Metabolic Disposition of [14C]Bazedoxifene in Healthy Postmenopausal Women

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Nonstandard abbreviations: SERM, selective estrogen receptor modulator; HPLC, high-performance liquid chromatography; LC/MS, liquid chromatography/mass spectrometry; C_max, maximum plasma concentration; AUC, area under the plasma concentration-versus-time curve; AUC_T, AUC to last measurable time; λz, terminal-phase elimination rate constants; C_T, last measurable concentration at time T; AUC_0-α, AUC_T extrapolated to infinity; Cl/F, estimate of apparent oral dose clearance; DPM, disintegrations per minute; Glu, glucuronide; CPM, counts per minute; m/z, mass-to-charge ratio; BZA, bazedoxifene.
ABSTRACT

Bazedoxifene is a selective estrogen receptor modulator under development for the prevention and treatment of osteoporosis. The disposition of [14C]bazedoxifene was determined in 6 healthy postmenopausal women following administration of a single oral dose of 20 mg (200 μCi). Following dosing, blood was collected at frequent intervals, and urine and fecal samples were collected for up to 10 days. Aliquots of plasma, blood, urine, and fecal homogenates were analyzed for concentrations of radioactivity. Bazedoxifene metabolite profiles in plasma and feces were determined by high-performance liquid chromatography with radioactivity flow detection; metabolite structures were confirmed by liquid chromatography/mass spectrometry. Bazedoxifene was rapidly absorbed, exhibiting a mean peak plasma concentration of 3.43 ng/mL at 1.2 hours post-dose. The total mean recovery of the radioactive dose in excreta was 85.6%, with the majority recovered in feces (84.7%) and only a small fraction (0.81%) in urine. Radiochromatograms of plasma revealed that glucuronidation was the major metabolic pathway; little or no P450-mediated metabolism was evident. The majority of circulating radioactivity was constituted by metabolites, with bazedoxifene-5-glucuronide being the predominant metabolite (up to 95%). Bazedoxifene-4′-glucuronide was a minor metabolite (up to 20%), and unchanged bazedoxifene represented 0% to 13% of the radioactivity in most plasma samples. Unchanged bazedoxifene was the major radioactive component in feces, however, reflecting unabsorbed drug and/or glucuronides that were hydrolyzed by intestinal bacterial enzymes. [14C]Bazedoxifene was generally well tolerated. These findings demonstrated that, following oral administration in healthy postmenopausal women, bazedoxifene was rapidly absorbed, metabolized via glucuronidation, and excreted predominantly in feces.
Bazedoxifene is a new selective estrogen receptor modulator (SERM) that is in clinical development for the prevention and treatment of postmenopausal osteoporosis (Komm et al., 2005; Ronkin et al., 2005), a disorder characterized by decreased bone mass and weakening of the microarchitecture of bone tissue, resulting in increased bone fragility and a propensity to fractures (Cummings and Melton, 2002). Bone loss and formation are mediated at the cellular level by the balanced activity of osteoclasts, which remove old bone, and osteoblasts, which form new bone (Gass and Dawson-Hughes, 2006). The decline in circulating estrogen levels associated with the menopausal transition is thought to shift this balance toward osteoclast-mediated bone breakdown (Riggs, 2000). Thus, the end result of estrogen deficiency is the development of osteopenia and, ultimately, osteoporosis (Gass and Dawson-Hughes, 2006). Consequently, postmenopausal women with undetectable serum estradiol concentrations are at an increased risk for hip and vertebral fractures (Cummings et al., 1998).

The clinical development of SERMs has enabled the selection of compounds that provide the favorable therapeutic effects of estrogen on bone while minimizing the undesirable effects of estrogen on breast and uterine tissue. SERMs are a chemically diverse set of compounds that lack the steroid structure of estrogen, but possess tertiary structure that facilitates binding to the estrogen receptor (Marín and Barbancho, 2006; Riggs and Hartmann, 2003), enabling their selective agonist or antagonist effects in a tissue-dependent manner (Deroo and Korach, 2006). Bazedoxifene is a chemically distinct SERM (Figure 1) that was selected for its proestrogenic effects on bone and lipid metabolism and its antiestrogenic effects on the breast and endometrium (Miller et al., 2002; Komm et al., 2005; Komm and Lyttle, 2001; Ronkin et al., 2005). Bazedoxifene represents a promising pharmacotherapy with a potentially enhanced safety
profile and may serve as a viable alternative to currently available therapies for the prevention and treatment of osteoporosis.

The absolute and relative bioavailability of bazedoxifene has been assessed in an open-label, randomized, three-way crossover study conducted in healthy postmenopausal women under fasting conditions (Patat et al., 2003). Subjects were administered 1 of the following formulations of bazedoxifene: two 5-mg capsules, one 10-mg tablet, or 3 mg given intravenously. The absolute bioavailability of both oral formulations was approximately 6.25, which is 3-fold higher than that of raloxifene, the only SERM currently available on the market. In addition, the concentration-time profiles of the oral formulations were superimposable, indicating that tablet and capsule formulations are bioequivalent with respect to the area under the curve (AUC). Dose proportionality has also been evaluated in postmenopausal women who received multiple oral doses of bazedoxifene 5, 20, or 40 mg in a randomized, crossover fashion for 14 days (Ermer et al., 2003). Maximum concentration (Cmax) was dose dependent and was reached within 1 to 2 hours. The half-life of bazedoxifene was approximately 28 hours. Bazedoxifene exhibited linear pharmacokinetics, with no unexpected accumulation. Plasma concentrations were approximately double at steady state, which was reached by Day 7; protein binding was greater than 99%.

In this study, we assessed the metabolic disposition of bazedoxifene in healthy postmenopausal women following oral administration of 20 mg (200 μCi) [14C]bazedoxifene.
METHODS

Compounds and Reagents

\[^{14}\text{C}]\text{Bazedoxifene, bazedoxifene-4'\text{-glucuronide, bazedoxifene-5-glucuronide, bazedoxifene-4', 5-di-glucuronide, and bazedoxifene-N-oxide were prepared by Wyeth Research (Pearl River, NY). Ascorbic acid and ammonium acetate were obtained from Sigma Chemical Co. (St Louis, MO). All solvents were analytical or high-performance liquid chromatography (HPLC)-grade. Solvents used for sample extractions and HPLC mobile phase preparation, including methanol, acetonitrile, and acetone were obtained from EMD Chemicals (Gibbstown, NJ) unless otherwise indicated. Ultima Gold and Ultima Flo M scintillation fluids were obtained from PerkinElmer (Downers Grove, IL). Glusulase was purchased from DuPont (Boston, MA).}

Study Design

This open-label phase I study designed to evaluate the metabolism and disposition of \[^{14}\text{C}]\text{bazedoxifene following a single oral dose (200 \(\mu\)Ci) was conducted at a single investigational study site (GFI Research Center, Evansville, IN). Healthy ambulatory postmenopausal women 48 to 65 years of age were enrolled in the study. On Day 1 of the inpatient phase (Day –1 to Day 11), subjects were administered a single dose of \[^{14}\text{C}]\text{bazedoxifene with a specific activity of 10 \(\mu\)Ci/mg (200 \(\mu\)Ci/capsule, 20 mg/capsule) at approximately 8 am. Dose was administered with water, and subjects abstained from food or drink (except water) for at least 10 hours prior to and 4 hours after dose administration. To facilitate daily bowel movements, subjects received 2 Senokot tablets at approximately 8 pm on days 1 through 10. Additionally, oral calcium and vitamin supplements at 100% or less of the recommended daily allowance were permitted throughout the study. At the time of \[^{14}\text{C}]\text{bazedoxifene administration, complete (including orthostatic) vital signs and a 12-lead ECG}

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were obtained, and blood, urine, and fecal samples were collected. Physical, laboratory, and ECG evaluations were also performed on Day 11. Subjects were discharged from the center when the measured radioactivity in urine was less than 3 times the background level (Day 11).

The protocol was approved by the Ohio Valley Institutional Review Board before the start of the study, which was conducted according to the Declaration of Helsinki and its amendments. Standard radiation safety procedures were followed according to procedures defined by the United States Nuclear Regulatory Commission (International Commission on Radiological Protection, 1977) and the investigator’s local radiation safety office (TBS, Inc).

Sample Collection

Venous blood samples were collected at 0, 0.25, 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 36, and 48 hours after administration of \([^{14}\text{C}]\text{bazedoxifene}\) and then once daily until Day 11 (total volume, approximately 560 mL). Blood samples were collected in 10-mL evacuated tubes containing sodium heparin, which were inverted gently 4 or 5 times and centrifuged in a refrigerated centrifuge within 15 minutes of collection. The resulting separated plasma for each sample was transferred to watertight, labeled polypropylene tubes, and one 2.5-mL aliquot was separated for analysis of parent drug (bazedoxifene). Whole blood was collected in 3 mL evacuated tubes containing EDTA and inverted gently 4 or 5 times. After 1 mL was used for hematocrit analysis, the remaining blood was frozen and stored. Additional 50-mL blood samples were collected at 1, 4, 8, 24, and 48 hours post-dose for metabolic profile analyses. These samples were collected in evacuated tubes containing sodium heparin, inverted gently 4 or 5 times, and centrifuged in a refrigerated centrifuge within 15 minutes of collection. All samples were stored at about –70°C in an upright position.
Urine samples were collected prior to $[^{14}\text{C}]$bazedoxifene administration at specified intervals following dose administration (0-4, 4-8, 8-24, and 24-48 hours) and once daily until Day 11. All urine voided during each specified interval was pooled and refrigerated (2°C-8°C) after the total volume and collection time were recorded. Fecal samples and bathroom tissues for each bowel movement were collected individually over each 24-hour interval after administration of $[^{14}\text{C}]$bazedoxifene and stored at –70°C until analysis.

**Measurement of Radioactivity**

Plasma, whole blood, urine, and feces were analyzed for total radioactivity using liquid scintillation spectrometry to determine mass balance by ABC Laboratories (Columbia, MO). The urine, feces (pre-dose to 48 hours), plasma, and blood samples were stored at –80°C upon receipt from the study site. Fecal samples (72-240 hours) were stored at –20°C. Concentrations of radioactivity were determined in plasma using duplicate 100-μL aliquots, which were directly assayed for radioactivity after addition of 10 mL Ultima Gold scintillation cocktail using a liquid scintillation counter (TriCarb liquid scintillation spectrometer Model 2750 TR/LL; PerkinElmer, Downers Grove, IL). A Packard oxidizer was used for combustion of blood and fecal homogenate samples. Blood samples were aliquoted, weighed, and combusted. Fecal samples were weighed and homogenized after the addition of liquid nitrogen using a Polytron blender (Brinkmann Instruments, Westbury, NY). Fecal homogenates were aliquoted, weighed, and combusted.

**Measurement of Unchanged Bazedoxifene in Plasma**

Concentrations of unchanged bazedoxifene in plasma were determined by Kansas City Analytical Services, Inc. (Shawnee, KS), using a validated HPLC method with fluorescence
detection. Plasma (1 mL) was processed using solid phase extraction, separation with a base-deactivated C-18 HPLC column, and fluorescence detection with 300 nm excitation and 380 nm emission. The lower limit of quantitation for bazedoxifene in plasma was 20 pg/mL. The method was validated and linear over a concentration range of 20 to 2000 pg/mL. Quality control samples were prepared and assayed with study samples as an independent assessment of accuracy and precision. The CV ranged from 5.8% to 12.4% and the recover (bias) ranged from 1.4% to 3.2% for control samples.

Metabolite Profiles in Plasma and Feces
Metabolite profiles in plasma were generated using HPLC with fraction collection and radioassayed using liquid scintillation spectroscopy (Wyeth Research, Monmouth Junction, NJ) because of the low concentration of radioactivity in plasma samples. Aliquots of plasma (9 mL) from each subject at each time point up to 24 hours was prepared for analysis. Metabolite profiles in feces were generated using HPLC with flow radioactivity detection. For each 1 mL of sample, protein was precipitated by the addition of 0.2 mL of chilled methanol followed by 5 mL of chilled acetonitrile. The protein precipitate was removed by centrifugation at 1800 g for 10 min at 4°C. To prevent oxidation of bazedoxifene and its metabolites to their respective N-oxides, 20 μL of 100 mM ascorbic acid was added to the supernatant, which was evaporated under nitrogen. The residue was then reconstituted in approximately 100 to 300 μL of mobile phase; of this, a 10-μL aliquot was removed for recovery calculations, and the remainder was injected onto the HPLC. Unlabeled standards of bazedoxifene and its phenyl and indole glucuronide metabolites (bazedoxifene-4'-glucuronide and bazedoxifene-5-glucuronide, respectively) were added before injection. While collecting fractions, UV profiles (254 nm) for unlabeled standards were monitored in order to match the retention times of the peaks in the
radiochromatograms. Fractions were collected every 1 min, 5 mL of scintillation cocktail (Ultima Gold) was added, and samples were radioassayed by liquid scintillation spectroscopy. HPLC was performed with a Waters Alliance HPLC system, which consisted of a Waters 2690 separations module and a 996 photo diode array detector. A Packard Radiomatic A525 Flo-one beta radioactivity flow detector was used to detect radioactivity. The scintillation fluid (Ultima Flo M) was pumped at 0.25 mL/min for all radioactive HPLC assays using a linear gradient with a mobile phase system consisting of 0.05 M ammonium acetate buffer (pH 4.5) and methanol and was pumped at 1 mL/min through a narrow bore Supelcosil column LC-18-DB (2.1 x 150 mm, 5 μm; Supelco, Bellefonte, PA). Millennium 32 software (Waters Corporation, Milford, MA) was used for data acquisition and processing. For feces, approximately 0.5 to 0.8 g of sample from each subject (through Day 4) was weighed, acetone (10 mL) was added, and samples were agitated for 10 min to form a homogeneous suspension. Samples were then centrifuged at 450g for 10 min at 4°C. The precipitate was re-extracted with 10 mL of acetone as described above. Supernatants from both extractions were combined, and ascorbic acid (20 μL of 100 mM) was added before evaporation to dryness under nitrogen. The residue was reconstituted in 200 to 400 μL of methanol/water (1:1). After sonication for 5 minutes, samples were centrifuged by microcentrifugation, and approximately 250 μL of supernatant was analyzed by HPLC with radioactivity flow detection as described above for plasma. The stability of the drug and its metabolites in feces was assessed by spiking 10 μg of reference [14C]bazedoxifene and its phenyl glucuronide and indole glucuronide metabolites separately into 0.3 to 0.5 g of control (Hour 0) fecal samples from Subjects 5 and 6. The samples were extracted and analyzed by HPLC as described above. The lower limit of detection for radiochromatography was twice the background (approximately 50 dpm). The identities of the metabolites were confirmed by liquid chromatography/mass spectrometry (LC/MS).
Glusulase® Hydrolysis of Plasma

Plasma (1 mL) collected at 1 and 4 hours post-dose was pooled for all 6 subjects, and protein precipitation was performed as described above. The supernatants were then evaporated to dryness under nitrogen. Sodium acetate (2 mL of 0.2 M), pH 5, was added to the residue. Glusulase® (400 μL; β-glucuronidase activity 90,000 U/mL; sulfatase activity 10,000 U/mL; DuPont, Boston, MA) was added to each tube to yield a final concentration of 15,000 U/mL of β-glucuronidase activity. The enzyme-treated samples were incubated at 37°C overnight, and hydrolysis was terminated by the addition of 200 μL of chilled methanol followed by 5 mL of acetonitrile. Precipitated protein was collected by centrifugation at 1800 g for 10 min at 4°C. Ascorbic acid (20 μL/100 mM) was added to the supernatants, which were then evaporated to dryness under nitrogen. The residues were reconstituted in approximately 300 μL methanol:water (1:1). A 100-μL aliquot was injected onto the HPLC under conditions described above, except the mobile phase flow rate was 1 mL/min, and the column diameter was 4.6 mm.

Mass Spectrometry

A Finnigan TSQ700 triple quadrupole mass spectrometer (Thermo Scientific, San Jose, CA) equipped with an electrospray ionization interface and operated in the positive ionization mode was used for metabolite characterization. Settings for the mass spectrometer were as follows: nebulizer gas, 90 psi; auxiliary gas, 30 arbitrary units; spray voltage, 4.5 kV; heated capillary temperature, 200°C; mass resolution, 0.7 ± 0.2 Da width at half height for Q1 and Q3; collision gas pressure, 1.9 to 2.2 mtorr; collision offset, –32 to –36 eV. Mass spectral data for bazedoxifene and its metabolites were recorded in full scan mass spectrum, selected ion monitoring, and selected reaction monitoring modes.
Calculations

The total radioactivity (dpm) excreted in the urine or feces was determined by multiplying the volume of urine or the weight of fecal homogenates by the radioactivity concentration of each sample (in dpm/mL or dpm/g, respectively). The dose recovered was determined by total dpm in the sample at any given time point, divided by total radioactivity (in dpm) of the dose received by each subject, and multiplied by 100. Concentrations of radioactivity in blood and plasma were determined using a specific activity of 23.5 dpm/ng of [14C]bazedoxifene. Total radioactivity concentrations in blood were converted to units of ng equivalents/mL using the specific activity value determined for each subject.

Pharmacokinetic Analysis

Plasma concentrations of bazedoxifene for each subject were analyzed using empirical, model-independent methods. Thus, values for maximum plasma concentration (C_max) and time to C_max were taken directly from the observed data. The area under the radioactivity-versus-time curve and the plasma concentration-versus-time curve (AUC) to last measurable time (AUCT) were calculated using the linear/log-linear trapezoidal method with the observed data. T varied for each subject, depending on when the last measurable sample was taken; on average, T was 48 for bazedoxifene and 192 for total radioactivity. The terminal-phase elimination rate constants (\( \lambda_z \)) were estimated by least-square regression of the terminal portion of the log-transformed radioactivity- and concentration-versus-time curves. AUC was calculated as AUCT + last measurable concentration at time T (C_T)/\( \lambda_z \), AUCT was extrapolated to infinity (AUC_{\infty}) by addition of C_T + C_T/\( \lambda_z \), where C_T was the last measurable concentration. The estimated half-life was 0.693/\( \lambda_z \). The estimate of apparent oral dose clearance (Cl/F) and apparent volume of
distribution (V/F) for bazedoxifene was calculated by dose/AUC_{0-\infty}/weight and dose/\lambda_z \cdot AUC_{0-\infty}/weight, respectively, in the appropriate units. All pharmacokinetic calculations were performed using WinNonlin Professional Version 4.1 (Pharsight Corporation, Mountain View, CA).

Statistical Analysis

Because all subjects received the same treatment, no statistical comparisons were performed. The mean S.D. and range for concentrations of radioactivity, concentrations of \([14C]bazedoxifene and its radiolabeled metabolites, and bazedoxifene pharmacokinetic parameters are presented.

RESULTS

Subjects

All 6 subjects enrolled in the study were healthy, postmenopausal women (mean age, 56). Each subject received a single dose of \([14C]bazedoxifene (200 \mu Ci). All of the women completed the study and were included in the analyses. The mean (S.D.) age of the subjects was 56 (6) years (range, 48-65 years; CV, 10.6\%), and the mean (S.D.) weight and height were 73.4 (9.4) kg (range, 60.0-84.5 kg; CV, 12.9\%) and 165.2 (5.3) cm (range, 158.0-174.0 cm; CV, 3.2\%), respectively. ECG data, clinical laboratory test results, and vital sign measurements showed no evidence of any safety issues related to bazedoxifene (data not shown).

Absorption and Elimination of Radioactivity

The plasma time course profile of radioactivity concentrations indicated rapid absorption and slow elimination of \([14C]bazedoxifene following oral administration (Figure 2). By 48 hours
after administration, concentrations of radioactivity in plasma (5.7 ng equiv/mL) were less than 10% of the concentrations seen at 1 hour (79.8 ng equiv/mL). The total radioactivity concentrations in whole blood paralleled those of plasma, but at lower levels. The blood:plasma ratio of radioactivity was below 0.55, suggesting no preferential binding of drug-derived materials to whole blood constituents.

The concentrations of unchanged drug in plasma were determined using a validated analytical method. Table 1 summarizes selected plasma pharmacokinetic parameters of bazedoxifene and total radioactivity in subjects. Bazedoxifene was rapidly absorbed in this population of postmenopausal women; a mean peak concentration of 3.43 ng/mL was achieved at 1.2 hours after dosing (Table 2). Elimination of bazedoxifene was slow, with a mean (S.D.) half-life of 32.8 (15.6) hours. Based on mean plasma concentrations of unchanged drug and total radioactivity, parent drug accounted for about 4% to 8% of the total radioactivity in plasma, suggesting that bazedoxifene was extensively metabolized following oral administration in women.

Elimination of Radioactivity

Ten days after administration of a single dose of [14C]bazedoxifene (200 μCi), the mean total recovery of radioactivity from the combined excreta (urine and feces) was 85.6% (S.D., 4.8%). The fraction of the radioactive dose excreted in each time interval is summarized in Table 2. The rate of excretion was variable, with 2.3% to 80.8% of the radioactive dose recovered in feces within 48 hours. The major route of excretion of radioactivity was the feces, with 84.7% (S.D., 4.9%) recovered after 10 days. Excretion in urine represented a minor route of elimination of
radioactivity, with 0.81% (S.D., 0.27%) recovered after 10 days. The time course of the mean cumulative excretion of radioactivity is shown in Figure 3.

Metabolite Profile

A representative radiochromatogram of plasma at 1 hour after administration of $[14C]$bazedoxifene is shown in Figure 4. The recovery of radioactivity in the plasma extracts was more than 80% for all samples. Metabolite profiles were generated at 1, 4, 8, and 24 hours after administration. Because these concentrations approached the lower limit of detection for radiochromatography, the 48-hour plasma samples were not evaluated for metabolic profiling. The chromatograms revealed the presence of a single major radioactive component (40%-95%), bazedoxifene-5-glucuronide (the indole glucuronide), in all samples and at all time points examined. Unchanged bazedoxifene was a minor component (0%-13%), and small amounts of bazedoxifene-4′-glucuronide (the phenyl glucuronide) were present in most samples (0%-20%). Based on the retention time of the unlabeled reference standard, a minor metabolite present in the 8- and 24-hour samples was identified as bazedoxifene-di-glucuronide. No other metabolites were apparent by radiochromatography, except for an early eluting peak at 8 and 24 hours, which tended to represent a small percentage (<10%) of the plasma radioactivity in most samples. The metabolite profiles in plasma were similar among the 6 subjects at each time point. The majority of radioactivity was the result of the indole glucuronide metabolite. Chromatography of an enzyme-hydrolyzed pooled plasma sample produced one radioactive peak, with the same retention time as that of the parent compound, confirming the hydrolysis of the glucuronides resulting in the release of aglycone.
The stability of bazedoxifene and its metabolites in fecal samples was assessed using control samples spiked with $[^{14}\text{C}]$bazedoxifene and its phenyl glucuronide and indole glucuronide metabolites separately. Chromatograms of spiked control fecal samples showed that the glucuronide metabolites were partially (20%-40%) hydrolyzed, releasing the parent compound, under the conditions used to extract samples. Fecal metabolite profiles were generated from samples collected from 3 subjects at 0 to 24, 24 to 48, 48 to 72, and 72 to 96 hours after $[^{14}\text{C}]$bazedoxifene administration. A representative radiochromatogram of fecal extracts from 0 to 24 hours after $[^{14}\text{C}]$bazedoxifene administration is shown in Figure 5. Unchanged bazedoxifene was the predominant radioactive component (>90%) at all time points. Small amounts of bazedoxifene-N-oxide (0%-8%) were observed in most samples, whereas the 2 glucuronides of bazedoxifene were not detected. Additional radioactive peaks observed in some of the samples had not co-eluted with either of the glucuronides of bazedoxifene and were considered to be minor metabolites (representing <5% of total radioactivity) or artifacts because spiked control fecal samples also produced these peaks.

**Mass Spectrometric Analysis of Bazedoxifene Metabolites**

The mass spectral characteristics of an authentic standard of bazedoxifene were examined for comparison with metabolites. In the LC/MS spectrum of bazedoxifene, a protonated molecular ion ([M+H]$^+$) was observed at mass-to-charge ration ($m/z$) 471. Figure 6 shows the proposed fragmentation scheme and product ions of [M+H]$^+$ mass spectrum for bazedoxifene. Structurally diagnostic product ions were observed at $m/z$ 345, 252, 239, 126, 112, and 99. Changes in the $m/z$ values for these ions provided information for structural elucidation. This permitted sites of metabolism to be localized to the azepan, ethoxy, benzyl, or 2-(4-hydroxy-phenyl)-3-methyl-1$H$-indol-5-ol moieties. Liquid chromatography/mass spectrometry/mass spectrometry...
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(LC/MS/MS) analysis in the selected reaction monitoring mode was used to confirm the presence of metabolites in samples with insufficient material for full-scan mass spectral analysis. In these samples, observation of chromatographic peaks at the expected retention times while monitoring the m/z 471→126 (bazedoxifene), m/z 487→126 (bazedoxifene-N-oxide), and m/z 647→471 (bazedoxifene glucuronides) transitions was used to confirm the presence of bazedoxifene and its metabolites. Table 3 provides a summary of the metabolite peaks identified in plasma and feces by LC/MS analysis.

Safety

Two subjects reported a total of 4 adverse events. One subject had mild dysuria, which may have been related to study drug administration. A second subject complained of sinusitis and a mild rash, which she had also experienced prior to test article administration. This subject also experienced a syncopal episode that may have been related to the protocol requirements of fasting accompanied by frequent blood draws for pharmacokinetic and laboratory analyses.

DISCUSSION

Bazedoxifene, a new SERM in development for the prevention and treatment of osteoporosis, was well tolerated by all six women given a single dose of 20 mg, and no discontinuations or serious adverse effects were observed. After test article administration, there were no effects on clinical laboratory values, vital signs, or ECGs. None of these subjects had any illnesses at baseline that might have interfered with the pharmacokinetics of the test article or the interpretation of the results. In postmenopausal women, bazedoxifene given at 20 mg/day has been shown to decrease bone turnover by 20% to 25%, with no occurrences of endometrial hyperplasia (Gruber and Gruber, 2004; Ronkin et al., 2005; Stump et al., 2007). Bazedoxifene
displayed straightforward pharmacokinetic properties following a single oral dose of $[^{14}\text{C}]$bazedoxifene in this study. After administration of $[^{14}\text{C}]$bazedoxifene, bazedoxifene was rapidly absorbed, with peak plasma concentrations of both unchanged drug and radioactivity reached around 1 hour post-dose. The mean plasma elimination half-life of unchanged drug was long (about 33 hours). The pharmacokinetics of bazedoxifene observed in this study were similar to those seen in a previous study (Ermer et al., 2003).

Bazedoxifene is extensively metabolized in postmenopausal women through the glucuronidation pathway; in this study, little or no P450-mediated metabolism was evident. In glucuronidation reactions, uridine diphosphate glucuronosyltransferases catalyze the transfer of glucuronic acid to aromatic and aliphatic alcohols, carboxylic acids, amines, and free sulfhydryl groups of both exogenous and endogenous compounds to form O-, N-, and S-glucuronides, respectively (Wilkinson, 2001). The proposed pathways for glucuronidation of bazedoxifene are shown in Figure 7. According to this paradigm, conjugation takes place at both the phenyl and indole moieties of the molecule; however, the major circulating metabolite was the indole glucuronide (bazedoxifene-5-glucuronide). The extensive glucuronidation of bazedoxifene is consistent with the reported low bioavailability and high oral clearance (Ermer et al., 2003; Patat et al., 2003) and indicates a high first pass effect. A long half-life and secondary peaks in the concentration-time profiles also suggest that bazedoxifene undergoes enterohepatic recirculation.

Approximately 85% of the radioactive dose was recovered in the feces within 10 days of administration of $[^{14}\text{C}]$bazedoxifene. Urine contained less than 1% of the radioactive dose. In feces, unchanged drug was the only major radioactive component, and the 2 glucuronides of bazedoxifene were not detected. The glucuronides of bazedoxifene are unstable in human feces,
giving rise to the aglycone, possibly due to rapid hydrolysis by intestinal bacterial enzymes (data not shown). Therefore, the predominance of unchanged bazedoxifene observed in the fecal sample chromatograms likely resulted from unabsorbed drug but may have also been the result of excretion of hydrolyzed glucuronides.

The metabolic disposition of bazedoxifene in women reported here is consistent with findings previously reported in preclinical studies of other species (Ahmad et al., 2006). Following oral administration of $[^{14}C]bazedoxifene$, the predominant circulating radioactive component in rats and monkeys was the 5-glucuronide. Both 4’-glucuronide and 5-glucuronide appear to be the major plasma metabolites in mice; however, glucuronidation was the major metabolic pathway for bazedoxifene in all species examined thus far. In preclinical studies, the major route of excretion of radioactivity following oral administration of bazedoxifene was the feces, which was consistent with our findings in postmenopausal women. Data from a metabolism study (Ahmad et al., 2006) conducted in bile-duct cannulated female rats indicated that approximately 55% of a 1-mg/kg oral dose of $[^{14}C]bazedoxifene$ was excreted in bile in the form of glucuronide metabolites. However, metabolite profiles of fecal samples from intact rats indicated that the majority of the radioactive component in the feces was unchanged parent drug (Ahmad et al., 2006). These preclinical data support the hypothesis that bazedoxifene is readily absorbed in women following administration, metabolized via glucuronidation, and excreted in the bile as glucuronides, which are hydrolyzed by bacterial enzymes back to bazedoxifene.

Little or no cytochrome P450-mediated metabolism was evident in metabolite profiles during in vitro studies in humans. In human metabolite studies (Ahmad et al., 2008), UGT1A1, UGT1A8, and UGT1A10 were the most active isoforms involved in the glucuronidation of bazedoxifene.
and that UGT1A3, UGT1A7, UGT1A9, UGT2B7, and UGT2B15 had more limited glucuronidation activity. A major effect on the population pharmacokinetics in women due to the polymorphic forms of UGT1A1 or other isoforms is considered unlikely, as multiple UGTs appear to be involved in the metabolism of bazedoxifene.

The metabolic disposition of raloxifene, a SERM indicated for the prevention and treatment of postmenopausal osteoporosis, has been evaluated using the radiolabeled drug (Dodge et al., 1997; Kemp et al., 2002). Although raloxifene and bazedoxifene have some structural similarities and both compounds possess two phenolic groups, raloxifene contains a benzothiophene core, whereas bazedoxifene is indole-based (Gruber and Gruber, 2004). As with bazedoxifene, raloxifene was shown to be extensively metabolized, and the majority of the circulating radioactivity was determined to result from a glucuronide metabolite (Hochner-Celnikier, 1999). Raloxifene was primarily excreted via feces, with unchanged raloxifene as the major radioactive component. Similar to the results of this study, the glucuronide conjugates of raloxifene were hydrolyzed when added ex vivo to control fecal samples.

In conclusion, results of this study demonstrated that an oral dose of 20 mg [14C]bazedoxifene was well tolerated in postmenopausal women. Bazedoxifene was rapidly absorbed and extensively metabolized. Bazedoxifene-5-glucuronide was the major circulating metabolite as well as radioactive component, indicating that glucuronidation was the major metabolic pathway for bazedoxifene in women. Unchanged bazedoxifene and bazedoxifene-4'-glucuronide were minor components in plasma. The majority of the radioactive dose (85%) was excreted via the feces as unchanged drug, representing both unabsorbed drug and/or hydrolyzed glucuronides.
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REFERENCES


Footnotes


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Legends for Figures

Figure 1. Structure of [14C]bazedoxifene. *indicates site of 14C label.

Figure 2. Mean plasma concentrations of total radioactivity and plasma concentrations of bazedoxifene in healthy postmenopausal women receiving 20 mg [14C]bazedoxifene (200 μCi). BZA, bazedoxifene.

Figure 3. Mean cumulative excretion of radioactivity after a single oral dose of 20 mg [14C]bazedoxifene (200 μCi) in healthy postmenopausal women.

Figure 4. Representative radiochromatographic profile of plasma sample taken at 1 h after dose administration of 20 mg [14C]bazedoxifene (200 μCi). DPM, disintegrations per minute; BZA, bazedoxifene; Glu, glucuronide.

Figure 5. Representative radiochromatographic profile of a 0- to 24-h fecal sample after dose administration of 20 mg [14C]bazedoxifene (200 μCi). CPM, counts per minute; BZA, bazedoxifene.

Figure 6. Proposed fragmentation scheme (A) and product ions of [M+H]+ mass spectrum for bazedoxifene (B).

Figure 7. Proposed metabolic pathways of bazedoxifene in healthy postmenopausal women.
Table 1. Pharmacokinetic Parameters of [14C]Bazedoxifene in Healthy Postmenopausal Women (N = 6) After a Single 20-mg Dose\(^a\)

<table>
<thead>
<tr>
<th>Pharmacokinetic parameter</th>
<th>[14C]Bazedoxifene</th>
<th>Total Radioactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± S.D.</td>
<td>Mean ± S.D.</td>
</tr>
<tr>
<td>C(_{\text{max}}) (ng/mL) or (ngEq/mL)</td>
<td>3.43 ± 1.14</td>
<td>75.7 ± 26.7</td>
</tr>
<tr>
<td>t(_{\text{max}}) (h)</td>
<td>1.2 ± 0.4</td>
<td>1.2 ± 0.4</td>
</tr>
<tr>
<td>Half-life (h)</td>
<td>32.8 ± 15.6</td>
<td>10.2 ± 3.6</td>
</tr>
<tr>
<td>AUC(_T) (ng·h/mL) or (ngEq/mL)(^b)</td>
<td>54 ± 14</td>
<td>732 ± 204</td>
</tr>
<tr>
<td>AUC (ng·h/mL) or (ngEq/mL)</td>
<td>56 ± 14</td>
<td>746 ± 193</td>
</tr>
<tr>
<td>Cl/F (L/h/kg)</td>
<td>5.2 ± 1.4</td>
<td>—</td>
</tr>
<tr>
<td>V/F (L/kg)</td>
<td>248 ± 134</td>
<td>—</td>
</tr>
</tbody>
</table>

\(^a\)Concentrations of bazedoxifene were determined using a validated analytical method.

\(^b\)T varied for each subject, depending on when the last measureable sample was taken.

C\(_{\text{max}}\), maximum plasma concentration; t\(_{\text{max}}\), time to C\(_{\text{max}}\); AUC\(_T\), area under the plasma concentration-versus-time curve to last measurable time; AUC, area under the plasma concentration-versus-time curve; Cl/F, estimate of the apparent oral dose clearance; V/F, estimate of the apparent oral dose volume of distribution.
Table 2. Mass Balance of Radioactivity (Percentage of Dose [Mean  S.D.] Excreted in 0-240 h)

Following a Single Oral Dose of \(^{14}C\)Bazedoxifene in Healthy Postmenopausal Subjects (N = 6)

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Urine</th>
<th>Feces</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-4</td>
<td>0.14 ± 0.04</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-8</td>
<td>0.17 ± 0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8-24</td>
<td>0.28 ± 0.07</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-24</td>
<td>0.54 ± 0.10(^a)</td>
<td>23.5 ± 25.2</td>
<td>24.0 ± 25.1</td>
</tr>
<tr>
<td>24-48</td>
<td>0.21 ± 0.13</td>
<td>21.3 ± 15.9</td>
<td>21.5 ± 15.8</td>
</tr>
<tr>
<td>48-72</td>
<td>0.05 ± 0.05</td>
<td>19.4 ± 12.6</td>
<td>19.4 ± 12.6</td>
</tr>
<tr>
<td>72-96</td>
<td>0.01 ± 0.02</td>
<td>8.8 ± 10.4</td>
<td>8.8 ± 10.4</td>
</tr>
<tr>
<td>96-120</td>
<td>0.01 ± 0.02</td>
<td>3.0 ± 3.8</td>
<td>3.0 ± 3.8</td>
</tr>
<tr>
<td>120-144</td>
<td>0.01 ± 0.01</td>
<td>2.8 ± 2.8</td>
<td>2.8 ± 2.9</td>
</tr>
<tr>
<td>144-168</td>
<td>0.00 ± 0.00</td>
<td>4.0 ± 7.7</td>
<td>4.0 ± 7.7</td>
</tr>
<tr>
<td>168-192</td>
<td>0.00 ± 0.00</td>
<td>0.2 ± 0.1</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>192-216</td>
<td>0.00 ± 0.00</td>
<td>1.1 ± 2.5</td>
<td>1.1 ± 2.5</td>
</tr>
<tr>
<td>216-240</td>
<td>0.00 ± 0.00</td>
<td>1.0 ± 2.4</td>
<td>1.0 ± 2.4</td>
</tr>
</tbody>
</table>

\(^a\)Cumulative.
Table 3. Liquid Chromatography/Mass Spectrometry Identification of Bazedoxifene Metabolites

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Mol. Wt.</th>
<th>Characteristic ion (m/z)</th>
<th>Site of metabolism</th>
<th>Metabolic name</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td>646</td>
<td>647, 471</td>
<td>Phenol ring</td>
<td>BZA-4′-glucuronide</td>
<td>Plasma</td>
</tr>
<tr>
<td>M2</td>
<td>646</td>
<td>647, 471, 126</td>
<td>Indole ring</td>
<td>BZA-5′-glucuronide</td>
<td>Plasma</td>
</tr>
<tr>
<td>M4</td>
<td>486</td>
<td>487, 126</td>
<td>Azepan ring</td>
<td>BZA-N-oxide</td>
<td>Feces</td>
</tr>
<tr>
<td>Parent</td>
<td>470</td>
<td>471, 345, 252, 239, 126</td>
<td>—</td>
<td>BZA</td>
<td>Plasma and feces</td>
</tr>
</tbody>
</table>

Mol. wt., molecular weight; BZA, bazedoxifene.
Figure 1
Figure 2
Figure 3

- Total
- Feces
- Urine

Percentage of dose

Day after dosing
Figure 4
Figure 5
Figure 7

Bazedoxifene-N-oxide → Bazedoxifene → Bazedoxifene-4’-glucuronide

Bazedoxifene-5-glucuronide → Bazedoxifene-5,4’-diglucuronide