EXCRETION AND METABOLISM OF TROVAFLOXACIN IN HUMANS

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ABSTRACT:

The metabolism and excretion of trovafloxacin was investigated in four healthy male volunteers after a single oral administration of 200 mg of [14C]trovafloxacin (118 µCi). Mean values of 23.1 and 63.3% of the administered dose were recovered in the urine and feces, respectively, after 240 hr. The Cmax of total radioactivity and unchanged trovafloxacin in serum was 3.2 µg-equiv/ml and 2.9 µg/ml, respectively, and peaked in 1.4 hr. The mean AUC6→∞ for radioactivity and trovafloxacin was 58.2 µg·equiv·hr/ml and 32.2 µg·hr/ml, respectively. This implied that unchanged trovafloxacin constituted 55% of the circulating radioactivity. Urine and fecal samples were analyzed by LC/MS/MS for characterization of the metabolites, and the quantity of each metabolite in the matrices was assessed by means of a radioactivity detector. The profile of radioactivity in urine showed three main metabolites that were identified as the trovafloxacin glucuronide (M1), N-acetyltrova- floxacin glucuronide (M2), and N-acetyltrovafloxicin (M3). The major fecal metabolites were M3 and the sulfate conjugate of trovafloxacin (M4). Analysis of circulating metabolites from pooled serum extracts obtained at 1, 5, and 12 hr indicated that M1 was the major circulating metabolite (22% of circulating radioactivity), whereas M2 and M3 were detected in minor amounts. The results of the present study revealed that oxidative metabolism did not play a significant role in the elimination of trovafloxacin, and phase II conjugation was the primary route of trovafloxacin clearance in humans.

Trovafloxicin, (1α,5α,6α)-7-(6-amino-3-azabicyclo[3.1.0]hex-3-y1)-1-(2,4-difluorophenyl)-6-(fluoro-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acid), is a new quinolone antibacterial agent with a broad spectrum of activity against Gram-positive and Gram-negative bacteria (1). It has shown several desirable characteristics and can be differentiated from ciprofloxacin, ofloxacin, and other marketed fluoroquinolones by its greater potency against clinically significant species of Gram-positive organisms, most notably against streptococci such as Streptococcus pneumoniae (2–4). In addition to its in vitro activity and in vivo efficacy, previous animal pharmacokinetic and toxicological studies have shown that trovafloxacin is rapidly absorbed, with an oral bioavailability of 88% and is well tolerated in rats, dogs, and monkeys (5). Biotransformation studies in rats and dogs using [14C]trovafloxicin have indicated that trovafloxacin is metabolized mainly via glucuronidation, and very little drug is excreted unchanged in the urine, in both species (6).

Phase I studies of trovafloxacin in healthy male volunteers after oral administration of single doses ranging from 30 to 600 mg have suggested that the drug is rapidly absorbed as indicated by tmax of ~1 hr. Terminal-phase elimination half-life of the drug is independent of the dose, with an overall mean of 10–12 hr and <10% of the dose is recovered unchanged in urine (7). Low renal excretion and moderate serum protein binding of trovafloxacin suggests extensive metabolism or biliary elimination. Because the metabolic fate of trovafloxacin in humans is not well characterized, the goal of the present study was to investigate the metabolism of trovafloxacin in four healthy male human volunteers after single oral administration of 200 mg of [14C]trovafloxacin.

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Materials and Methods

Chemicals. Radiolabeled trovafloxacin, labeled in the 2-position of the naphthyridine ring, was synthesized as a mesylate salt at Pfizer Central Research by the radiochemistry group. Administration and sampling of [14C]trovafloxacin mesylate was conducted at Pharmaco LSR (Austin, TX), as a clinical study. [14C]Trovafloxicin mesylate had a specific activity of 0.47 µCi/mg and a radiochemical purity of >99%. All doses and concentrations of trovafloxacin provided herein are expressed as free base equivalents. For HPLC and extraction of the matrices, acetonitrile and methanol were purchased from Fisher Scientific (Pittsburgh, PA). Ammonium formate was purchased from Aldrich (Milwaukee, Wi) and dissolved in deionized water obtained by milli-Q reagent water system (Millipore Corp., Bedford, MA) to give a 50 mM solution. The pH of the solution was adjusted with formic acid (96%), which was purchased from Eastman Kodak (Rochester, NY). All reagents and solvents were used as such without further purification. Chromatography was performed on Zorbax C8 columns obtained from Mac-Mod Analytical, Inc. (Chadds Ford, PA). For liquid scintillation counting, Eco-lite scintillation cocktail was purchased from ICN (Costa Mesa, CA). Carbosorb and Permafluor V scintillation cocktails were purchased from Packard Instrument Co. (Downers Grove, IL).

Dosing and Sample Collection. The protocol for this study was approved by the local Institutional Review Board before initiation. A 200 mg dose of [14C]trovafloxacin containing a total activity of 118 µCi was administered to four healthy male volunteers between ages 26.8±4.3 years and weighing 72.4±8.3 kg, in an open fashion, as a single oral suspension followed by a standard meal 4 hr later.

Urine. After dosing, urine samples were quantitatively collected over a period of 0–240 hr postdose at 24-hr intervals, and the total volumes of the urine voided were recorded after each collection. All samples were immediately frozen at −20°C and were shipped over dry ice.

Feces. The fecal samples were collected as passed from the time of dosing until at least 240 hr postdose. The collected samples were immediately frozen and shipped over dry ice.

Blood. Blood samples sufficient to yield 2 ml of serum were collected from each subject at 0 (just before dosing), 0.5, 1, 2, 4, 8, 12, 24, 36, 48, 72, 96, 120, 144, 168, 192, 216, and 240 hr postdose. In addition, blood sufficient to yield 5 ml of serum was collected at times 1, 5, and 12 hr after dosing.
**Determination of Total Radioactivity.** The concentrations of total radioactivity in urine and serum samples were determined in triplicate by mixing 100 μl aliquots with Ecolite (5 ml) and counting on a Packard #2200CA liquid scintillation counter. Feces collected at each time point were weighed and homogenized in water using a Lab-Blender 400 stomacher. Aliquots of 100–150 mg of the homogenate were air-dried overnight and combusted in a Packard Oxidizer Model 307. The radioactivity in the combustion products was determined by trapping the liberated 14CO2 in 9 ml Carbosorb and 9 ml Permafluor V in a scintillation vial. Each sample vial was counted for 10 min, and the counting efficiency was determined by external standardization.

Serum samples were assayed for unchanged trovafloxacin by a reversed-phase HPLC-UV assay as reported previously (8). Briefly, after solid-phase extraction, the chromatographic separation was accomplished using C18 column and a phosphate mobile phase. Trovafloxacin and the internal standard \(\text{[7-(2-methyl-6-amino-3-azabicyclo[3.1.0]hexyl)-naphthyridine, also synthesized at Pfizer Central Research]}\) were detected by UV absorbance at 275 nm.

**Quantitation of Metabolites in Biological Fluids.** Urinary and fecal samples obtained from four subjects over 0–72 and 0–120 hr, respectively, were pooled. Pooled urine was lyophilized, and pooled fecal homogenates (0.7–1.0 g) were extracted 5 times consecutively with methanol (1 ml). The combined methanolic washes were evaporated to dryness. About 75% of the fecal radioactivity was extracted in the four subjects by this procedure. The residues obtained from lyophilized urine and methanolic fecal extract was reconstituted in mobile phase. Serum samples obtained at 1, 5, and 12 hr postdose were pooled, deproteinized with 2 volumes of acetonitrile, and centrifuged. The clear supernatant was evaporated under vacuum, and the residue was reconstituted in 200 μl of 50 mM ammonium formate. An aliquot (50 μl) of the supernatant was counted as described previously. The recovery of radioactivity in the supernatant after precipitation was 99%.

Aliquots of the reconstituted samples were analyzed by reversed-phase HPLC as described previously (6). Chromatography was conducted on a Zorbax R-P_C8 column (4.6 mm × 150 mm; 5 μm) using binary mobile phase consisting of a mixture of 50 mM ammonium formate (pH was adjusted to 3 with formic acid, solvent A) and acetonitrile (solvent B) at a flow rate of 1 ml/min. The gradient conditions used to resolve the metabolites were as follows: solvent A:solvent B 90:10 for 5 min; changed from 90:10 to 80:20 from 5 to 10 min; held at 80:20 from 10 to 20 min; changed from 80:20 to 70:30 from 20 to 25 min; held at 70:30 from 25 to 30 min; changed from 70:30 to 60:40 from 30 to 35 min; held at 60:40 from 35 to 40 min; changed from 60:40 to 20:80 from 40 to 45 min. The solvent A:solvent B ratio was then changed to 90:10 over a period of 10 min and allowed to equilibrate for 10 min before the next injection. A quantitative assessment of the metabolites in urine and the fecal extracts was made by measuring the percentage radioactivity in the individual peaks that were separated by HPLC. The Z-RAM was operated in a homogeneous liquid scintillation counting mode with the addition of 4 ml/min of Ecolite to the eluent post-UV detection. Metabolites in the extracted serum sample were quantified by fractionating and counting the effluents (0.3 min).

**Identification of Metabolites.** Characterization of the metabolites was performed on a Perkin-Elmer Sciex API III triple quadrupole mass spectrometer (Thornhill, Ontario, Canada) using the same HPLC gradient system and the column described previously. A portion of the effluent from the HPLC column was split and introduced into the atmospheric ionization source via an ion spray interface at a rate of 50 μl/min. The remaining effluent was directed into a 500 μl flow cell of the Z-RAM. The RAM response was recorded in real time by the mass spectrometer data system that provided simultaneous detection of radioactivity and total ion chromatogram. The ion spray interface was operated at 5000 V, and the mass spectrometer was operated in the positive-ion mode. Collision induced dissociation studies were performed using argon gas at a collision energy of 28 eV and collision gas thickness of 3.00 × 1014 molecules/cm².

**Calculation of Pharmacokinetic Parameters.** The pharmacokinetic parameters were calculated using PK PARAM, a program that was developed in house. The program is based on standard equations (9). \(\text{Kel}^1\) was determined from the beginning of the terminal phase to the last sampling time postdose by least squares regression analysis of the serum concentration-time data

\(\text{mean terminal log-linear phase. Mean terminal phase half-life (t_{1/2}) was calculated as } 0.693/\text{mean Kel}. \text{AUC from 0 to the last time (t) was calculated by linear trapezoidal approximation. The area beyond the last concentration point to infinity was estimated by C_{i0}/\text{Kel, where C}_{i0}(t)/\text{Kel represents the estimated concentration at time t based on the regression analysis. The total area under the curve was estimated as the sum of the two (i.e. AUC}_{i0} = AUC_{i0} + AUC_{i0}^\infty). C}_{i0} was the first occurrence of the peak serum concentration, and t_{i0} was the earliest time at which C}_{i0} was observed.\)

**Results**

**Excretion Studies.** The time course of mean cumulative urinary and fecal elimination for the four subjects studied is shown in fig. 1. Urinary and fecal excretion accounted for 23.1 ± 3.7 and 63.3 ± 2.2%, respectively, leading to a total excretion of 86.3 ± 4.2% over a period of 240 hr. The excretion pattern was similar in all subjects. Greater than 90% of the total radioactivity excreted in the urine and feces was recovered in the initial 72 and 120 hr, respectively.

**Metabolism Studies.** Because a significant portion of radioactivity was recovered in the urine within 72 hr, the samples collected over 0 to 72 hr were pooled. A representative HPLC radiochromatogram of the pooled urine is shown in fig. 2A. Fecal samples from collections at 0 to 120 hr postdose (maximum fecal excretion) from each subject were pooled on weight basis and extracted with methanol. A representative chromatogram of a methanolic fecal extract after HPLC analysis is shown in fig. 2B.

The metabolites in the urine and fecal extracts were characterized by liquid chromatography/ion spray mass spectrometry and tandem MS, such as precursor ion scan using fragment ion at m/z 330 and neutral loss scan of 18 amu. The precursor ion scan was useful in identifying metabolites with modifications on the 6-amino-3-azabicyclohexane moiety, and the neutral loss scan of 18 amu suggested the presence of a free carboxyl group (6). Each metabolite was further characterized by obtaining the product ion mass spectrum of its protonated molecular ions (M+H)+.

The mean percentage of trovafloxacin and its metabolites in urine and methanolic feces extracts of the four male human volunteers is depicted in table 1. Unchanged trovafloxacin accounted for 5.9% of

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1 Abbreviations used are: \(\text{Kel}^1\), terminal phase elimination rate constant; AUC, area under the serum concentration vs. time curve.
the dose. The major metabolites in the urine were trovafloxacin glucuronide M1 and N-acetyl trovafloxacin M3 representing 12.8 and 1.9% of the administered dose, respectively. N-Acetyl trovafloxacin glucuronide M2 was identified as the minor metabolite and accounted for 0.4% of the administered dose. Hydrolysis of the glucuronide conjugates with β-glucuronidase and base (0.1 M sodium hydroxide) confirmed that M1 and M2 were ester and not N-glucuronides (data not shown). In the fecal extracts, the major peaks were due to trovafloxacin, M3, and sulfate conjugate of trovafloxacin M4, and represented 43.3, 3.9, and 9.2% of the administered dose, respectively.

**TABLE 1**

<table>
<thead>
<tr>
<th>Metabolites</th>
<th>Urine (%)</th>
<th>Feces (%)</th>
<th>Serum (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trovafloxacin</td>
<td>5.9</td>
<td>43.3</td>
<td>52</td>
</tr>
<tr>
<td>M1</td>
<td>12.8</td>
<td>ND</td>
<td>22</td>
</tr>
<tr>
<td>M2</td>
<td>0.4</td>
<td>ND</td>
<td>2.7</td>
</tr>
<tr>
<td>M3</td>
<td>1.9</td>
<td>3.9</td>
<td>2.5</td>
</tr>
<tr>
<td>M4</td>
<td>ND</td>
<td>9.2</td>
<td>ND</td>
</tr>
</tbody>
</table>

*ND, not detected.

Values represent mean ± SD of total radioactivity and unchanged trovafloxacin from four healthy male volunteers. The quantifiable count rate for radioactivity was chosen to be twice the background (0.3 μg-equiv/ml). The assay dynamic range for trovafloxacin in serum was 0.1–20 μg/ml.

**Discussion**

The present study demonstrated the excretion and metabolism of trovafloxacin in humans after a single oral administration of [14C]trovafloxacin at a dose of 200 mg. Fecal elimination constituted the primary route of trovafloxacin excretion in humans. This result was consistent with a previous study which indicated that renal excretion was a minor route of elimination for trovafloxacin. The proposed metabolic scheme for trovafloxacin is depicted in fig. 4. Glucuronidation was the major route of trovafloxacin clearance. Trovafloxacin could potentially form N-glucuronides either with the free amino group of 6-amino-3-azabicyclohexane moiety or the nitro-
gen of the naphthyridone ring (quaternary \( N \)-glucuronide), especially in humans (10). However, base hydrolysis of \( M1 \) indicated that the metabolite was an ester glucuronide, because \( N \)-glucuronides, if formed, are resistant to base hydrolysis (11). Furthermore, identical mass spectral characteristics and similar retention time of \( M1 \) in humans and the other species (6) indicated that the metabolite was an ester glucuronide. Total disappearance of the glucuronide peak following \( \beta \)-glucuronidase treatment suggested that the glucuronide did not undergo significant intra-acyl migration (12). There was no indication of the presence of \( M1 \) and \( M2 \) in the feces. The absence of these metabolites was attributed to the hydrolysis of glucuronides by gut microflora (13). Metabolite \( M3 \) was detected in the urine and feces of all subjects. Even though the site of \( N \)-acylation is the liver or the intestinal wall (14), the role of intestinal microflora in the formation of \( M3 \) could not be ruled out. Previous studies in dogs (6) and reports in the literature (13, 15) have suggested that the intestinal microflora are capable of acylating amines. Metabolite \( M4 \) has been previously observed in the bile of rats (6); however, its presence in human fecal extracts was rather surprising because it suggested that the metabolite was not hydrolyzed by intestinal microflora. Analysis of \(^{14}\text{C} \) drug-related materials in pooled serum samples indicated that trovafloxacin and its glucuronide were the major components during the initial 12 hr after drug administration.

In summary, the present study demonstrates that phase II conjugation plays a major role in the metabolism of trovafloxacin in humans. Comparison of trovafloxacin with other fluoroquinolone antibiotics suggests marked differences in their excretion and metabolism. Although conjugative metabolism is observed for most fluoroquinolones, their biotransformation occurs mainly by oxidative mechanisms (pefloxacin and norfloxacin), or the drug is excreted unchanged in urine (ofloxacin or ciprofloxacin) (16). In contrast, urinary excretion does not play a major role in trovafloxacin clearance, and oxidative metabolism seems to be an insignificant route of trovafloxacin elimination in man. This metabolic profile, coupled with its pharmacokinetic properties and improved antibacterial spectrum, makes trovafloxacin an interesting new member of this class of compounds.

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References


