that in the rat, a pseudo-gene is present in the UGT1 gene complex that encodes for an incomplete UGT1A4 protein, leaving the rat without a functional UGT1A4 enzyme (Green and Tephly, 1998). This could potentially provide an explanation for the lack of N\textsuperscript{-}glucuronidation observed in this species. With the exception of rabbit, few data on UGT1A4 expression levels in cynomolgus monkey and other laboratory species are available, and thus the inability of the other species to catalyze the formation of a quaternary N\textsuperscript{-}glucuronide of cediranib remains unexplained (Green and Tephly, 1998).

Functional polymorphisms of hepatic UGT1A4 that are predicted to alter the hepatic metabolism and detoxification of selective xenobiotics and steroids have been identified in German Caucasian and Japanese populations (Ehmer et al., 2004; Mori et al., 2005; Saeki et al., 2005). The frequencies of two genetic variations, P\textsuperscript{T} (UGT1A4.2) and L\textsuperscript{494V} (UGT1A4.3) were up to 8 and 17%, respectively (Benoit-Biancamano et al., 2009). Whether these UGT1A4 polymorphisms could potentially influence the interindividual variability in cediranib disposition in vivo and have an impact on an individual’s response to cediranib treatment remains to be assessed, given that functional investigations of UGT1A4 polymorphisms to date have been limited to in vitro systems. In addition, it has also been suggested that the effects of these functional polymorphisms may be dependent on the xenobiotic administered (Mori et al., 2005).

In this study, the human FMO enzymes were shown to be primarily involved in the N-oxidation of cediranib to metabolite M4. Among the FMO enzymes tested, only human FMO1 and FMO3 were able to catalyze this reaction in vitro, with FMO1 being most efficient in catalyzing this reaction. Although FMO1 is the most prominent isoform in adult kidney, its expression is very low in adult liver, with FMO3 being the major isoform in this tissue (Cashman and Zhang, 2006). Based on the kinetic parameters and absolute levels of each enzyme, it is conceivable that in vivo both enzymes may be involved to a similar extent in the N-oxidation of cediranib. A role for P450 enzymes in the formation of this metabolite was excluded, because of a lack of significant inhibition of cediranib N-oxide formation by 1-ABT.

The enzyme(s) responsible for the metabolism of cediranib to metabolite M2 were CYP450 enzymes. As for metabolite M1, the enzymes involved in its formation are unknown and have yet to be determined. However, it is clear that CYP450 enzymes do not play a role, given the lack of inhibition of metabolite formation in the presence of 1-ABT. Based on the structure of cediranib, a possible role for aldehyde oxidase may exist (Beedham, 2001). Although no metabolism of cediranib was observed after incubation with human cytosolic fractions, it should be noted that this finding may be due to the instability of aldehyde oxidase upon preparation and storage of the human hepatic tissue (Lang and Kalogutkar, 2003).

For the formation of metabolite M11, which was only produced in rat and cynomolgus monkey, a potential mechanism involves the hydroxylation of the methylene group adjacent to the pyrrolidine nitrogen, followed by the intermediate formation of the aldehyde through N-dealkylation, which would then be rapidly oxidized to the carboxylic acid M11.

In conclusion, the present study has shown that the major biotransformation pathways of cediranib in humans involve UGT1A4-mediated N\textsuperscript{-}glucuronidation and non-P450-mediated oxidations, with the N-oxidation of cediranib catalyzed by the FMO enzymes. With the exception of the N\textsuperscript{-}glucuronide, all other metabolites of cediranib found in humans were also detected in rat and cynomolgus monkey. As the metabolism of cediranib proceeds via conjugative and non-P450 oxidative pathways, it is predicted that cediranib may be less prone to potential drug-drug interactions from other coadministered xenobiotics. The FMO enzymes are not readily inhibited or induced by xenobiotics (Cashman and Zhang, 2006; Mitchell, 2008), and the magnitude of UGT-based drug interactions is significantly less compared with that of P450-mediated interactions (Williams et al., 2004; Kiang et al., 2005).

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**References**


and regulation of cytochrome P450 enzymes in primary cultures of human hepatocytes. 

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