DISPOSITION AND METABOLISM OF FINASTERIDE IN DOGS


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ABSTRACT:
Finasteride (FIN) is a potent 5α-reductase inhibitor that has shown clinical success in treating men with benign prostatic hyperplasia. In the study of biological effects and metabolism of FIN in animals, the dog serves as the primary modality. This study was conducted to determine the pharmacokinetics and fate of FIN after oral administration of single doses of [14C]FIN to dogs at 10 and 80 mg/kg (N = 2 and 3, respectively), and also after intravenous infusion at 5 mg/kg (N = 2). Plasma, urine, and feces were analyzed for total 14C content. Parent drug and metabolites in plasma and excreta were measured by HPLC/UV/radioassay and identified by NMR spectroscopy and MS. FIN was subject to extensive biotransformation before excretion. Structures were determined for the major metabolites in plasma, urine, and feces. The primary metabolic events for FIN were hydroxylation of the 1-butyl side chain to give hydroxymethylene-FIN (metabolite I), which is oxidized further to form the carboxylic acid derivative (metabolite IV), and hydroxylation at positions 6α and 15. Terminal half-life of FIN after the intravenous dose was 3.4 hr. Plasma clearance and volume of distribution at steady-state were 48 ml/min/kg and 1.1 liter/kg. Dogs showed rapid absorption after oral administration of the low dose, with Cmax reached in the 1–2 hr; bioavailability was estimated to be >90%. After either dosing route, 45% of the plasma radioactivity (as represented by AUC) was parent drug, 43% was metabolite I, and 1% was metabolite IV. After oral administration, the 80 mg/kg dose was absorbed slowly, with the highest levels of radioactivity in plasma reached in 4–30 hr. Average Cmax value for FIN and metabolite I increased in a dose-related, but nonproportional, manner. Compared with the 10 mg/kg dose, it seems the higher dose was reasonably well-absorbed, as indicated by the nearly proportional increase of AUC values of total radioactivity and FIN. Composition of plasma metabolites observed at the 80 mg/kg dose level was similar to that observed previously for the low dose, suggesting that an increase in plasma exposure was effected in dogs receiving FIN at 80 mg/kg in toxicity studies. Most of the administered radioactivity was recovered in feces after all doses. Little of the intravenous and low oral doses, but >50% of the 80 mg/kg oral dose, was excreted as intact FIN, suggesting that metabolism might have been saturated at the high dose.

FIN1 (Proscar; fig. 1) is an orally active 4-azasteroid synthesized by MRL that has been demonstrated to be effective in the clinical management of BPH (1–3), one of the most common diseases exhibited by middle-aged and older men (4). Abnormalities of prostate growth are common only to humans and male dogs. Although there are differences between human and canine BPH, the condition in the dog has many features in common with the human disease, and this model is widely accepted as the best animal model for studying BPH (5, 6).

FIN is a potent, mechanism-based inhibitor of human type 2 5α-reductase (7), the enzyme expressed in prostate, liver, genital skin, and male accessory sex glands, whereas type 1 5α-reductase is expressed in the sebaceous glands of human skin and is poorly inhibited by FIN (8–10). In animal studies in both rats (11) and dogs (12–14), treatment with FIN resulted in a significant reduction in the size of the prostate gland. These findings provide further support for the use of the dog as a model for the human disease.

The metabolism and excretion of FIN in rats and human subjects and its in vitro metabolism have been reported (15–18). FIN undergoes extensive hepatic metabolism through oxidative pathways giving metabolites that are eliminated primarily through bile. After an oral dose of [14C]FIN (38.1 mg) to humans, 39% of the dose was excreted renally. The major radiolabeled component recovered from the urine was the carboxylic acid metabolite (17). Recently, CYP3A has been shown to be the major enzyme involved in the biotransformation of FIN (19). However, only limited data have been published on the in vivo metabolism of FIN in the dog (20). The aim of the present study was to describe the metabolic fate and pharmacokinetics of FIN in the dog after an intravenous dose and oral doses of 10 and 80 mg/kg. Metabolic patterns are of interest because they demonstrate the dog resembles humans in major metabolic pathways. This is important...
FIN used in this study was radiolabeled with $^{14}$C (*) and $^3$H (†).

because the dog is the relevant animal model for the study of BPH, and in addition served as one of the primary species used for investigation of the pharmacology and toxicology of FIN.

Materials and Methods

**Chemicals.** FIN (molecular weight: 372), the IS (the 4-\textit{N}-methyl analog of FIN), and the oxidative metabolites (\textit{o}-OH-FIN, \textit{o}-carboxy-FIN, FIN-\textit{o}-al, and Me FIN-\textit{o}-oate) were prepared at MRL (Rahway, NJ). $[1,2-^3$H$/^1$C$]$FIN and $[^\text{[r-buty1-stem]}^1$C$]$FIN (radio purities >98\% at specific activities of 18.5 and 9.5 $\mu$Ci/mg, respectively) were used for preparation of the intravenous dose and the 10 mg/kg oral dose. For the 80 mg/kg oral dose, $[^1$C$]$FIN was diluted to a specific activity of 1 $\mu$Ci/mg. Both radiolabeled compounds were prepared at MRL. All organic solvents (EM Science, Gibbstown, NJ) were HPLC grade. Water was purified in a Millipore Milli-Q system (Bedford, MA). Carbosorb, Permafluor, and Monophase S (Packard, Downers Grove, IL) were used for tissue combustions. All other chemicals and reagents were used as described previously (21).

**Dosing and Sample Collection.** Five male beagle dogs (body weight 10–16 kg, 2–7 years old) were obtained from either White Eagle Farms (Doylestown, PA) or Marshall Farms (North Rose, NY). Animals were housed and maintained according to MRL IACUC-approved guidelines. Dogs were fasted (water \textit{ad libitum}) for \textasciitilde{}16 hr before dosing. Three dogs were dosed with $[^1$C$]$FIN (10 mg/kg po): one dog in the group received $[^3$H$/]^{14}$C$]$FIN. In a separate study, two of the three dogs received $[^1$C$]$FIN at 80 mg/kg po. The drug was suspended in 0.5\% methylcellulose and given by gavage. Two dogs were dosed intravenously with $[^1$C$]$FIN at 5 mg/kg (one animal received the doubly labeled drug). For administration of the intravenous dose, the dog was restrained in a sling, and a catheter was implanted in the brachiocephalic vein. For administration of the intravenous dose, the dog was restrained in a sling, and a catheter was implanted in the brachiocephalic vein.

**Analysis of Unchanged FIN and Metabolites in Plasma, Urine, and Fecal Samples.** Concentrations of FIN in plasma and urine were determined by an HPLC method with UV and/or radiometric detection as previously reported (21), then modified to include the analysis of metabolites (17). The $N$-methyl analog of FIN was used as the IS to monitor the procedural recovery and reproducibility of the assay. UV response of the IS in each analyte was compared with that of an equivalent amount injected on-column. Typically, extraction efficiencies averaged 84\%, with \textless{}5\% variation among the samples. The LOQ of $[^3$H$/]^{14}$C$]$FIN (1 ng-eq/ml) was based on radioactivity data.

**Analysis of Radioactivity.** Plasma and urine samples were assayed for total radioactivity by direct LSC (Packard Tri-Carb Scintillation Spectrometer model 1900 TR) using a liquid scintillant (Insta-Gel, Packard). Before analysis, a separate set of plasma samples, blood and fecal homogenates (1:4 dilutions of feces), were air-dried, combusted in a tissue oxidizer (Packard model B-306), and the resultant $^{14}$CO$_2$ and/or tritiated water was trapped and then mixed with scintillant. Concentrations of radioactivity were expressed as drug equivalents/milliliter (or gram) of sample. Difference in the total radioactivity obtained by the two methods (direct counting \textit{vs} combustion) was taken as evidence for the presence of a volatile tritiated species in plasma. Alternately, the presence of volatile tritium in a sample was determined by the difference of radioactivity in the sample determined before and after evaporation under a stream of nitrogen.

**Fecal Samples.** Concentrations of FIN in plasma and urine were determined by an HPLC method with UV and/or radiometric detection as previously reported (21), then modified to include the analysis of metabolites (17). The $N$-methyl analog of FIN was used as the IS to monitor the procedural recovery and reproducibility of the assay. UV response of the IS in each analyte was compared with that of an equivalent amount injected on-column. Typically, extraction efficiencies averaged 84\%, with \textless{}5\% variation among the samples. The LOQ of $[^3$H$/]^{14}$C$]$FIN (1 ng-eq/ml) was based on radioactivity data.

**Plasma:** One- to 2-ml aliquots of plasma were prepared for analysis by dilution to 15 ml with water and addition of 4.1 $\mu$g of the IS. The sample was passed through a Sep-Pak C$_{18}$ cartridge (Waters Associates, Milford, MA), followed by elution with methanol/H$_2$O (70:30 \textit{v/v}); the eluate was taken to dryness with nitrogen, diluted with H$_2$O, and passed through a Sep-Pak CN (cyanopropyl) cartridge. Typically, 1–4\% of the total radioactivity was not retained on the cyano cartridge; thus, it was characterized as acidic based on the selectivity of the phase. Unretained fractions were stored frozen until...
analysis. Retained radioactivity was eluted from the cyano cartridge with methylene chloride. The solvent was evaporated and the residue was redissolved in methanol for analysis by HPLC. To recover the radioactivity quantitatively, samples were applied to C18 cartridges. Retained radioactivity was desorbed with 100% methanol. The eluate was concentrated by evaporation, redissolved in methanol, and analyzed by HPLC method 3.

Urine. A 5-ml aliquot of urine (0–24 and 24–48 hr) was mixed with 5 ml 0.2 M Na2HPO4 (pH 7.4) buffer and 4.1 μg of the IS. These samples were then extracted sequentially at pH 7.4 and pH 2 with methylene chloride and ethyl acetate; solvent fractions and the aqueous layer were assayed for total radioactivity. In a separate study, incremental specimens collected from 0 to 72 hr after 80 mg/kg FIN were pooled on a proportional basis before fractionation as described. Before HPLC analysis, each sample of neutral and acidic urinary radioactivity was purified further by use of serial adsorptions-desorptions from C18 cartridges. The pH of the aqueous layers was adjusted to 5, and the sample was stored frozen until used in enzymatic deconjugation experiments. Incubation with β-glucuronidase. Before incubation, the aqueous fraction from 0 to 72 hr dog urine (50 ml, containing on average 18% of the total urinary radioactivity) was extracted with ethyl acetate to remove products resulting from nonenzymatic hydrolysis. Each of the resulting aqueous samples was applied to eight C18 Sep-Paks: radioactivity was desorbed quantitatively with solvent A as methanol/acetonitrile/H2O [21:14:65 (v/v/v)] and solvent B as 100% methanol. The effluent was evaporated and the residue dissolved in 0.2 M sodium acetate buffer (pH 5.0). Aliquots of samples (50 μg equivalents of radioactivity) were incubated overnight at 37°C with and without β-glucuronidase (2000 units, Helix aspersa, Type HA-4; Sigma Chemical Co., St. Louis, MO). In a parallel incubation, α-sarcosyr-1,4-lactone (0.5 mM) was added. After incubation, samples were extracted as described previously for urine. The resulting extracts, containing neutral products, were analyzed by HPLC using method 1 (described herein); acidic extracts, containing little radioactivity, were not assayed.

Feces. Ten-milliliter- aliquots of feces were diluted with pH 7.4 buffer and extracted at neutrality and at pH 2 in the same way as described previously for urine, except that the aqueous fractions of feces contained little radioactivity and were not saved.

HPLC Analysis. The HPLC system consisted of two pumps (Spectroflow 400), a UV detector and gradient controller (Spectroflow 783) from Kratos/ Applied Biosystems (San Jose, CA), and Rythdene 7125 injector (Cotati, CA). Data acquisition and integration were performed by use of a PE Nelson Analytical Model 2600 Data System, 760 series interface (Cupertino, CA). All analyses were conducted on a Zorbax C8 analytical column (4.6 × 250 mm, Mac-Mod Analytical, Inc., Chadd’s Ford, PA) with a LC–8 packed guard column (Supelco, Bellefonte, PA). Flow rate was 1 ml/min. Column effluent was monitored by UV absorbance at 210 nm. HPLC separations were conducted at ambient temperature. Four HPLC methods were used as follows: methods 1 and 2 for separation of the major and minor neutral metabolites, respectively; method 3 for separation of acidic metabolites; and method 4 for purification of those metabolites partially resolved by method 1. In method 1, the column was eluted isocratically with a mobile phase consisting of methanol/acetonitrile/H2O [39:26:35 (v/v/v)]. Typical retention times for FIN, its metabolites, and the IS with this system are as follows (in minutes): FIN, 13.5; α-OH-FIN, 7.5; β-OH-FIN, 6.1; 6α,6β-(OH)2-FIN, 4.5; and IS, 21. In method 2, the column was eluted with a gradient from 0% to 100% B in 25 min with solvent A as methanol/acetonitrile/H2O [21:14:65 (v/v/v)] and solvent B as methanol/acetonitrile/H2O [45:30:25 (v/v/v)]. The retention times of FIN-α,ω- and Me FIN-ω-oxide were 21 and 22 min, respectively. In method 3, the mobile phase consisted of solvent A [acetonitrile/H2O, 10:90 (v/v)] containing 0.2% H3PO4 and solvent B [acetonitrile/H2O, 90:10 (v/v)] containing 0.2% H3PO4, and the column was eluted with a linear gradient from 0 to 100% B in 60 min. Typical retention times were ω-carboxy-FIN, 26.8 min (25–28) and FIN, 36–37 min. Method 4 used two C8 columns connected in series and eluted at a flow rate of 0.7 ml/min with a mobile phase of methanol/acetonitrile/H2O [33:22:45 (v/v/v)]. Retention times were noted for ω-OH-FIN (30.3–32.3 min) and the di-OH-FIN metabolites (13–18 min). For off-line radioactivity detection and metabolite isolation, fractions of column effluent were collected at 0.5– or 1-min intervals (ISCO Retriever II). Radioactivity content of each fraction was determined by LSC, and the results were expressed as a percentage of the total radioactivity that was recovered from the column. For each extract of plasma, urine, and feces, the percentage of the total radioactivity contained in each fraction was plotted to establish an elution profile of radioactivity. Concentrations of FIN and metabolites were calculated from the elution profiles as described previously (17). Percentage-based values obtained by HPLC were converted to radioactivity and subsequently converted to amounts by use of the appropriate specific activity; thereafter, amounts were reported as nanograms (or micrograms) of drug and metabolites. In performing this conversion, the small difference in the molecular weights of the various compounds was not taken into account. A comparison of concentrations measured by this specific HPLC assay and the percentage-based total radioactivity method revealed a very close correlation (17, 22).

Metabolite Isolation and Identification. Larger amounts of plasma, urine, and feces were processed to obtain metabolites, using essentially the same methods employed to obtain the respective metabolic profiles. Identification of metabolites was determined by cochromatography with authentic metabolite standards, NMR spectroscopy, and MS.

Spectral Studies. MS analysis of metabolites was performed by several techniques. DCI mass spectra (98 eV) were acquired on a Nermag R 1010C quadrupole mass spectrometer (Houston, TX) operated as follows: source temperature of 120°–130°C, reagent gas either methane or ammonia, and source pressure 0.2 torr. Mass spectra were acquired from m/z 250–500 at 1 scan/sec. Data reduction and analysis were conducted using SIDAR software. Samples were introduced into the ionization chamber by a direct insertion probe on a platinum filament, which was heated rapidly (current gradient of 50–550 mA at 20 mA/sec) to desorb the sample. Low-resolution EI mass spectra were obtained using a LKB 9000 mass spectrometer (Rockville, MD) operated as follows: electron energy, 70 eV; source temperature, 270°C; accelerating potential, 3.5 kV; and trap current, 60 μA. Molecular mass measurements and collision-induced dissociation to produce product ion spectra were performed on a SCIEX API III tandem mass spectrometer (Toronto, Canada) using the heated nebulizer interface and positive-ion detection. Metabolites were introduced into the instrument by flow injection with a mobile phase of acetonitrile/m3 ammonium acetate (1:1, v/v) at 40 μl/min. Mass spectra were acquired from m/z 150–600 at a scan rate of 1.25 sec/scan, with the orifice potential set at 60 V. NMR spectra were recorded at either 400 MHz for a Varian XL-400 spectrometer or at 500 MHz on a Varian VXR500S instrument (Palo Alto, CA) using CDCl3 as solvent. Data were collected using a 45° flip angle and a 1-sec acquisition time. Chemical shifts are given in ppm relative to Si tetramethylsilane at 0 and the residual CHCl3 signal set at 7.26 ppm.

Plasma Protein Binding of [14C]FIN. Fresh hepatopan dog plasma samples were mixed with [14C]FIN (specific activity: 24.7 μCi/mg) at concentrations of 0.02, 0.2, 0.5, and 2.0 μg/ml, and incubated at 37°C for 30 min. Immediately after incubation, 1 ml of sample was pipetted into an ultrafiltration device (Amicon Centrifilter micropartition system; Amicon, Danver, MA) and then centrifuged at 2000g for 1 hr. An aliquot of the filtrate (plasma water) and an aliquot of the initial plasma to which the drug had been added were assayed for radioactivity. fu was calculated from the ratio of drug concentration in the ultrafilterate to the initial drug concentration in the plasma; the ratio was expressed as a percentage (fu%). Nonspecific binding of [14C]FIN was determined using the same method in the absence of plasma.

Data Analysis. The principal pharmacokinetic parameters were estimated by model-independent methods from plasma concentration–time data. Cmax and tmax were the observed values. Ke was determined by least squares linear regression of the terminal concentration–time data points (7–24 or 48 hr); t1/2 was calculated as 0.693/Ke. AUC from time of dosing to the 24- or 48-hr sampling time was determined by the trapezoidal rule and was extrapolated to infinite time by addition of the term CKpC, where C is the concentration at the last quantifiable sampling time. After correcting for dose, the oral bioavailability of FIN was calculated as AUCf/AUCo. AUCf was calculated from the plasma data using the trapezoidal rule and extrapolated to time infinity (23). MRT was determined from the intravenous data by the expression MRT = AUCf/AUCo. CLp was Dose/AUC, and Vd = CLp·MRT. CLp of unchanged FIN was estimated from the fecal data by the expression CLp = FINf/Plasma AUC, wherein FINf is the amount (μg) of FIN excreted in feces over 24 or 72 hr.
Plasma radioactivity declined with time, concentrations of $^{14}$C FIN at 10 and 80 mg/kg to the same two dogs. Although the 10 mg/kg dose was absorbed rapidly by both dogs, the high dose seemed to be absorbed slowly as $C_{\text{max}}$ of total radioactivity ($\sim 12 \mu g/ml$) was reached in 4 hr in one animal, but not until 30 hr in the other. After the 80 mg/kg dose, FIN levels declined slowly. Compared with the 10 mg/kg dose, it seems that the higher dose was reasonably well-absorbed. An 8-fold increase in dose resulted in 5- and 8.5-fold increases in the AUC values for total radioactivity, and 5- and 7-fold increases in that of the AFR. Peak times of FIN and its metabolites in plasma nearly paralleled those of total radioactivity with dose-related increases, but less than proportionally higher $C_{\text{max}}$ values as follows: FIN $6.7$ and $8.1 \mu g/ml$; $\omega$-OH-FIN, $2.7$ and $5.6 \mu g/ml$; and $\omega$-carboxy-FIN, $0.07$ and $0.1 \mu g/ml$. In contrast, AUC values of parent drug and the $\omega$-OH metabolite increased 12- and 7-fold, respectively, in one dog and only 5-fold in the other. Of the circulating radioactivity in plasma after the high dose, average AUC values indicated 45% was FIN, 42% was $\omega$-OH-FIN (metabolite I), and nearly 3% was AFR, with 0.8% identified as metabolite IV, $\omega$-carboxy-FIN (table 1).

Characterization of Plasma Radioactivity. After either dosing route and for up to 7 hr postdose, the $^3$H/$^{14}$C-labeled radioactivity in plasma exhibited similar characteristics. Procedural recoveries and quantification of FIN and its metabolites based on either radiisotope were essentially the same. A time-dependent increase in the amount of volatile tritium present in plasma has been observed after administration of $^3$H FIN. In the present study, when plasma samples from the two dogs given 10 mg/kg $[{}^3$H]/$[{}^{14}$C]FIN either orally or intravenously were passed through a C$_{18}$ Sep-Pak, the fraction of radioactivity not adsorbed increased from $<3\%$ over the 5-min to 7-hr interval and to $>15\%$ at 24 hr and $>70\%$ at 48 hr, with a less than corresponding increase in $^{14}$C-labeled material. Tritium radioactivity declining slowly in plasma was not due to polar metabolites, but rather tritiated water; the mechanism for its formation from $^3$H FIN is unknown. The small but increasing percentage of $^{14}$C-label not retained on the cartridge suggested the time-dependent formation of very polar metabolites.

Distribution of FIN in blood and plasma was constant at a ratio of $1.14 \pm 0.02$ in samples over the 0.5- to 24-hr interval after either dosing route at 10 mg/kg. At an average hematocrit value of 0.45, results based on $^3$H or $^{14}$C indicated that $88\%$ of the radioactivity in blood was associated with the plasma; however, the ratio increased to 1.3 and 2.2 in the 48- and 72-hr blood samples, suggesting that a slight change in the distribution pattern of radioactivity occurred at later time points.

Plasma Protein Binding. The in vitro protein binding of $[{}^{14}$C]FIN in dog plasma seems to be independent of concentration over the range (0.02-2.0 $\mu g/ml$) investigated. Mean $\mu$ was $17.8 \pm 0.8\%$. Nonspecific binding of FIN to the ultrafiltration device could not be determined due to the hydrophobicity of the compound. In the absence of plasma, relevant concentrations of FIN could not be maintained in an aqueous solution.

Excretion of $[{}^{14}$C]FIN and Its Metabolites. The recoveries of total radioactivity in urine and feces are presented in table 2. For all dose groups, most of the administered radioactivity was excreted in the feces over a 72-hr period, with the majority excreted during the first 48 hr. Less than 5% of the oral doses and $<10\%$ of the intrave-
Use of one column (method 1) resolved the monohydroxy metabolites and were qualitatively similar after oral and intravenous dosing.

Doses. Radiochromatographic profiles demonstrated extensive metabolism and were qualitatively similar after oral and intravenous dosing.

Urine. Individual values of two dogs are shown. Values are the means ± SD (N = 3).

Individual values of two dogs are shown.

TABLE 1
Pharmacokinetic parameters of FIN and metabolites I and IV in dogs after single 10 and 80 mg/kg oral doses of [14C]FIN

<table>
<thead>
<tr>
<th>Dose (mg/kg) and Parameter</th>
<th>Total Radioactivity</th>
<th>AFR</th>
<th>FIN</th>
<th>Metabolite I</th>
<th>Metabolite IV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10</td>
<td>80</td>
<td>10</td>
<td>80</td>
<td>10</td>
</tr>
<tr>
<td>C_max (µg/ml)</td>
<td>3.4</td>
<td>12.5</td>
<td>0.11</td>
<td>0.36</td>
<td>2.1</td>
</tr>
<tr>
<td>t_max (hr)</td>
<td>1.5</td>
<td>4</td>
<td>30</td>
<td>5.5</td>
<td>4.3</td>
</tr>
<tr>
<td>AUC (µg·hr/ml)</td>
<td>66.3</td>
<td>474.5</td>
<td>2.7</td>
<td>12.4</td>
<td>25.4</td>
</tr>
<tr>
<td>AUC (%) change</td>
<td>—</td>
<td>616</td>
<td>—</td>
<td>439</td>
<td>748</td>
</tr>
<tr>
<td>% of total 14C′</td>
<td>3.5</td>
<td>2.6</td>
<td>38.5</td>
<td>45.4</td>
<td>49.5</td>
</tr>
</tbody>
</table>

Results are average values from the same two dogs given both doses.

a Total radioactivity (TotalRAD, undifferentiated) is expressed as µg FIN-eq/ml.
b Defined as that fraction of radioactivity not retained on a solid-phase extraction cartridge (cyano Sep-Pak).
c ND, not determined. Individual plasma samples of dogs at the low dose were not analyzed for metabolite IV; however, analysis of a pooled plasma sample showed that the monocarboxylic acid metabolite accounted for 0.8% of the total 14C in plasma.
d Due to the prolonged absorption of the high dose in one dog, both t_max values are shown.
e Estimated by use of the following expression: (AUC/AUC_Total RAD) × 100.

TABLE 2
Excretion of radioactivity in the urine and feces of dogs after single oral doses (10 and 80 mg/kg) and an intravenous dose (5 mg/kg) of [14C]FIN

<table>
<thead>
<tr>
<th>Dose Group</th>
<th>Collection Interval</th>
<th>% of Radioactive Dose Excreted in Urine</th>
<th>% of Radioactive Dose Excreted in Feces</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral dose</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 mg/kg</td>
<td>0–72</td>
<td>3.1 ± 3.8</td>
<td>86.2 ± 18.2</td>
</tr>
<tr>
<td>Oral dose</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>80 mg/kg</td>
<td>0–72</td>
<td>4.7 ± 2.9</td>
<td>77.6, 88.4</td>
</tr>
<tr>
<td></td>
<td>0–192</td>
<td>5.3 ± 3.5</td>
<td>85.8, 95.8</td>
</tr>
<tr>
<td>IV dose</td>
<td>5 mg/kg</td>
<td>9.7 ± 8.9</td>
<td>76.7, 76.7</td>
</tr>
</tbody>
</table>

a Values are the means ± SD (N = 3).
b Individual values of two dogs are shown.

dose were excreted renally. Radioactivity excreted in urine and feces was characterized by its solvent-partitioning properties. Results indicated that the major urinary metabolite excreted after the 10 mg/kg oral dose were divided between neutral (44%) and polar (i.e. not extractable) compounds (39%). After the 80 mg/kg oral dose, renal excretion of neutral material increased to >70%, with a concomitant decrease in the polar fraction of radioactivity (17%). At both dose levels, only 12% of the urinary radioactivity was characterized as acidic, which is consistent with the small amount of acidic fraction radioactivity found in dog plasma. After intravenous dosing, dogs exhibited a similar pattern of urinary excretion, but inverse amounts of neutral and acidic metabolites. The major fraction (94%) of fecal radioactivity excreted over the 0- to 72-hr interval was characterized as neutral, whereas 4.5% was acidic and only 1.5% was nonextractable. Partially fractionated radioactivity in urinary and fecal extracts was also characterized according to its chromatographic properties. Representative urinary (0–72 hr) and fecal (24–48 hr) profiles of neutral metabolites are shown in fig. 3, and quantitative information on the cumulative excretion of FIN and its major neutral and acidic metabolites is presented in table 3 (urine) and table 4 (feces).

Urine. Unlike plasma, urine contained only a small amount of parent drug (0.02–0.15% of the dose) after the 10 and 80 mg/kg oral doses. Radiochromatographic profiles demonstrated extensive metabolism and were qualitatively similar after oral and intravenous dosing. Use of one column (method 1) resolved the monohydroxy metabolites (the ω-OH and 6α-OH derivatives of FIN), but only partially resolved the neutral, dihydroxylated metabolites eluting between 4–5 min.

When radioactivity in these column fractions was purified further by use of serial columns (method 4), the metabolite profiles were relatively complex, with three dihydroxylated metabolites identified (6α-OH, 6β-OH and 15-OH derivatives of ω-OH-FIN) and a number of trace metabolites unidentified. ω-Carboxy-FIN was the major metabolite present in the acidic urinary extracts. Treatment of the polar urinary fraction with β-glucuronidase resulted in a substantial increase in the amount of neutral radioactivity. The acid profile of the latter was essentially the same as that obtained from the original free fraction of neutral metabolites. Most of the radioactivity obtained as consequence of the enzymatic hydrolysis eluted at the retention time of ω-OH-FIN, with a minor fraction corresponding to the diols. Hydrolysis was partially inhibited by d-saccharo-1,4-lactone, a spe-
specific inhibitor the β-glucuronidase. Spontaneous hydrolysis of urinary radioactivity resulted in an increase in the quantity of diol metabolites. Results indicated the presence of stable and labile conjugates in urine.

**Feces.** The majority of administered radioactivity was excreted in the feces. Unchanged FIN was a prominent component in all fecal samples, accounting for 5% of the 10 mg/kg oral dose and increasing to 53% (dog 1) and 77% (dog 2) when the dose was increased to 80 mg/kg. Consistent with these data and also reflecting the increase in dose, CL_{d} of unchanged FIN in dog 1 increased from 0.4 to 2.5 ml/min/kg, whereas after administration of the high dose to dog 2, the CL_{d} value was 7 ml/min/kg. HPLC results showed that the neutral and acidic metabolites present in fecal extracts corresponded to those observed in urine.

**Identification of Metabolites.** In dogs, FIN was transformed to at least six metabolites that were detected in various amounts by radiochromatographic analysis of plasma, urine, and feces samples. These metabolites were isolated and purified to allow comparison with authentic standards for structural identification (see Materials and Methods). Structures of metabolites of FIN and the proposed pathways are shown in fig. 1. Where possible, LC/MS/MS fragmentation further supported the structures of metabolites after the pattern of product ions was compared with that produced by reference compounds or previously identified metabolites. As summarized in table 5, MS results show ions consistent with the metabolite structures. Spectral features of FIN and its metabolites are described herein.

FIN. The most highly retained radiographic component in plasma, urine, and feces was determined to be FIN. The EI/MS spectrum of FIN showed the [M]+ ion at m/z 372 and an ion m/z 357 consistent with the loss of the methyl group [M-CH_{3}]^{+} and ions at m/z 317 [M-C_{3}H_{5}]^{+} and m/z 300 [M-NHC(CH_{3})_{3}]^{+} resulting from the stepwise fragmentation of the side chain. Cleavage of the entire side chain resulted in a diagnostic fragment ion at m/z 272 corresponding to an unaltered tetracyclic nucleus. The positive-ion DCI/MS and LC/MS spectra exhibited the [M+H]^{+} ion at m/z 373. No fragment ions were produced during the LC/MS ionization process, whereas the MS/MS spectrum showed an intense product ion at m/z 317. Loss of ring A (C1-C3 and the NH group) from the molecular ion yielded the ions observed at m/z 305 and m/z 69. The chemical shifts obtained in the NMR spectrum were: 6.78 d, 1H, H1; 5.15 br s, 1H, NH; 5.07 br s, 1H, NH; 3.34 m, 1H, H5; 1.36 s, 9H, (CH_{3})_{9}; 0.98 s, 3H, 19-CH_{3}; 0.70 s, 3H, 18-CH_{3}.

ω-OH-FIN (I). ω-OH-FIN was the principal metabolite in plasma and a major metabolite in urine and feces. Its EI/MS spectrum showed a mean [M]+ at m/z 388, 16 mass units greater than that of FIN and contained ions at m/z 370 [M-H_{2}O]^{+}, m/z 357 [M-CH_{3}OH]^{+}, m/z 317 [M-C_{3}H_{5}O]^{+}, m/z 300 [M-NHC(CH_{3})_{2}CH_{2}OH]^{+}, and m/z 272—the ion indicating no transformation had taken place on the ring system. The positive-ion DCI/MS and LC/MS spectra exhibited the [M+H]^{+} ion at m/z 389. MS/MS of the parent ion gave a product ion spectrum containing a base ion at m/z 272, as well as ions at m/z 317, m/z 73, and m/z 321—the latter resulting from cleavage through ring A. MS fragmentation patterns indicated that oxidation had occurred on one of the methyl groups of the t-butyl side chain. The NMR spectrum is distinguished from that of FIN by a —CH_{2}OH and two methyl peaks at 1.30 ppm and 1.28 ppm. These findings are consistent with hydroxylation of one of the t-butyl methyl groups. In other resests, the spectrum closely resembles that of FIN. NMR spectral signals for the metabolite were observed at 6.78 d, 10.0, 1H, H1; 5.82

<table>
<thead>
<tr>
<th>Animal</th>
<th>Dose Group</th>
<th>Interval</th>
<th>% of Dose Excreted as</th>
<th>Metabolite</th>
<th>I</th>
<th>II</th>
<th>Diols^{b}</th>
<th>IV</th>
<th>Acidic^{c}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dog 1</td>
<td>10</td>
<td>0–24</td>
<td>4.4</td>
<td>FIN</td>
<td>0.02</td>
<td>0.63</td>
<td>ND</td>
<td>1.4</td>
<td>0.1</td>
</tr>
<tr>
<td>Dog 2</td>
<td>80</td>
<td>0–72</td>
<td>4.7</td>
<td>FIN</td>
<td>0.15</td>
<td>1.3</td>
<td>0.2</td>
<td>1.7</td>
<td>0.1</td>
</tr>
<tr>
<td>Dog 2</td>
<td>80</td>
<td>0–72</td>
<td>2.9</td>
<td>FIN</td>
<td>0.05</td>
<td>0.23</td>
<td>0.1</td>
<td>1.5</td>
<td>0.1</td>
</tr>
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</tr>
</tbody>
</table>

TABLE 3

Cumulative urinary excretion of FIN and its metabolites after oral administration of [14C]FIN

<table>
<thead>
<tr>
<th>Animal</th>
<th>Dose Group</th>
<th>Interval</th>
<th>% of Dose Excreted as</th>
<th>Metabolite</th>
<th>Total [14C]</th>
<th>FIN</th>
<th>I</th>
<th>II</th>
<th>Diols^{b}</th>
<th>IV</th>
<th>Acidic^{c}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dog 1</td>
<td>10</td>
<td>0–24</td>
<td>46.2</td>
<td>FIN</td>
<td>5.0</td>
<td>5.6</td>
<td>1.7</td>
<td>14.6</td>
<td>2.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dog 2</td>
<td>80</td>
<td>0–72</td>
<td>77.6</td>
<td>FIN</td>
<td>52.8</td>
<td>6.5</td>
<td>2.4</td>
<td>18.6</td>
<td>1.6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

TABLE 4

Cumulative fecal excretion of FIN and its metabolites in dogs after oral administration of [14C]FIN

<table>
<thead>
<tr>
<th>Animal</th>
<th>Dose Group</th>
<th>Interval</th>
<th>% of Dose Excreted as</th>
<th>Metabolite</th>
<th>Total [14C]</th>
<th>FIN</th>
<th>I</th>
<th>II</th>
<th>Diols^{b}</th>
<th>IV</th>
<th>Acidic^{c}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dog 1</td>
<td>10</td>
<td>0–24</td>
<td>46.2</td>
<td>FIN</td>
<td>5.0</td>
<td>5.6</td>
<td>1.7</td>
<td>14.6</td>
<td>2.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dog 2</td>
<td>80</td>
<td>0–72</td>
<td>77.6</td>
<td>FIN</td>
<td>52.8</td>
<td>6.5</td>
<td>2.4</td>
<td>18.6</td>
<td>1.6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Values represent the recoveries of the radioactive doses in the collection intervals.
b Values show represent the total excretion of the three dihydroxy metabolites of FIN (V, VI, and VII) that are not separated by HPLC using method 1.

c Values represent the recoveries of the radioactive doses in the collection intervals.
d Metabolite V in this fraction accounted for nearly 60% of the radioactivity.
e Metabolite V in this fraction was identified tentatively.
f Metabolites V, VI, and VII each accounted for 20–30% of the radioactivity in this fraction.
g Metabolite VII in this fraction accounted for nearly 80% of the radioactivity.
TABLE 5

Mass spectrometric fragmentation patterns observed for FIN and its metabolites.

<table>
<thead>
<tr>
<th>Compound</th>
<th>EI</th>
<th>LC/MS/MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>FIN M</td>
<td>372</td>
<td>357</td>
</tr>
<tr>
<td>Me FIN-voate</td>
<td>416</td>
<td>401</td>
</tr>
<tr>
<td>-Carboxy-FIN</td>
<td>402</td>
<td>384</td>
</tr>
<tr>
<td>6,15-(OH)2-FIN</td>
<td>404</td>
<td>386</td>
</tr>
<tr>
<td>VI,7-(OH)2-FIN</td>
<td>405–333, 270</td>
<td></td>
</tr>
</tbody>
</table>

a Ions shown represent the protonated molecular ion and its significant product ions.

b Ions shown represent the protonated molecular ion and its significant product ions.

d, 10.0, 2.0, 1H, H2; 5.32 br s, 1H, NH; 5.16 br s, 1H, NH: 3.60 dd, ~10.0, 6.0, 1H: 3.57 dd, ~10.0, 6.0, 1H, ω-CH2OH: 1.30 s, 3H, ω-CH3: 1.28 s, 3H, ω-CH3: 0.98 s, 3H, 19-CH3: 0.70 s, 3H, 18-CH3.

Spectral data and the HPLC retention time (using method 1) of this metabolite matched that of an authentic standard of ω-OH-FIN.

6α-OH-FIN (II). This was a minor metabolite. Its EI/MS spectrums showed an intense ion at m/z 388 [M]+ and contained fragment ions at m/z 373 [M-CH3]+, m/z 357 [M-CH2-OH]+, m/z 333 [M-C4H9]+, and m/z 316 [M-NHC4H9]+. The ion observed at m/z 288 indicated the presence of an oxidized ring system, whereas the ion at m/z 270 was consistent with the loss of the elements of water from m/z 288.

The exact position and stereochemistry of the hydroxyl group was established by NMR spectroscopy. Analysis was conducted using a specimen of the urinary metabolite, because authentic material was not available. Key NMR features include the appearance of a new signal at 3.75 ppm consistent with a CHOH and the loss of the smaller of the two coupling constants that characterize a normal H5 resonance.

These two findings provided strong evidence for replacement of the C6 equatorial proton by OH. NMR spectral signals for the metabolite were observed at 6.74 d, 10.0, 1H, H1; 6.05 br s, 1H, NH; 5.84 dd, 10.0, ~2, H2; 5.10 br s, 1H, NH; 3.75 m, 1H, H6; 3.15 d, 10.0, 1H, H5; 1.35 s, 9H, (CH3)3; 0.99 s, 3H, 19-CH3; 0.70 s, 3H, 18-CH3. The HPLC retention time and MS fragmentation patterns of a radiolabeled component isolated from plasma were essentially identical to those of the urinary metabolite, and thus it was postulated that 6α-OH-FIN was present as a minor metabolite in plasma. Subsequently, the same metabolite was identified in vitro by Ishii et al. (18).

ω-Carboxy-FIN (I). Formation of this carboxylic acid metabolite presumably involves further oxidation of the hydroxymethyl group of ω-OH-FIN. The presence of ω-carboxy-FIN in the acidic fraction of plasma radioactivity and in urinary and fecal extracts (obtained by extraction of samples with methylene chloride at pH 2) was inferred by HPLC retention time comparison (using method 3) with the synthetic standard. The EI/MS of the metabolite showed an [M]+ at m/z 402, 30 amu greater than that of FIN, and contained ions m/z [M-COOH]+ and at m/z 272 indicating the lack of metabolic change on the tetracycline ring system. The positive-ion DCl/MS and LC/MS spectra exhibited [M+H]+ ions at m/z 403. MS/MS of the parent ion gave a product ion spectrum containing an ion at m/z 272 and many of the ions produced from FIN, as well as the ion at m/z 335, diagnostic for cleavage of this molecule through ring A. The NMR spectrum was characterized by the presence of two methyl peaks at 1.58 ppm and 1.57 ppm and by the absence of the t-buty1 signal. In other respects, the spectrum closely resembled that of the parent drug. Compared with the spectrum of the ω-CH2OH metabolite, further downfield displacement of the remaining two methyls of the original t-buty1 group would be expected by a carboxy group. NMR signals were observed at 6.73 d, 10.0, 1H, H1; 5.81 dd, 10.0, ~2.0, 1H, H2; 5.65 br s, 1H, NH; 3.33 m, 1H, H5; 1.58 s, 3H, ω-CH3; 1.57 s, 3H, ω-CH3; 0.97 s, 3H, 19-CH3; 0.70 s, 3H, 18-CH3.

Dihydroxyxylated Metabolites (V–VII). These compounds were found as unconjugated metabolites obtained by extraction of urine and feces with methylene chloride at pH 7, and were liberated following spontaneous or enzymatic hydrolysis of the polar urinary radioactivity. Taken together, they accounted for a substantial amount of radioactivity. Dihydroxy metabolites also were observed in dog plasma (fig. 4); however, they were isolated from the acidic fraction of radioactivity that suggests they, too, may have been present initially as labile (possibly sulfate) conjugates. Urinary HPLC (method 1) isolate containing metabolites V, VI, and VII, when examined under the second HPLC system (method 4) using the dual-column tech-
addition to the signals for metabolite V, \( \omega_6 \delta_6 -(OH)_2 \)-, the spectrum of metabolite fraction VI shows the presence of a closely related species. The 6\( \beta \)-hydroxyl analog is indicated both by the loss of the 10.0-Hz coupling constant associated with H5 and by the 0.2 ppm downfield displacement of the 19-methyl resulting from a 1.3-diaxial interaction. A broad singlet at 4.10 ppm is assigned to the equatorial 6\( \alpha \)-proton. The NMR signals for the 6\( \alpha \)-hydroxy analog are presented herein. The NMR signals for the 6\( \beta \)-hydroxy analog were observed at 6.73 d, 10.0, 1H; 5.80 dd, 10.0, 2.0, 1H; H2; 5.56 s, 1H; 4NH; 5.32 s, 2H; side chain NH in 6\( \alpha \) and 6\( \beta \)-ol; 4.10 q, 3.0, 1H; H6; 3.37 br s, 1H; H5; 1.32 s, 3H, \( \omega \)-CH\(_3\); 1.31 s, 3H, \( \omega \)-CH\(_3\); 1.17 s, 3H, 19-CH\(_3\); 0.71 s, 3H; 18-CH\(_3\).

On the basis of NMR, metabolite VII has been identified tentatively as \( \omega_6 15-(OH)_2 \)-FIN. In addition to the recognizable \( \omega \)-hydroxylation \( [i.e. \text{two } \text{methyls at } 1.31 \text{ ppm and } 1.29 \text{ ppm and signals centered at } 3.59 \text{ ppm (CH}_2\text{OH)}, \text{the spectrum shows a new multiplet at } 3.50 \text{ ppm reasonably assigned to a } \text{CHOH}. \text{Although the location of the hydroxyl has not been uniquely determined, the choice was narrowed to C15 or 12\( \alpha \) based on the following arguments. Although the angular methyl chemical shifts are unchanged from those of FIN, it follows that the sites that induce displacements of those signals (e.g. 6\( \beta \), 11\( \alpha \), 11\( \beta \), 12\( \beta \), and 16\( \beta \) can be excluded). Moreover, because the 3.50 ppm multiplet is unaffected when the 6\( \beta \) proton is irradiated, C7 is also excluded as a possible site. A triplet at 2.06 ppm (J = 9 Hz) represents H17, and its multiplicity is compelling evidence that both protons are present at C16. Finally, the tertiary carbon sites—C8, C9, and C14—need not be considered, because hydroxylation would not result in a \( \text{CHOH} \) signal. The NMR signals were observed at 6.72 d, 10.0, 1H; H1; 5.84 dd, 10.0, 2.0, 1H; H2; 5.34 s, 1H; NH; 5.14 s, 1H, NH; 4.79 t, 6.0, 1H, \( \omega \)-OH; 3.60 dd, 11.0, 6.0, 1H, \( \omega \)-CHO; 3.58 dd, 11.0, 6.0, 1H, \( \omega \)-CHO; 3.52 m, 1H, H12\( \alpha \) or H15; 3.35 dd, 13.4, 3.5, 1H, H5; 1.31 s, 3H, \( \omega \)-CH\(_3\); 1.29 s, 3H, \( \omega \)-CH\(_3\); 1.01 s, 3H, 19-CH\(_3\); 0.74 s, 3H, 18-CH\(_3\). Hydroxylation at C15 is favored, because a metabolic transformation of FIN at this site has been observed in rats (16).

Me FIN-\( \omega \)-oate (III). Trace amounts of this derivative of \( \omega \)-carboxy-FIN were detected in plasma. The HPLC (method 1) isolate corresponding to the region of compound III, when examined under a second HPLC system (method 2), showed the major radiolabeled component was \( \omega \)-OH-FIN. Negligible amounts of radioactivity coluted at the retention time (21.2 min) of the side chain aldehyde derivative of FIN, indicating the aldehyde metabolite was not present in plasma. About 4% of the partially purified radioactivity eluted at 22 min, the retention time of the authentic methyl ester derivative of \( \omega \)-carboxy-FIN. The EI/MS of this compound showed an [M\(^+\)]\(^+\) ion at m/z of 416 (44 amu greater than that of FIN and \( \omega \)-carboxy-FIN, respectively), and key features of FIN and its identified metabolites, along with fragment ions corresponding to losses of OCH\(_3\) and COOCH\(_3\). Its DCI/MS spectrum exhibited the [M+H\(^+\)]\(^+\) ion at m/z 417. Authentic Me FIN-\( \omega \)-oate gave the same mass spectrum as radiolabeled component III. The mechanism for its formation is unknown, and possibly it is formed \textit{ex vivo} as an artifact of sample handling. Compound III was not formed by reaction of the aldehyde or carboxy derivatives of FIN in methanol. Authentic compounds were stable in methanol; however, when the carboxylic acid was treated with diazomethane in ethereal methanol, it gave Me FIN-\( \omega \)-oate.

Discussion

FIN was labeled with carbon-14 at the tertiary carbon of the t-buty1 side chain, because previous studies in animals (data not shown) and results obtained subsequently in humans confirmed that the label
position was stable metabolically (17). HPLC-based assays allowed the monitoring of concentrations of drug and metabolites in plasma, urine, and feces for a minimum of 24 hr after dosing.

Absorption estimates based on plasma radioactivity (equivalents) indicated that FIN at 10 mg/kg was well absorbed. In contrast, the 80 mg/kg dose when given to the same two dogs was absorbed slowly; peak levels of radioactivity and parent drug were reached in 4 hr in one dog, but not until 30 hr in the other. It is possible that, when a large amount of FIN is administered, its low aqueous solubility may increase its dissolution time, resulting in a reduction in the rate of absorption. Thus, the 8-fold increase in dose resulted in less than proportional increases in Cmax values for both radioactivity and FIN. On the other hand, it seems the higher dose was reasonably well absorbed in both animals, as indicated by the nearly proportionate increase in AUC values of total radioactivity and FIN.

FIN was eliminated rapidly in all dose groups, with the majority of the administered radioactivity excreted in feces during the first 48 hr, providing indirect evidence of excretion via the bile. Only a small fraction of the oral and intravenous doses, 5 and 10%, respectively, was excreted renally. Elimination of FIN was governed entirely by metabolism, as negligible amounts of parent drug were detected in urine after either dosing route or in feces of intravenous-dosed dogs. After oral dosing, 5% of the 10 mg/kg dose was excreted in feces as intact FIN, confirming the lower dose was well absorbed. On the other hand, a large amount (53–77%) of the 80 mg/kg dose was excreted unchanged in contrast to that observed at the 10 mg/kg dose, which was eliminated mainly as metabolites. These results suggested that, at the high dose, a saturation of metabolic pathways may have occurred and was compensated for by increased biliary excretion of intact FIN. Investigation of the underlying mechanism for the difference in patterns between the 10 and 80 mg/kg doses was beyond the scope of the present study.

In dog plasma, ~82% of [14C]FIN was bound to protein with no indication of concentration dependency over the range 0.02–2.0 μg/ml; whereas at that range in human plasma, protein binding was higher (90%) and seemed to be slightly concentration-dependent (17). In both species, most of the carbon-14 measured in blood was associated with the plasma. The blood-to-plasma ratio of radioactivity remained constant in samples up to 24 hr postdose, indicating that the biotransformation of FIN did not give rise to metabolites that changed the selective distribution of radioactivity to either the plasma or red blood cells.

Fractionation of the urinary and fecal radioactivity from dogs into neutral and acidic metabolites provided a basis for comparing the difference in the excretion patterns and profiles of FIN metabolites exhibited by dogs with those observed in humans (17). Also, preliminary purification of the sample by solvent extraction was an advantage, because it would have been more difficult to achieve chromatographic separation of positional isomers (dihydroxylated metabolites V and VII) in the presence of the acidic metabolite(s).

At all time points and for all dose groups, parent drug was a major component in plasma with ω-OH-FIN (metabolite I) as the major metabolite. This also was observed in humans after oral administration of FIN. Plasma concentration profiles of unchanged FIN after intravenous dosing showed that the drug declined plasma in a multieponential fashion. The relatively low value of Vdss (1.1 liter/kg) suggests that FIN was not extensively distributed to the tissues. Peak concentrations of ω-OH-FIN after the oral dose were reached ~4 hr later than those of FIN, suggesting the metabolite was formed subsequent to the first pass. AUC values for FIN and the ω-OH metabolite were similar after intravenous and oral dosing at 10 mg/kg, indicating a low first-pass effect that is in agreement with the high bioavailability determined from dose-normalized plasma data. The metabolic pathway may have been started with the high dose, as evidenced by the small increase in the amount of plasma radioactivity accounted for as parent drug and the small decrease accounted for as ω-OH-FIN.

When 38 mg of [14C]FIN was administered to human subjects in a metabolism study, human plasma contained an AFR, accounting for ~27% of the total radioactivity, with the major acidic component identified as ω-carboxy-FIN, metabolite IV with an AUC value of 1.4 μg·hr/ml (17); however, canine plasma exhibited a minor AFR (<4%), with the ω-carboxy metabolite accounting for ~1%. Plasma metabolic profiles from the dog and human are compared in fig. 4. The possible difference in systemic exposure to this acidic material between humans at the therapeutic dose of 5 mg and dogs undergoing toxicity testing at 80 mg/kg prompted the metabolism study in dogs at 80 mg/kg. An 8-fold increase in dose from 10 to 80 mg/kg resulted in less than proportional increases in the AUC values obtained for AFR, but no change was observed in the percentage of plasma radioactivity identified as ω-carboxy-FIN. It can be concluded from these data that, at 80 mg/kg of FIN, systemic levels of the ω-carboxy metabolite in dogs were nearly 3-fold higher than those found circulating in the plasma of humans who received 38 mg of FIN in the metabolism study, and it was estimated that the levels of metabolite circulating in the dog may be ~20-fold higher than those present in plasma of clinical subjects receiving 5 mg of drug.

FIN was metabolized extensively through oxidative pathways (fig. 1). The major biotransformations included hydroxylation of the t-butyl side chain to give ω-OH-FIN with subsequent oxidation of the side chain to ω-carboxy-FIN or hydroxylation of the tetracyclic ring system to the major dihydroxylated—ω,6α-(OH)2, ω,6β-(OH)2, and ω,15-(OH)2—derivatives of FIN. Hydroxylation of the t-butyl side chain is not uncommon and, for example, was observed for timolol by Tocco et al. (24). The pathway leading to the formation of ω-carboxy-FIN involves formation of an aldehyde intermediate, which is then metabolized to the acid. The in vitro formation of the aldehyde from FIN was detected by Ishii et al. (18) using rat liver microsomal preparations and by Huskey et al. (19) using human liver microsomal preparations. By use of human liver microsomes containing recombinant CYP enzymes, Huskey et al. demonstrated that CYP3A catalyzed each step of the biotransformation of FIN to ω-carboxy-FIN. Although we were able to confirm the formation of the aldehyde intermediate in vitro using rat liver microsomes (unpublished data), we were not able to detect this metabolite in vivo (plasma).

The 6α-OH derivative of FIN was identified as minor metabolite in dog plasma, whereas the 6α- and 6β-hydroxylated metabolites were identified as the additionally hydroxylated derivatives of ω-OH-FIN. Derivatives of FIN hydroxylated at the 6α-position were detected in human samples (15) and in the rat (16). The stereospecificity in hydroxylation of FIN at the 6-position differs from that of testosterone which, in most species, including the dog (25, 26), is usually hydroxylated at the 6β-position. In the rat, 6β-hydroxylation is conducted by the members of CYP3A family of isozymes (27), whereas the 6α-OH metabolite has been found as a minor metabolite of testosterone catalyzed by CYP2A1 (28). Characterization of the isozyme responsible for the hydroxylation of FIN at the 6α-position rather than 6β-position of the ring system is beyond the scope of the present study, but the explanation may involve the difference in the conformation of the A–B ring of the azasteroid, compared with that of testosterone, resulting in the α side of FIN being more accessible to the enzymes.
In summary, the absorption, plasma distribution, metabolism, and elimination of FIN have been studied in male beagle dogs after oral (10 mg/kg) and intravenous (5 mg/kg) administration, and also after the oral route at the dose level (80 mg/kg) used in the toxicity testing of the compound. The high systemic bioavailability of FIN indicates that the drug is well absorbed and the first-pass metabolism is low. Despite quantitative differences in the amounts of neutral vs. acidic metabolites formed, we have shown the drug is metabolized by dog and humans via similar pathways. The physiological disposition of FIN in the dog seems to be a reasonable paradigm for humans.

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References