Disposition of Lasofoxifene, a Next-Generation Selective Estrogen Receptor Modulator, in Healthy Male Subjects

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ABSTRACT:

Disposition of lasofoxifene, a next-generation selective estrogen receptor modulator, was investigated in male volunteers after p.o. administration of a single 20-mg dose of [14C]lasofoxifene. Approximately 72% of the administered dose was recovered from the urine and feces, with majority of dose excreted in the feces, probably via bile. The absorption of lasofoxifene in humans was slow with Tmax values typically exceeding 6 h. The Cmax and area under plasma concentration–time profile from time 0 to the last quantifiable time point values of lasofoxifene were lower than those determined for total radioactivity, indicating presence of circulating metabolites. The primary clearance mechanisms for lasofoxifene in humans were direct conjugation (glucuronide and sulfate conjugates) and phase I oxidation, each accounting for about half of the metabolism. Several oxidative metabolites were identified by liquid chromatography/tandem mass spectrometry. The primary phase I metabolites were the result of hydroxylations on the tetra line moiety and the phenyl rings attached to the tetraline, and oxidation on the pyrrolidine moiety. Considering the numerous metabolites seen in vivo, additional in vitro studies using human liver and intestinal microsomes, recombinant cytochromes P450 (P450s), and UDP glucuronosyltransferases (UGTs) were performed. The turnover of lasofoxifene was very slow in liver microsomes, and only two metabolites were identified as two regioisomers of the catechol metabolite. The results from in vitro experiments with recombinant isofoms and P450 isoform-selective inhibitors suggested that the oxidative metabolism of lasofoxifene is catalyzed primarily by CYP3A and CYP2D6. In addition, its glucuronidation is catalyzed by UGTs that are expressed in both the liver (UGT1A1, UGT1A3, UGT1A6, and UGT1A9) and the intestine (UGT1A8 and UGT1A10).

Postmenopausal women have an increased risk of developing osteoporosis as their levels of endogenous estrogen decrease (Luietto et al., 1993; National Institutes of Health Consensus Development Panel on Optimal Calcium Intake, 1994; Gambacciani and Ciaponi, 2000; Riggs et al., 2002; Sowers et al., 2003a,b). In the past, osteoporosis has been effectively treated with estrogen-progestin replacement therapy, but recent findings from the Women’s Health Initiative study have shown that the risks outweigh the benefits (Rossouw et al., 2002; Chlebowski et al., 2003; Hods et al., 2003; Manson et al., 2003; Wassertheil-Smoller et al., 2003). The long-term use of estrogen-progestin replacement therapy is no longer recommended except under special circumstances, although its short-term use for the relief of vasomotor and urogenital symptoms is considered acceptable. As a consequence, alternative long-term antosteoporotic therapies are being actively sought, and agents that can mimic the positive effects of estrogen without increasing the risk of breast or endometrial cancer are of particular interest.

Lasofoxifene (Fig. 1), a next-generation selective estrogen receptor modulator (SERM), is being developed for the prevention and treatment of osteoporosis. Studies have shown that lasofoxifene is an estrogen receptor (ER) agonist in bone and cholesterol regulation and an ER antagonist in the mammary gland and uterus. It binds with high affinity to the human ERα, has good oral bioavailability in preclinical species (Rosati et al., 1998), prevents bone loss, and maintains bone mass and strength in ovariectomized rats without stimulating the uterus (Ke et al., 1998, 2004). In both nonclinical and clinical human studies, lasofoxifene has shown a favorable safety profile and a proven efficacy in preventing bone loss and lowering cholesterol levels (Gennari et al., 2006), and it is now under submission for regulatory approval.

It is important to understand how a new therapeutic agent is metabolized and excreted by the body. Metabolism and excretion studies in humans provide valuable information about any potential drug-drug interactions that may need to be considered when coadministering a long-term treatment such as lasofoxifene with other drugs frequently used in postmenopausal women. A thorough understanding of the clearance mechanisms of lasofoxifene should also help to predict whether extra care, such as dose adjustment, will need to be taken when treating special patient groups, for example, those with renal or liver disease. Preliminary in vitro studies suggested that lasofoxifene was stable in rat, monkey, and human liver microsomes. These findings indicated that lasofoxifene is unlikely to undergo rapid oxidative metabolism in vivo in the rat, monkey, or human. However, in vivo studies in rats and monkeys have shown that lasofoxifene is metabolized by phase I oxidation and phase II conjugation reaction

ABBREVIATIONS: SERM, selective estrogen receptor modulator; ER, estrogen receptor; P450, cytochrome P450; UGT, UDP glucuronosyltransferase; LC/MS/MS, liquid chromatography/tandem mass spectrometry; HPLC, high-performance liquid chromatography; SAM, S-adenosyl methionine; ABT, aminobenzotriazole; AUC, area under the curve; SPE, solid phase extraction; MRM, multiple reaction monitoring.
The objective of the present study was to evaluate the metabolism and excretion of radiolabeled lasofoxifene in healthy men and to identify and characterize the enzymes [cytochromes P450 (P450s) and UDP glucuronosyltransferases (UGTs)] involved in the metabolism. The metabolites were characterized by liquid chromatography/tandem mass spectrometry (LC/MS/MS) using a single reaction monitoring technique and by comparison of their high-performance liquid chromatography (HPLC) retention times with those of metabolites identified in preclinical species.

Materials and Methods

General Chemicals. Commercially obtained chemicals and solvents of HPLC or analytical grade. Ecolite (+) scintillation mixture was obtained from ICN (Irvine, CA). Carbosorb and Permafluor E+ scintillation mixtures were purchased from Packard Instrument Company (Downers Grove, IL). HPLC grade acetonitrile, methanol, and water and certified aqueous counting scintillant grade ammonium acetate and acetic acid were obtained from J.T. Baker (Phillipsburg, NJ). S-Adenosyl methionine (SAM) and isoform-specific inhibitors ketoconazole, quinidine, sulfaphenazole, furafylline, and aminobenzotriazole (ABT) were obtained from Sigma-Aldrich Chemicals (St. Louis, MO).

Human liver samples were obtained from 20 organ donors (both male and female between the ages of 18–65 years) and purchased from Tissue Transformation Technology (Edison, NJ) on approval from the ethics committee of the vendor. Liver and microsomal samples were stored at −70°C until used. Human liver and intestinal microsomes and other subcellular fractions (S-9 and cytosol) were prepared and characterized for P450 isoforms at Pfizer Global Research and Development (Groton, CT) using standard procedures (Prakash et al., 2000). HL-mix 13 was prepared by mixing liver microsomes from several donors to represent the P450 in normal humans. Recombinant human cytochromes P450 (P450s) and UDP glucuronosyltransferases (UGTs) were expressed in a baculovirus-insect cell expression system.

Radioisotopes. [14C]-0-methyl-lasofoxifene, 7-methoxy-lasofoxifene, 7-hydroxy-lasofoxifene, 7-hydroxy-6-O-methyl-lasofoxifene, and 5-methoxy-5-hydroxy-6-O-methyl-7-methoxy-lasofoxifene were synthesized at Pfizer Global Research and Development using standard procedures (Rosati et al., 1998).

Subject and Dose Administration. Four healthy male subjects between the ages of 18 and 45 (inclusive) years participated in the study. All the subjects provided written, informed consent before participation in the study. The study protocol, consent documents, consent procedures, and subject recruitment procedures were approved by the Independent Institutional Review Board. The study was conducted in compliance with the International Conference on Harmonization Good Clinical Practices guidelines, the ethical principles that have their origin in the Declaration of Helsinki, and in compliance with the U.S. Food and Drug Administration regulations for informed consent and protection of patient rights. Subjects entered the Clinical Research Facility at least 12 h before dosing and remained there for at least 480 h after dosing under continuous medical observation. All the subjects had fasted for at least 8 h before morning dosing. The subjects were given a single 20-mg dose of [14C]-0-methyl-lasofoxifene (80 μCi/subject). The drug was administered in an open fashion as a single p.o. dose in the morning. A standard meal was provided 4 h later. The dosing solution was prepared by dissolving the radiolabeled drug in water (100 ml). Subjects were required to refrain from lying down during the first 4 h after drug administration. Subjects were prohibited to consume caffeine-containing foods and/or beverages for 24 h before dosing and for 48 h after dosing. Administration of the study drug by or under the supervision of medical personnel ensured adherence.

Plasma, Urine, and Feces Collection. Venous blood samples (enough to yield 6 ml of plasma) for lasofoxifene concentrations and total radioactivity were collected from each subject in heparinized glass vacuum blood collection tubes before dosing (0) and at 1, 2, 4, 8, 12, 16, 24, 36, 48, 72, 120, and 168 h following dosing. At 216, 264, 336, 408, and 480 h postdose, blood samples sufficient to yield 3 ml of plasma were collected. All the blood samples were separated in a refrigerated centrifuge within 1 h of collection. Blood samples collected up to 168 h postdose were divided into two 3-ml aliquots and stored frozen at or below −20°C. One 3-ml aliquot from each time point was used for analysis of lasofoxifene concentrations. The remaining 3-ml sample was used for analysis of total radioactivity.

For the analysis of lasofoxifene metabolites, venous blood samples (sufficient to yield 20 ml of plasma) were collected from each subject as described above at 6, 24, and 48 h postdose administration. These were stored at or below −20°C until analyzed.

Each subject emptied his bladder and provided a 50-ml sample of urine immediately before dosing, which was frozen at or below −20°C. Urine was collected from hours 0 to 12, 12 to 24, and at 24-h intervals for 24 days. All the urine collected during each time period was mixed, the total volume recorded, and an aliquot removed for storage at or below −20°C until analysis. Fecal samples were collected as passed from the time of dosing until at least 480 h after dosing.

Measurement of Total Radioactivity in Plasma, Urine, and Feces. All the measurements of total radioactivity were performed by liquid scintillation counting at Phoenix International Life Sciences Inc. (Montreal, QC, Canada). Samples of plasma and urine (1 ml) from each sampling time point were added to 10 ml of Ecolite (+) scintillation fluid and counted in a liquid scintillation counter (Beckman, Fullerton, CA). Fecal samples were transferred into tared Stomacher 3500 bags, hydrated with equal amounts of water, and homogenized using a Stomacher homogenizer (Cooke Laboratory Products, Alexandria, VA). Triplicate aliquots (0.25–0.5 g) of each fecal homogenate were weighed into oxidizer sample cups and combusted in an oxidizer (model 307; Packard). The liberated 14CO2 was trapped by Carbosorb E (PerkinElmer Life and Analytical Sciences, Boston, MA). Scintillation mixture Permafluor V (PerkinElmer Life and Analytical Sciences) was added to the samples and counted in a PerkinElmer Wallac 1409 liquid scintillation counter (PerkinElmer Life and Analytical Sciences). Combustion efficiency was determined by combustion of the 14C-Spec Check standard in an identical manner. Radioactivity less than twice the background value was considered to be below the limit of determination. Fecal samples collected before dosing were used as the control samples and provided the background count rate.

The dose of radioactivity administered to each subject was determined by measuring the residual radioactivity in the dosing container following dose administration and subtracting this from the total radioactive dose in the dosing container. When determining the amount of radioactivity excreted in urine and feces at each time point as a proportion of the amount administered, the net radioactivity in the dose was considered to be 100%. The amount of radioactivity in plasma at each time point was calculated using the specific activity of

![Image](https://example.com/image.png)
the dose administered and was expressed as nanogram-equivalent of parent drug per milliliter.

**Pharmacokinetic Analysis.** Concentrations of unchanged lasofoxifene in plasma were determined at CEDRA Corporation (Austin, TX) using a validated LC/MS/MS assay. The analytical method was validated for analysis of lasofoxifene in 1 ml of heparinized human plasma samples over a concentration range of 0.025 to 6.0 ng/ml. The pharmacokinetic parameters were determined using the WinNonlin-Pro version 1.5 program (Pharsight, Mountain View, CA) by noncompartmental approach. The pharmacokinetic parameters for lasofoxifene and total radioactivity were calculated from the plasma concentration data. The apparent maximum plasma concentration ($C_{\text{max}}$) and the time at which this concentration was achieved ($t_{\text{max}}$) were taken directly from the concentration data. The area under plasma concentration–time profile from time 0 to the last quantifiable time point ($AUC_{0-t_{\text{last}}}$) was calculated using linear trapezoidal approximation. The plasma terminal phase rate constant ($K_t$) was estimated using least square regression analysis of the plasma concentration–time data obtained during the terminal log-linear phase. Terminal elimination half-life ($t_{1/2}$) was calculated by ln2/$K_t$ relationship. The area under plasma concentration–time profile from time $t_{\text{last}}$ to infinity ($AUC_{t_{\text{last}}-\infty}$) was estimated as $C_{\text{last}}/K_t$, where $C_{\text{last}}$ represented the estimated concentration at the last time point in which drug was quantitated based on the regression analysis. The $AUC_{0-t_{\text{last}}}$ was estimated as the sum of the $AUC_{0-t_{\text{last}}}$ and $AUC_{t_{\text{last}}-\infty}$ values. For the purpose of calculating mean drug concentrations at each sampling time, concentrations less than the lower limit of quantification were considered to be 0 ng/ml; a mean was not calculated when 50% or more of the concentrations were less than the lower limit of quantification.

**Extraction of Metabolites from Biological Samples.** Plasma samples (20 ml) taken from each subject on day 24 and 48 h postadministration were mixed with 40 ml of acetonitrile, vortexed, and sonicated. The mixture was centrifuged and the supernatant collected. The remaining pellet was mixed with 20 ml of acetonitrile and centrifuged, and the supernatant was combined with the first supernatant. The overall recovery of radioactivity in plasma was about 78 to 85%. The combined supernatants were evaporated to dryness in nitrogen Turbo Vap LV evaporator (Caliper Life Sciences Corporate, Hopkinton, MA). The residue was reconstituted in 300 µl of methanol/water (1:1) and centrifuged to remove insoluble matter, and an aliquot (100 µl) was injected into the HPLC system for analysis.

The majority of radioactivity (>74%) excreted in urine was recovered during 240 h postdose. Therefore, urine (approximately 50 ml) from each subject taken between 0 and 240 h postdose administration was pooled. Approximately 500 ml of urine from each subject was lyophilized; the residue was reconstituted in 30 ml of water and passed over a conditioned C-18 SPE column (Supelco, Bellefonte, PA). The column was washed with water, and the radioactivity was eluted with 5 ml of methanol. The overall recovery of radioactivity was about 82 to 88%. The methanolic eluate was concentrated under nitrogen, the residue reconstituted in 0.5 ml of methanol/water (1:1), and a 50 µl aliquot injected into the HPLC column for analysis.

Aliquots of fecal homogenates from 24 to 336 h for subjects 1 through 3 and from 24 to 408 h for subject 4 were pooled, and the pooled samples were lyophilized. The dried residue was reconstituted in 120 ml of acetonitrile and stirred for 2 h using a magnetic stirrer. The mixture was centrifuged and the supernatant collected. The process of extraction was repeated three times with 120 ml of acetonitrile each time. The supernatants were collected, and 250-µl aliquots were counted in a liquid scintillation counter. The overall recovery of radioactivity in feces was about 78 to 85%. The organic solvent was evaporated to dryness in a rotary evaporator, the residue reconstituted in 10 ml of methanol/water (1:1), and a 100-µl aliquot injected into the HPLC system for analysis.

**In Vitro Studies of Lasofoxifene Metabolism.** Human liver microsomes and recombinant human P450 incubations. Human liver microsomes and recombinant P450 isoforms were reconstituted in 100 mM potassium phosphate buffer, pH 7.4, before analysis. [14C]Lasofoxifene (1 µM) was preincubated with micromoles (0.5 mg/ml protein microsomes) or recombinant enzyme (20 pmol/ml) for 3 min at 37°C in a shaking water bath. The incubation was initiated with the addition of 100 µl of cofactor (1.1 mM NADPH, 10 mmol of MgCl2) per 1 ml of incubation mixture. After 30 min, the incubations were terminated by the addition of 100 µl of cold acetonitrile and ascorbic acid (50 µl of a 20 mM stock solution in water). Isomorf-specific inhibitors were used in conjunction with microsomal experiments to determine the isomorf responsible for the metabolism of lasofoxifene. The inhibition studies used P450 isoform-selective inhibitors (1 µM ketoconaizole, 1 µM quinidine, 40 µM quercetin, 40 µM sulfaphenazole, 40 µM furafylline, and 100 µM ABT). These were added to the microsomal incubations at the start of the incubation, except for furafylline and ABT, which were preincubated with NADPH for 5 min before the addition of lasofoxifene. Metabolites of lasofoxifene were extracted from the incubation mixtures by liquid/liquid extraction using two volumes of methyl-tert-butyl ether.

**Human liver S-9 and cytosol incubations.** 5-Hydroxy-lasofoxifene and 7-hydroxy-lasofoxifene (5 µM) were incubated at 37°C (30 min) in human cytosolic fractions with SAM (1 mM) to show these compounds as substrates for in vivo phase II reactions. Before use, subcellular fractions were thawed on ice and diluted 1:1 with 100 mM potassium phosphate, pH 7.4. The substrates were dissolved in acetonitrile (1 mM) and added to the incubation mixture to a final substrate concentration of 1 µM. Incubations were stopped after 30 min by the addition of 100 µl of cold acetonitrile and ascorbic acid (50 µl of a 20 mM stock solution in water).

**Glucuronidation by human liver and intestinal microsomes and expressed UGTs.** Before use, microsomes and UGTs were thawed on ice and reconstituted using 100 mM potassium phosphate, pH 7.4. Lasofoxifene was dissolved in dimethyl sulfoxide (5 mM) and added to the incubation mixture to a final substrate concentration of 25 µM. Samples were preincubated for 3 min (0.5 mg/ml protein) at 37°C in a shaking water bath. Incubations were initiated with the addition of 100 µl of cofactor (25 µg/ml amelamin, 3 mM UDP-glucuronic acid, 25 µM saccharalactone, 8 mM MgCl2) per 1 ml of incubation mixture. Incubations were stopped after 30 min by the addition of an equal volume of cold acetonitrile. Precipitated protein was removed by centrifugation (14,000 rpm for 5 min). Supernatants were transferred to HPLC injection vials without further purification.

Microsomal and cytosolic incubations were passed over C8 SPE for sample concentration. Spec C8 SPE cartridges (0.5 mg) were conditioned by the passing methanol (1 ml) followed by water (1 ml) over the column bed under slight vacuum. Samples were aspirated through the column under vacuum (<5 mm Hg). The column beds were then washed with water (1 ml), and the samples were eluted with acetonitrile (2 × 250-µl aliquots). Following elution, ascorbic acid (50 µl of a 20 mM stock solution in water) was added, and the samples were placed under nitrogen for concentration and reconstituted in 100 µl of mobile phase (10 mM ammonium formate/acetonitrile, 70:30).

**HPLC.** The HPLC system consisted of an HP-1050 solvent delivery system, an HP-1050 membrane degasser, an HP-1050 autoinjector (Hewlett Packard, Palo Alto, CA), a Thermo Separations spectromonitor 3200 UV (San Jose, CA), and a radioactive monitor (β-RAM: IN/US, Tampa, FL). Chromatography was performed on a Beckman Ultrasphere C-18 column (4.6 × 250 mm, 5 µm) with a mobile phase containing a mixture of 10 mM ammonium acetate (solvent A) and methanol (solvent B). The mobile phase was initially composed of solvent A/solvent B (80:20), held for 1 min, and then it was linearly programmed to solvent A/solvent B (20:80) over 30 min followed by a short gradient to solvent A/solvent B (5:95) over 5 min. Chromatography was conducted under isocratic conditions for 10 min. The mobile phase composition was returned to the starting solvent mixture over 5 min. The system was allowed to equilibrate for approximately 15 min before making the next injection. A flow rate of 1.0 ml/min was used for all the analyses.

For microsomal incubations, chromatography was performed on a Metasil AQ column (4.6 × 250 mm, 3 µm). The mobile phase was initially composed of 0.05% formic acid/0.1% ammonium hydroxide in water containing 30% acetonitrile (solvent A) and acetonitrile (solvent B). The mobile phase was initially composed of solvent A/solvent B (80:20) held for 12 min. The mobile phase composition was then linearly programmed to solvent A/solvent B (20:80) over 30 min followed by a short gradient to solvent A/solvent B (5:95) over 5 min. Chromatography was conducted under isocratic conditions for 10 min. The mobile phase composition was returned to the starting solvent mixture over 2 min. The system was allowed to equilibrate for 10 min before the next injection. A flow rate of 1.0 ml/min was used for all the analyses.

For cytosolic incubations, chromatography was performed on a Metasil AQ column (4.6 × 250 mm, 3 µm). The mobile phase was initially composed of 50:50 solvent A/solvent B. It was linearly ramped to 60:40 over 20 min and then to 20:80 in 5 min. It was held for 1 min and changed to 50:50 in 2 min.
The feces (Table 1), with a mean total of 66.1 \[14C\]lasofoxifene. The majority of the radioactivity was excreted in the urine and feces in relation to the administered dose of lasofoxifene, as shown in Fig. 2. Excretion of radioactivity via urine and feces continued for a considerable period, with 22.1% being excreted during the period 240 to 576 h postdose administration.

<table>
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<th>Subject Number</th>
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<td>70.8</td>
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<td>3</td>
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<td>74.6</td>
</tr>
<tr>
<td>4</td>
<td>5.94</td>
<td>67</td>
<td>72.9</td>
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<td>6.39</td>
<td>66.1</td>
<td>72.4</td>
</tr>
<tr>
<td>S.D.</td>
<td>0.87</td>
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Pharmacokinetics. The mean (± S.D.) concentration-time profiles of lasofoxifene and total radioactivity in plasma of humans following p.o. administration [14C]lasofoxifene were graphically depicted in Fig. 3. Plasma concentrations for lasofoxifene and total radioactivity peaked at 12 and 4 h, respectively, after p.o. administration (Table 2). The concentration-time profiles for lasofoxifene were graphically depicted in Fig. 3. Plasma concentrations for lasofoxifene and total radioactivity were determined for radioactivity, indicating presence of circulating metabolites in plasma (Table 2). The AUC\(_{0-168}\) values for the total radioactive material, as well as the peak representation. The \(C_{max}\) values for lasofoxifene ranged from 11.5 to 20.3 ng/ml with a mean value of 17.1 ng/ml. The mean terminal phase (\(t_{1/2}\)) of lasofoxifene was estimated as 153 ± 28 h. The mean AUC\(_{0-168}\) values for the parent drug ranged from 2280 to 3110 ng·h/ml with a mean value of 2620 ng·h/ml. Mean AUC\(_{0-168}\) total for radioactivity was 3290 ng·Eq·h/ml. The mean terminal phase (\(t_{1/2}\)) of lasofoxifene was estimated as 153 ± 28 h. The mean AUC\(_{0-168}\) and AUC\(_{0-240}\) values for lasofoxifene and total radioactivity were lower than those determined for radioactivity, indicating presence of circulating metabolites in plasma (Table 2). The AUC\(_{0-168}\) and AUC\(_{0-240}\) values for lasofoxifene and total radioactivity were lower than those determined for radioactivity, indicating presence of circulating metabolites in plasma (Table 2). The AUC\(_{0-168}\) and AUC\(_{0-240}\) values for lasofoxifene and total radioactivity were lower than those determined for radioactivity, indicating presence of circulating metabolites in plasma (Table 2). The AUC\(_{0-168}\) and AUC\(_{0-240}\) values for lasofoxifene and total radioactivity were lower than those determined for radioactivity, indicating presence of circulating metabolites in plasma (Table 2).

Identification of Metabolites. Urine. HPLC analysis of pooled urine collected 0 to 240 h postdose administration identified five metabolites of lasofoxifene: the monohydroxylated (M21) and methylated catechol (M17) metabolites and a glucuronide conjugate (M7), with its monohydroxy and dihydroxy derivatives (M9 and M23/M24) (Fig. 4). Identification of these metabolites was performed by LC/MS/MS using multiple reaction monitoring (MRM) and by comparison of their HPLC retention times with the metabolites identified in rat and monkey (Johnson and Prakash, 1998). The mean percentages of each of these metabolites in urine in relation to the administered dose of lasofoxifene were shown in Table 3. Unchanged lasofoxifene accounted for only 1.77% of the dose in urine collected from 0 to 240 h postdose, and the glucuronide conjugate M7 was the most abundant metabolite (1.47%).

Feces. Six metabolites of lasofoxifene were found in pooled samples of feces collected 24 to 336 h (three subjects) and 24 to 408 h (one subject) postdose administration: the catechol intermediate (M21), its sulfate conjugate M10, and its methylated conjugate M17; the sulfate conjugate of lasofoxifene (M11); M15, the product of the oxidation of the pyridoline moiety; and M12, which resulted from the aromatic hydroxylation of lasofoxifene (Fig. 5). Table 3 shows the mean percentages of each of these metabolites in feces in relation to the administered dose of lasofoxifene. The greatest proportion of radioactivity in feces was attributable to unchanged lasofoxifene (21.8%), with similar proportions for each of the metabolites (5.10–8.42%).
Radioactivity in humans (n = 4) following p.o. administration of a single 20-mg dose of \([^{14}C]\)lasofoxifene.

**FIG. 3.** Mean (± S.D.) plasma concentration-time curves for lasofoxifene and total radioactivity in humans (n = 4) following p.o. administration of a single 20-mg dose of \([^{14}C]\)lasofoxifene.

**TABLE 2**

Pharmacokinetic parameters for lasofoxifene and total radioactivity in humans (n = 4) following a single 20-mg p.o. dose of \([^{14}C]\)lasofoxifene

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<th>Analyte</th>
<th>Subject Number</th>
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<td></td>
<td></td>
<td>(T_{\text{max}})</td>
<td>(C_{\text{max}})</td>
<td>AUC(_{0-168})</td>
<td>AUC(_{\text{BSM}})</td>
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<tr>
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<td>14</td>
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</table>

**CV%**, percentage coefficient of variation.

\(C_{\text{max}}\) and AUC values for total radioactivity are expressed as ng-Eq/ml and ng-Eq.h/ml, respectively.

Total radioactivity could not be measured past 168 h and consequently \(t_{1/2}\) and AUC\(_{\text{BSM}}\) values were not estimated.

**Plasma.** Plasma samples pooled at 24 and 48 h postdose for each subject contained lasofoxifene and three metabolites as shown by HPLC analysis. The three metabolites detected were the glucuronide conjugate of lasofoxifene (M7), its hydroxylated metabolite (M9), and a methylated catechol (M17). These metabolites were identified by LC/MS/MS using MRM and by comparison of their HPLC retention times with the metabolites identified in rat and monkey (Fig. 6). Table 3 shows the percentage of each of these metabolites and lasofoxifene circulating in plasma for each subject. Approximately one third of the radioactivity in plasma was attributable to unchanged lasofoxifene, and the most common metabolite was the glucuronide conjugate M7 (21.8%).

**In Vitro Metabolism of Lasofoxifene.** Human liver microsomes. HPLC-selected ion chromatograms of metabolites of lasofoxifene in human liver microsomes are shown in Fig. 7. Lasofoxifene has a very low turnover in HL-mix 13 (∼6%); the two metabolites formed were M21A (83%) and 7-hydroxy-lasofoxifene M21B (100%), whereas 5-hydroxy-lasofoxifene (M21A; 5.11%) and 7-hydroxy-lasofoxifene (M21B; 1.19%). These metabolites were not detected in the incubations devoid of NADPH, suggesting that the metabolism of lasofoxifene was mediated by P450 isozymes.

**Recombinant P450 isozymes.** The ability of specific P450 isozymes to metabolize lasofoxifene was determined using recombinant CYP1A1, CYP1A2, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4, and CYP3A5. Incubations of lasofoxifene with CYP3A5 and CYP2D6 formed 7-hydroxy metabolite (M21B) as the major product (44.7 and 58.0%, respectively). It was also observed in the incubations with CYP2C19 and CYP1A1 but at a much lesser extent (1.1 and 5.8%, respectively). 5-Hydroxy-lasofoxifene (M21A) was formed primarily by CYP3A4 (57.6%) and CYP3A5 (11.1%). These oxidative metabolites were not detected in incubations containing recombinant CYP1A2, CYP2C8, CYP2C9, or CYP2E1. As the enzyme activities of the recombinant P450s were selected to reflect activities typical of a human liver microsomal preparation, it can be concluded that these latter isoforms do not contribute significantly to lasofoxifene metabolism.

**Inhibition Studies.** Inhibition of metabolite formation by each inhibitor of specific P450 (isomor) is summarized in Table 4. Ketoconazole inhibited the formation of both 5-hydroxy-lasofoxifene M21A (83%) and 7-hydroxy-lasofoxifene M21B (100%), whereas
quinidine inhibited 7-hydroxy and 5-hydroxy metabolite formation by 61.3 and 10.2%, respectively. ABT showed 100% inhibition of these two metabolites. Sulfaphenazole (37.8%), furafylline (45.4%), and quercetin (60.5%) showed moderate inhibition for the formation of 7-hydroxy-lasofoxifene. The formation of 5-hydroxy-lasofoxifene was also inhibited by sulfaphenazole (3.13%), furafylline (14.9%), and quercetin (22.5%). Addition of the isoform-specific inhibitors ketoconazole to CYP3A4 and CYP3A5 or quinidine to CYP2D6 isoform incubations showed 100% inhibition of metabolite production.

Cytosolic Fraction. HPLC-selected ion chromatograms of metabolites of 5-hydroxy-lasofoxifene and 7-hydroxy-lasofoxifene with human liver cytosols in the presence of SAM are shown in Fig. 8. 5-Hydroxy-lasofoxifene and 7-hydroxy-lasofoxifene each yielded a mixture of two monomethylated regioisomers. These data suggest that in vivo, a mixture of 5-hydroxy-lasofoxifene and 7-hydroxy-lasofoxifene may form four regioisomers of the monomethylated catechol metabolite (M17).

Lasofoxifene Glucuronidation by Human Liver and Intestinal Microsomes and Expressed UGTs. Human liver and intestinal microsomes and various UGT isoforms (UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A7, UGT1A8, UGT1A9, UGT1A10, UGT2B4, UGT2B7, UGT2B15, and UGT2B17) were tested for their ability to catalyze lasofoxifene glucuronidation. Human liver and intestinal microsomes produced a major glucuronide metabolite M7 that was identified in vivo in humans. From the 12 UGTs tested, six (UGT1A1, UGT1A3, UGT1A6, UGT1A8, UGT1A9, and UGT1A10) were able to catalyze the formation of the lasofoxifene glucuronide (M7).

Discussion

The aims of this study were to characterize the metabolism and excretion of lasofoxifene in humans and to identify the enzymes (P450s and UGTs) involved in its metabolism. A single 20-mg dose of [14C]lasofoxifene was well tolerated by all four subjects in this study. There were no serious adverse effects or withdrawals as a result of adverse events.

Recovery of radioactive dose in humans was 72.4% on average over a period of 24 days and ranged from 64.3 to 78.0% in the four subjects. Small amounts of radioactivity were excreted up through the last day of collection with 0.6 to 1.5% recovered in feces on day 24. From days 11 to 24, 20.3% of the dose was excreted in feces, and 1.8% was excreted in urine. Examination of the rate of excretion of radioactivity suggests that if the study could have been continued beyond 24 days, recovery would have increased. The majority (66.1%) of radioactive dose was excreted in the feces, which is likely the consequence of biliary excretion of metabolites. In the bile duct–cannulated rats, 83.0% of the dose was excreted in the bile over 48 h, of which 56% was identified as glucuronide conjugate (K.A. Johnson and C. Prakash, unpublished data). Therefore, the unchanged drug in feces of humans is thought to be caused by hydrolysis of lasofoxifene glucuronide by gut microflora rather than incomplete absorption. The excretion of lasofoxifene radioactivity primarily via the feces was similar to that of raloxifene, a SERM already used for the treatment and prevention of osteoporosis in postmenopausal women (Hochner-Celnikier, 1999; Snyder et al., 2000; Heringa, 2003; Morello et al., 2003). In humans, only 6.4% of the dose was excreted in urine, with <2% as unchanged lasofoxifene. This indicates renal elimi-


FIG. 7. Extracted ion chromatograms of lasofoxifene metabolites produced by human liver microsomes.
Sulfation to form M10 or underwent the catechol intermediate M21, which was either conjugated via conjugation. For example, hydroxylation of the tetraline ring produced the catechol intermediate M21. The catechol intermediate M21 was also hydroxylated and conjugated with glucuronic acid to form M23/M24. How- ever, in feces, conjugated metabolites were identified as the aglycones, possibly as a result of hydrolysis of conjugates by gut microflora (Scheline, 1973). A significant portion of the administered radioactivity was excreted as unchanged lasofoxifene. It could be envisioned that this is likely to be the result of hydrolysis of lasofoxifene glucuronide by gut microflora and not unabsorbed drug (Scheline, 1973). Metabolite M15, only found in feces, was formed by the oxidation of the pyrrolidine moiety. Metabolite M12 was formed by the hydroxylation on the 5-phenyl group of lasofoxifene.

In addition to lasofoxifene, three metabolites were identified in the pooled 24- to 48-h plasma samples. Lasofoxifene, its glucuronide (M7), and the methylated catechol metabolite (M17) were identified as the major circulating metabolites in humans. The hydroxylation at the tetraline ring could form two regiosomers of catechol intermediates [5-hydroxy-lasofoxifene (M21A) and 7-hydroxy-lasofoxifene (M21B)]. Catechol-O-methyltransferase could then generate four isomeric monomethyl ethers from these catechol intermediates (i.e., 5-methoxy-lasofoxifene, 5-hydroxy-6-O-methyl-lasofoxifene, 7-methoxy-lasofoxifene, and 7-hydroxy-6-O-methyl-lasofoxifene). Because of low abundance of these metabolites in humans, the exact position of methylation could not be determined. Analysis of plasma from postmenopausal women confirmed that the lasofoxifene metabolites seen in male plasma were also present in the plasma of postmenopausal women (not shown).

Oxidative metabolites, most likely the result of P450-dependent activity and therefore in vitro metabolism of lasofoxifene, were studied in human liver microsomes and recombinant P450 isoforms to determine the P450 isoform(s) responsible for its oxidative metabolism. Incubation of lasofoxifene with human liver microsomes resulted in the formation of only two catechol metabolites, 5-hydroxylasofoxifene (M21A) and 7-hydroxy-lasofoxifene (M21B). Further results from experiments with recombinant P450 isoforms and inhibition studies suggested that 5-hydroxy-lasofoxifene (M21A) was formed primarily by CYP3A4 and CYP3A5, whereas 7-hydroxy-lasofoxifene (M21B) was formed by CYP2D6 and CYP3A5 and to a much lesser extent by CYP2C19 (1.1%). The in vitro metabolism studies of these catechol metabolites with human S-9 fraction in the presence of SAM formed a mixture of two monomethylated regioisomers of each standard compound. These data suggest that metabolite M17 identified in vivo in humans may represent a mixture of four regiosomers of the monomethylated catechols.

Because glucuronidation also plays an important role in the metabolism of lasofoxifene, in vitro studies were conducted in human liver and intestinal microsomes and expressed UGTs. The results suggested that glucuronidation of lasofoxifene is catalyzed by UGTs that are expressed in both the liver (UGT1A1, UGT1A3, UGT1A6, and UGT1A9) and the intestine (UGT1A8 and UGT1A10). The relative contribution of various UGTs is now under investigation and will be reported separately.
The major circulating metabolites M17 (four regioisomers) and M7 were synthesized and tested in vitro for the ability to bind to either ERα or ERβ and were found to have significantly lower affinity than lasofoxifene (not shown). Based on the in vitro findings and the observed circulating concentrations in human subjects, it is not likely that any of these metabolites contribute to the pharmacologic activity of lasofoxifene.

Although lasofoxifene is extensively hydroxylated in humans, the nonconjugated catechol metabolite was not detected in systemic circulation. It is probably because of rapid conjugative metabolism (O-methylation and subsequent glucuronidation or sulfation) followed by urinary excretion. It is well established that the catechols are metabolized to highly reactive O-quinone by monooxygenase or peroxidase enzymes, metal ions, or molecular oxygen (Monks et al., 1992; Bolton et al., 2000). However, unlike raloxifene, no glutathione conjugate of lasofoxifene was detected either in humans or preclinical species, suggesting that the formation of catechol is rate-limited (Chen et al., 2002).

This study has clearly shown that the most important route of elimination of lasofoxifene was via metabolism and excretion in the feces. The study presented evidence that both conjugation and oxidation reactions were involved in the metabolism of lasofoxifene in humans. The results from in vitro experiments with recombinant isoforms and P450 isozyme-selective inhibitors suggested that the oxidative metabolism of lasofoxifene is catalyzed primarily by CYP3A4/3A5 and CYP2D6 and that its glucuronidation is catalyzed by UGTs that are expressed in both the liver (UGT1A1, UGT1A3, UGT1A6, and UGT1A9) and the intestine (UGT1A3, UGT1A8, and UGT1A10) (Tukey and Strassburg, 2000). Because oxidation accounts for about half of total lasofoxifene clearance and this pathway is further divided among multiple P450 isozymes, inhibition of a single enzyme will not have a clinically significant impact on the pharmacokinetics of lasofoxifene. Phase 2 clinical studies showed only modest increase in both the AUC and Cmax of lasofoxifene with concomitant administration of either ketoconazole, a potent inhibitor of CYP3A4 (Ouellet et al., 2006), or paroxetine, a potent inhibitor of CYP2D6 (Ouellet et al., 2006). Considering the large safety margin for lasofoxifene, no dosage adjustment is needed when coadministered with either CYP3A or CYP2D6 inhibitors (Ouellet et al., 2006). However, as a result of these data, there is the potential for drug–drug interactions with any coadministered drug that is also eliminated by the same metabolic pathways, particularly those involving phase I oxidation by microsomal P450 enzymes.

In conclusion, this metabolism and excretion study in healthy subjects has shown that lasofoxifene, a new generation SERM, is primarily eliminated via metabolism and fecal excretion comparable with that of other SERMs, such as tamoxifen, toremifene, and raloxifene (Morello et al., 2003). The primary clearance mechanisms for lasofoxifene in humans appear to be the result of direct conjugation and P450-mediated oxidation, each accounting for half of the metabolism. CYP3A4/5 and CYP2D6 are the primary P450 isoforms involved in the oxidative metabolism, and several UGTs of UGT1A family are involved in the glucuronidation of lasofoxifene.

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