Residue Depletion Studies on Danofloxacin in the Chicken

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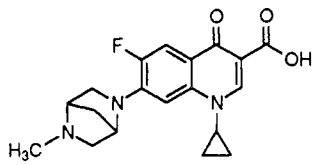
Penetration of danofloxacin into tissues is consistent with oral bioavailability and pharmacokinetic plasma data derived from chickens given danofloxacin by iv bolus and oral gavage administration at 5 mg/kg. This pharmacokinetic assessment was confirmed when [^{3}H]danofloxacin was administered to broilers in drinking water at 25 μg/mL for 5 days, and tissues were assayed for residues. Under this regimen, total residues of danofloxacin were higher in liver than other tissues and declined in liver from 0.612 μg/g at 6 h to 0.056 μg/g by 48 h of withdrawal, suggesting rapid depletion. In kidney and muscle total residues depleted in parallel with liver but were 2- and 10-fold lower, respectively. The major residue in all tissues was unchanged danofloxacin. An *N*-demethyl metabolite was found in liver and excreta but was not detected in muscle or fat/skin, indicating that it is an excretory metabolite. The depletion of unchanged and *N*-demethyl metabolite residues from edible tissues of the chicken was confirmed by HPLC with fluorescence detection when danofloxacin was given to 3-week-old chickens under a commercial use condition, i.e., in drinking water for 3 days at 5 mg/kg of body weight per day.

Danofloxacin is a synthetic antibacterial agent of the fluoroquinolone class, which emerged from a program designed to identify a member of this class of compounds for the treatment and control of bacterial and mycoplasmal infections in poultry, swine, and cattle (McGuirk et al., 1989a,b, 1992). The synthesis and antibacterial activity



danofloxacin

of danofloxacin (McGuirk et al., 1989a,b, 1992), its efficacy in food-producing animal disease models (Mann, 1989; Kemp et al., 1992; Tanner et al., 1992), its pharmacokinetics in cattle, swine, and poultry (Frame et al., 1989; Giles et al., 1991), and its metabolism in the chicken, rat, and dog (Nowakowski et al., 1993) have been described. For use in poultry, the highly water soluble mesylate salt of danofloxacin is formulated with nonactive excipents to form a free-flowing water-soluble powder containing 16.7% danofloxacin. The product is given to broiler chickens at 5 mg/kg per day.

In a review of the pharmacokinetics of quinolone compounds with respect to their structure and residues in poultry, it was concluded that quinolones have rapid per os absorption, attain serum concentrations above the minimum inhibitory concentration (MIC) for most Gram-negative and Gram-positive organisms, and have relatively long plasma half-lives (Anadon et al., 1990). These distributional and antimicrobial properties make them potentially useful in veterinary medicine (Vancutsem et al., 1990). The pharmacokinetics and tissue distribution of the fluoroquinolones flumequine, norfloxacin, enrofloxacin, and sarafloxacin have been described in the chicken (Samaha et al., 1991; Anadon et al., 1992; Scheer, 1987; Velagaleti et al., 1993). The pharmacokinetic disposition of quinolones in human body fluids and tissues also shows most tissue concentrations exceed plasma levels (Sorgel et al., 1989).

For animal health drugs, information on bioavailability as well as excretion, distribution, and depletion from edible tissues is needed when their utility is assessed. Thus, the studies reported here were designed to assess the absorption, distribution, and rate of elimination of danofloxacin residues in chickens under laboratory and use conditions utilizing radiotracer and chemical methods.

EXPERIMENTAL PROCEDURES

Instruments. The chromatographic system used consists of a Perkin-Elmer ISS-100 autoinjector, a Milton Roy Model CM 4000-1 pump, a Waters Novapak 4-μm, 15-cm C_{18} column equipped with a Supelco C_{18} guard column, a Kratos Model 980 fluorescence detector, and a Spectra Physics Model 4200 integrator.

Reagents and Reference Compounds. All reagents were of reagent grade or glass-distilled. Reference compounds consisted of danofloxacin mesylate [1-cyclopropyl-6-fluoro-1,4-dihydro-7-[(1*S*,4*S*)-5-methyl-2,5-diazabicyclo[2.2.1]hept-2-yl]-4-oxo-3-quinolinecarboxylic acid monomethanesulfonate], demethyldanofloxacin mesylate [1-cyclopropyl-6-fluoro-1,4-dihydro-7-[(1*S*,4*S*)-2,5-diazabicyclo[2.2.1]hept-2-yl]-4-oxo-3-quinolinecarboxylic acid monomethanesulfonate], and CP-71,755-27 [1-ethyl-6-fluoro-1,4-dihydro-7-(8-methyl-3,8-diazobicyclo[3.2.1]-oct-2-yl)-4-oxo-3-quinolinecarboxylic acid monomethanesulfonate].

Preparation of Tritium-Labeled Danofloxacin. This compound was synthesized from [1-^{3}H]cyclopropylamine hy-

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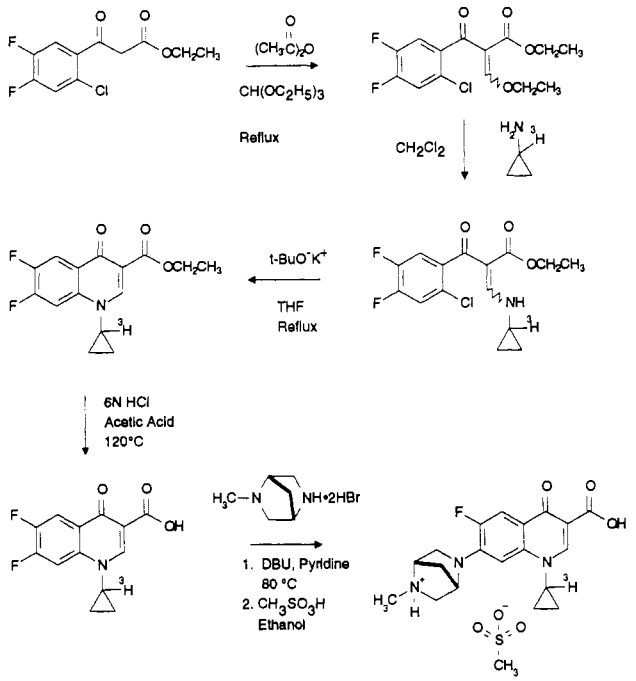
Scheme 1



drochloride by a multistep procedure according to labeling conditions and steps listed in Scheme 1. Starting from the β-keto ester (CP-84,192), this material was reacted with 1.5 equiv of triethylorthoformate and 2.5 equiv of acetic anhydride at 90 °C for 90 min to give CP-84,193. [^{3}H]Cyclopropylamine hydrochloride (25 mCi/mM, Amersham) was added to a methanolic solution of CP-84,193 to give the tritium-labeled aminoacrylate, [^{3}H]-CP-84,194. Cyclization of [^{3}H]-CP-84,194 to [^{3}H]-CP-84,-195 with potassium *tert*-butoxide in tetrahydrofuran, followed by acid hydrolysis of [^{3}H]-CP-84,195 with 6 N HCl in glacial acetic acid, gave the difluoroquinolone acid, [^{3}H]-CP-70,335. Coupling of [^{3}H]-CP-70,335 with (1*S*,4*S*)-5-methyl-2,5-diazabicyclo[2.2.1]heptane dihydrochloride in pyridine and 1,8-diazabicyclo[5.4.0]undec-7-ene yielded [^{3}H]danofloxacin. The addition of 1.1 equiv of methanesulfonic acid in water to [^{3}H]-danofloxacin converted the free base to [^{3}H]danofloxacin mesylate. The pure material was synthesized from [1-^{3}H]cyclopropylamine hydrochloride in 29% yield with a specific activity of 4.3 mCi/mmol.

Analysis of [^{3}H]-1′-Danofloxacin Mesylate. A melting point with decomposition was observed (Hoover apparatus) at 313 °C for [^{3}H]danofloxacin mesylate. Chromatography by TLC (chloroform/methanol = 85:15, ethyl acetate/ methyl ethyl ketone/formic acid/water = 3:3:1:1 on silica gel) of [^{3}H]danofloxacin mesylate revealed traces of fluorescent impurities (365 nm) but no (<1%) radiochemical byproducts or precursors. HPLC [Novapak C_{18}, 4 μm, 15 cm using a mobile phase of 7% acetonitrile/93% buffer (0.01M H_3PO_4, 0.008 M tetrabutylammonium hydroxide, 0.005 M Waters D-4 reagent, adjusted to pH 3.0); flowing at 1 mL/min] revealed no impurities (<1%) by UV (280 nm) or fluorescence (excitation: 280 nm, emission cutoff at 440 nm). The proton magnetic resonance spectrum and the retention time (R_t = 4.1 min) of [^{3}H]danofloxacin mesylate were identical to those of a working standard of danofloxacin mesylate. Also, identical ultraviolet (200–400 nm) spectra in water were obtained for [^{3}H]danofloxacin mesylate and a standard of danofloxacin mesylate. The specific activity of [^{3}H]danofloxacin mesylate was 12.1 μCi/mg (base) or 4.3 mCi/mmol. The metabolic stability of the tritium label in the test substance was demonstrated by the absence of tritium water in the 0–24-h urine collection containing 44% of an intraperitoneal injection of 2.13 mg of [^{3}H]danofloxacin mesylate, administered to a rat.

Animal Experiments with [^{3}H]-1′-Danofloxacin Mesylate. The first experiment was designed to determine the depletion of [^{3}H]danofloxacin total residues from plasma, bile, edible tissues, and excreta in broiler chickens that received radiolabeled drug in medicated water for 5 days. Levels of the unchanged drug and the major metabolite were also determined in liver, the tissue containing the highest residues. For this experiment, water medicated with [^{3}H]danofloxacin mesylate at 25 μg/mL activity was provided *ad libitum* to 12 male and 12 female healthy, uniform, 3-week-old broiler chickens for 5 days. Plasma, bile, muscle, liver, kidney, and skin with adhering fat were collected from three birds per sex at 6, 12, 24, and 48 h after the medication was withdrawn. Samples of excreta (pooled by sex and homogenized) were collected for each 24-h period of the 5 days of drug administration and for the 2 days of withdrawal until sacrifice of all birds was complete. Due to the death of one male during the second day of medication, the cause of which was unrelated to treatment, two males were available for sampling at 48 h following treatment. Tissues from one male and one female were also harvested to provide negative control tissues. Except for the loss of the one bird, all other birds consumed feed and water and grew normally through the study. Average daily drug consumption via medicated water was 5.28 mg/kg of body weight.

Animal Experiments with Danofloxacin Mesylate. On the basis of the results of the radiotracer experiment, 3-week old Hubbard broilers were medicated daily with danofloxacin soluble powder (SP) in drinking water for 3 days at 5 mg/kg of body weight, and then the drug was removed from the water supply to monitor the depletion of danofloxacin and its *N*-demethyl metabolite in edible tissues. Twenty cockerels and 20 pullets housed in four pens of 10 were medicated; an additional pen of 5 cockerels and 5 pullets served as nonmedicated controls. Control and medicated birds were weighed daily, and the amount of SP required to provide the daily target dose was calculated. The SP was mixed in a quantity of water so that the total daily dose would be consumed within the 24-h period. Water samples (medicated and nonmedicated) were taken daily during the medication period for assay and refrigerated pending shipment. Nonmedicated water was offered for approximately 2 h at the end of each medication day and for the duration of the study following withdrawal of the drug. Water consumption was determined daily. Liver, muscle (breast and thigh), and skin with adhering fat were harvested for assay from three medicated birds per sex at 6, 12, 18, 24, and 36 h following withdrawal of the drug. Samples were also harvested from the same number of nonmedicated birds at the 6-h postwithdrawal time. During the 3- day medication period, the average body weights of cockerels and pullets treated with danofloxacin were 852 and 739 g, respectively. The control group of five pullets and five cockerels averaged 792 g. The average daily doses of danofloxacin consumed by cockerels and pullets were 4.8 and 4.7 mg/kg, respectively.

The plasma pharmacokinetics of danofloxacin were also determined in chickens given 5 mg/kg either by a single intravenous (iv) dose in the basilic vein or by per os (po) administration of a 2.5% solution in water. Plasma samples were collected at 0, 1, 2, 4, 8, 12, and 24 h after treatment from five birds per treatment per collection time. The birds were bled via cardiac puncture for provision of individual plasma samples and then euthanized.

Determination of ^{3}H Radioactivity in Tissues and Excreta of Poultry. Approximately 1.0-g samples of liver, kidney, and muscle, 0.3-g samples of fat and skin (with adhering fat), or 0.5-g samples of excreta were burned with cellulose powder and Combustaid on a Packard TriCarb Model 306 oxidizer. The burn gases were absorbed in 15 mL of Monophase-S scintillation cocktail in 20-mL Kimble scintillation vials. Plasma (0.5 mL) and bile (0.25 mL) were mixed with 15 mL of Ecoline scintillation cocktail. Samples were counted using a DPM program on a Packard TriCarb 2000CA scintillation analyzer.

Determination of Danofloxacin and Its Demethyl Metabolite in Tissues. Samples of liver, muscle, and skin with adhering fat were collected from the birds and frozen under normal atmosphere at 0 °C for 3–6 weeks prior to analysis. The individual samples were thawed and homogenized, and triplicate aliquots of each (0.5 g) were weighed into separate, disposable glass tubes. To each tube was added 50 μL of CP-71,755 (10 μg/mL), a closely related analog (McGuirk et al., 1989a,b), which served as an internal standard for the quantitation process. This mixture was extracted with 5.0 mL of a mixture of 150 mM perchloric acid and 150 mM phosphoric acid in a solution of 1:1

Table 1. Determination of Danofloxacin in Fortified Poultry Tissues

level (μg/g) fortified	concn (±1 SD) of danofloxacin found in tissue		
	liver	muscle	fat/skin
0.010	0.011 (0.0014)		
0.025	0.024 (0.0011)	0.026 (0.0025)	0.025 (0.0024)
0.050	0.049 (0.0021)	0.050 (0.0049)	0.050 (0.0037)
0.100	0.101 (0.0029)	0.100 (0.0053)	0.101 (0.0056)
0.150			0.149 (0.0033)
0.200	0.199 (0.0041)	0.196 (0.0088)	
0.250		0.250 (0.0052)	
0.400	0.397 (0.0078)	0.395 (0.0050)	
0.600	0.602 (0.0110)	0.605 (0.0155)	

Table 2. Determination of Demethyldanofloxacin in Fortified Poultry Tissues

level (μg/g) fortified	concn (±1 SD) of demethyldanofloxacin found in tissue		
	liver	muscle	fat/skin
0.010	0.011 (0.0024)		
0.025	0.025 (0.0021)	0.026 (0.0017)	0.025 (0.0031)
0.050	0.049 (0.0028)	0.051 (0.0020)	0.048 (0.0071)
0.100	0.099 (0.0032)	0.102 (0.0040)	0.104 (0.0070)
0.150			0.148 (0.0059)
0.200	0.201 (0.0055)	0.193 (0.0129)	
0.250		0.250 (0.0055)	
0.400	0.397 (0.0106)	0.397 (0.0073)	
0.600	0.602 (0.0083)	0.604 (0.0179)	

methanol/water (Burdick and Jackson B&J brand). The components were vortexed briefly and homogenized with a Polytron tissue homogenizer. The sample tube was then capped and heated to 50 °C for 90 min to effect extraction of the analytes from the sample matrix.

Following centrifugation, 30–75 μL of the supernatant was injected into the liquid chromatograph. The analytical mobile phase consisted of ca. 5:95 acetonitrile/0.01 M phosphate buffer, pH 3.5, containing 0.008 M tetrabutylammonium hydroxide and 0.005 M Waters D-4 reagent, at a flow rate of 1.0–1.5 mL/min. The exact ratio of acetonitrile to buffer solution, the flow rate, and the injection volume were varied as required by the nature of the sample background. Subsequent to the elution of the internal standard peak, the composition of the mobile phase was increased to 22:78 acetonitrile/buffer to rapidly elute well-retained peaks. This composition was replaced by the analytical mobile phase prior to the next sample injection. The detector was operated with λ_{ex} = 280 nm and λ_{em} = 440 nm. For each tissue, the concentration of drug and *N*-demethyl metabolite in each study sample was determined by comparison of the ratio of analyte/internal standard to a linear plot of known values obtained by adding known concentrations of analyte to blank homogenized tissue. The limits of quantitation of the assay were typically 0.010–0.600 μg/g for the liver samples and 0.025–0.200 μg/g for the muscle and skin/fat samples. Correlation coefficients (r) exceeded 0.999, and standards produced values within 5% of the amount taken. The mean recovery of drug was 89.3% and for demethyldanofloxacin was 93.7% in liver, the target tissue. A summary of results for validation of the method for the determination of danofloxacin and its demethyl metabolite in liver, muscle, and fat/skin is given in Tables 1 and 2.

Determination of Danofloxacin and Its Demethyl Metabolite in Plasma. For each plasma sample, an Interaction Polysorb MP-1 solid-phase extraction column (100 mg) was wetted with 1 mL of methanol, followed by 1 mL of 0.025 M KH_2PO_4, pH 3.0. An aliquot of the plasma sample (0.25 mL) was then passed through the extraction column, followed by 0.1 mL of an aqueous solution of 4.0 μg/mL CP-71,755 (McGuirk et al., 1989a,), which served as an internal standard for the quantitation process. The column was then washed with 1.0 mL of 0.025 M KH_2PO_4, pH 3.0, followed by elution of the analytes with 1.0 mL of methanol. The methanol eluent was evaporated to dryness under N_2 at 50 °C, and the residue was reconstituted in 0.3 mL of 90:10 water/acetonitrile. This was centrifuged briefly to precipitate particulates, and 50 μL of the clear solution was injected into the liquid chromatograph as described above.

Table 3. Mean Concentrations (Micrograms per Milliliter) of Danofloxacin in Plasma of Five Chickens following Intravenous or Oral Administration at 5.0 mg/kg, SID × 1

	0.5 h	1 h	2 h	4 h	8 h	12 h	24 h
iv administration	0.69	0.64	0.53	0.32	0.14	0.08	0.02
SD	0.12	0.10	0.09	0.07	0.03	0.02	0.01
po administration	0.34	0.61	0.73	0.36	0.21	0.10	0.05
SD	0.13	0.11	0.29	0.07	0.06	0.02	0.01

Concentrations of danofloxacin in plasma samples were determined by comparison of the ratio of drug/internal standard to a linear plot of known values obtained by adding appropriate concentrations of drug to blank plasma. The limits of quantitation of this assay were 0.01–0.4 μg/mL, with a typical r = 0.996 and a relative standard deviation (RSD) of 3.2% at 0.01 μg/mL (n = 4).

Determination of Danofloxacin and Its Demethyl Metabolite in Excreta. Samples of excreta were thawed, and duplicate aliquots of each (1.0 g) were weighed into separate 50-mL screw-cap tubes. To each tube was added CP-71,755, which served as an internal standard. This mixture was extracted with 10 mL of a mixture of 150 mM perchloric acid and 150 mM phosphoric acid in a solution of 1:1 methanol/water (Burdick and Jackson B&J brand). The components were shaken for 1 h on a reciprocating shaker and then centrifuged. Following centrifugation, 200 μL of the supernatant was diluted with 5 mL of extraction solvent (150 mM $HClO_4$, 150 mM H_3PO_4 in CH_3OH/water 1:1), and 15 μL was injected into the liquid chromatograph. The chromatographic system consisted of the same components listed for assay of tissues and plasma. The analytical mobile phase consisted of ca. 6.5:93.5 acetonitrile/0.01 M phosphate buffer, pH 3.5, containing 0.008 M tetrabutylammonium hydroxide and 0.005 M Waters D-4 reagent, at a flow rate of 1.0 mL/min. The detector was operated with λ_{ex} = 280 nm and λ_{em} = 440 nm. For each sample, the concentration of danofloxacin and *N*-demethyl metabolite was determined by comparison of the ratio of analyte/internal standard to a linear plot of known values obtained by adding known concentrations of analyte to blank homogenized excreta. The limits of quantitation of the assay were typically 5–50 μg/g for danofloxacin and 1–5 μg/g for *N*-demethyldanofloxacin in excreta. Correlation coefficients exceeded 0.999, and standards produced values within 5% of the amount taken.

Calculation of Pharmacokinetic Parameters. The area under the plasma concentration–time curve from time zero to the last time (t) with a measurable concentration (C_p) was estimated using linear trapezoidal approximation. The area from time t to infinity was estimated as (1) C_p/k_{el}, where k_{el} represents the terminal elimination rate constant. The total area under the curve was estimated as the sum of (2) $AUC_{(0-\infty)} = AUC_{(0-t)} + AUC_{(t-\infty)}$. k_{el} was estimated using least-squares regression analysis of the log concentration–time data obtained during the terminal log-linear elimination phase. The elimination half-life ($t_{1/2}$) was calculated as (3) $0.693/k_{el}$. The maximum plasma concentration (C_{max}) was estimated directly from the experimental data, with T_{max} defined as the time of the first occurrence of C_{max}. On the basis of data generated by intravenous administration of danofloxacin, the systemic plasma clearance (CL_p) was estimated as (4) dose/$AUC_{(0-\infty)}$. The volume of distribution at steady state (Vd_{ss}) was calculated as (5) Vd_{ss} = Dose × $(AUMC_{(0-\infty)})/(AUC_{(0-\infty)})^2$. The bioavailability after oral administration (F) was calculated as (6) the ratio of $AUC_{(0-\infty)}$ for the oral dose divided by $AUC_{(0-\infty)}$ for the intravenous treatment times $(t_{1/2})_{iv}/(t_{1/2})_{po}$ (Gibaldi and Perrier, 1982).

RESULTS

The plasma levels of danofloxacin following intravenous and per os administration are displayed in Table 3, and the pharmacokinetic parameters derived from these data are shown in Table 4. The values of the plasma clearance (CL_p), elimination half-life, and volume of distribution at steady state (Vd_{ss}) are 1.23 L/h·kg, 5.76 h, and 8.0 L/kg, respectively. These values are consistent with drug penetration into the vascular compartment and rapid

Table 4. Pharmacokinetic Parameters following Intravenous and Per Os Administration of 5.0 mg/kg Danofloxacin SID × 1 to Chickens

treatment	C_{max} (mg/mL)	T_{max} (h)	k_{el} (h^{-1})	harmonic $t_{1/2}$ (h)	$AUC_{(0-\infty)}$ (mg·h/mL)	$AUMC_{(0-\infty)}$ (mg·h/mL)	CL_p (L/h·kg)	Vdss (L/kg)	F^a (%)
iv			0.120	5.76	4.07	26.4	1.23	8.0	
po	0.73	2.0	0.096	7.21	5.20				102

[a] F = Bioavailability [expressed as the percent po AUC to iv AUC × $(t_{1/2})_{iv}/(t_{1/2})_{po}$].

Table 5. Mean (±1 SD) Total Residues of [^{3}H]Danofloxacin (Micrograms per Milliliter or Micrograms per Gram Equivalents of Drug) in Tissues, Plasma, and Bile of 3-Week-Old Chickens (3/Sex/Time Point) after Continuous Administration of Tritiated Danofloxacin in Drinking Water at 25 μg/mL for 5 Days

withdrawal time (h)	residues					
	liver	kidney	muscle	skin/fat	plasma	bile
6	0.612 (0.134)	0.406 (0.138)	0.099 (0.037)	0.054 (0.018)	0.044 (0.006)	7.6 (7.5)
12	0.298 (0.086)	0.134 (0.034)	0.033 (0.010)	0.042 (0.020)	0.023 (0.006)	2.9 (1.9)
24	0.103 (0.023)	0.051 (0.013)	0.011 (0.002)	0.029 (0.010)	0.011 (0.002)	2.4 (1.8)
48	0.056 (0.025)	0.020 (0.004)	0.003 (0.001)	0.011 (0.001)	0.007 (0.001)	0.1 (0.02)

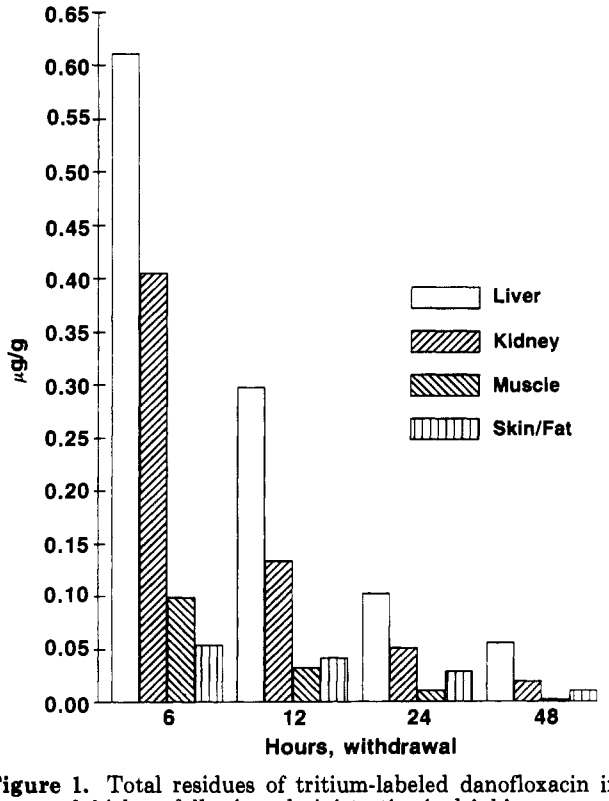


Figure 1. Total residues of tritium-labeled danofloxacin in tissues of chickens following administration in drinking water at 25 μg/mL for 5 days.

attainment of steady-state plasma levels. On the basis of a comparison of AUC values following iv and oral administration, 102 percent of an oral dose of danofloxacin is absorbed into the systemic circulation.

This pharmacokinetic assessment was confirmed when [^{3}H]danofloxacin was administered to broilers in drinking water at 25 μg/mL for 5 days and tissues were assayed for residues. Mean total residues in tissues are shown in Table 5. Among tissues, liver consistently showed the highest concentrations of total residues with a mean level of 0.612 μg/g at 6 h decreasing to 0.056 μg/g at 48 h (Figure 1). In kidney and muscle, total residues declined at the same rate as liver but were 2- and 10-fold lower, respectively. Total residues in skin with adhering fat declined from a mean of 0.054 μg/g at 6 h to 0.011 at 48 h.

In parallel with a determination of residues in tissues, mean plasma levels for total residues of [^{3}H]danofloxacin declined from 0.044 μg/mL at 6 h postdose to 0.007 μg/mL by 48 h. Radioactivity in bile was variable as total residues decreased from means of 7.6 μg/mL at 6 h to 0.1 μg/mL at 48 h (Table 5). Overall mean excreta radioactivity ranged from 35.0 to 46.5 μg/g during each of the 5 days of dosing but declined during the first to the second day of withdrawal from 20.1 to 6.7 μg/g (Table 6).

Table 6. Mean (±1 SD) Excreta Radioactivity (Micrograms per Gram of Danofloxacin Equivalents) Collected from Male and Female Chickens Receiving *ad Libitum* Access to Water Medicated at 25 μg/mL for 5 Days[a]

period (h)	males	females	mean by period
dosing			
0–24	40.3 (6.0)	34.3 (18.2)	37.3 (12.6)
24–48	32.1 (2.0)	37.9 (7.6)	35.0 (5.9)
48–72	33.4 (4.7)	59.5 (17.4)	46.5 (18.2)
72–96	40.9 (3.1)	39.9 (2.1)	40.4 (2.4)
96–120	46.5 (5.7)	38.7 (3.1)	42.6 (6.0)
mean by gender (±1 SD)	38.6 (5.9)	42.1 (10.0)	40.4 (4.5)
withdrawal			
0–24	19.2 (6.4)	21.0 (7.6)	20.1 (6.4)
24–48	7.3 (1.6)	5.8 (4.0)	6.7 (2.1)

[a] Limit of quantitation for 0.5-g excreta samples is 0.05 μg/g on the criterion that the lowest measurable residue should display radioactivity equal to twice background.

The development of a chemical method for the determination of danofloxacin and the demethyl metabolite in tissues, fluids, and excreta was guided by the analytical methods described for this class of compounds (Edelson et al., 1977) and specific methods for ciprofloxacin (Scholl et al., 1987) and enrofloxacin (Waggoner and Bowman, 1987; Tyczkowska et al., 1989; Rogstad et al., 1991). Utilization of HPLC in combination with fluorometric detection permitted the determination of residues of danofloxacin down to 0.010 μg/g. The incubation of tissues at 50 °C in a solution of perchloric acid and phosphoric acid in methanol/water provided extracts containing analytes suitable for HPLC analysis.

When specific chemical assays based on HPLC with fluorescence detection for unchanged danofloxacin and its *N*-demethyl metabolite were applied to excreta and pooled liver samples (Tables 7 and 8), ca. 85% of labeled material in excreta consisted of unchanged drug and ca. 4–6% was the *N*-demethyl metabolite. The high recovery of the dose as unchanged drug and metabolite confirmed stability of the radiolabel. In liver, the unchanged drug accounted for 47–61% of total radioactivity and the metabolite 20% or less over the 6–48 h withdrawal period. Thus, the unchanged drug is a suitable marker for monitoring residues of danofloxacin.

Table 7. Concentrations (Micrograms per Gram) of Total, Unchanged, and Demethyl Metabolite Levels of Danofloxacin in Excreta of Chickens following Administration of [^{3}H]Danofloxacin in Drinking Water at 25 μg/mL for 5 Days

sex	24-h collection	μg/g total residue	μg/g danofloxacin	% drug	μg/g demethyl metabolite	% metabolite
male						
dosing	24–48	32.1	33.4		2.3	
dosing	48–72	33.4	28.8		2.3	
dosing	72–96	40.9	34.0		2.3	
dosing	96–120	46.5	38.4		2.5	
mean (±SD)		38.2 (6.7)	33.7 (3.9)	88.2	2.4 (0.1)	6.3
withdrawal	24–48	7.3	8.7	119.2	<1.0	
female						
dosing	24–48	37.9	41.5		1.8	
dosing	48–72	59.5	27.9		1.9	
dosing	72–96	39.9	30.6		1.8	
dosing	96–120	38.7	36.2		2.2	
mean (±SD)		44.0 (10.3)	34.1 (6.1)	77.5	1.9 (0.2)	4.3
withdrawal	24–48	5.8	4.9	84.5	<1.0	

Table 8. Total Residues, Unchanged Drug, and Demethyl Metabolite Levels (Micrograms per Gram) and Percentages of Danofloxacin in Pooled Livers of 21-Day-Old Chickens (3/Sex/Time Point) after Continuous Administration of Tritiated Danofloxacin in Drinking Water at 25 μg/mL for 5 Days

withdrawal (h)	total residues	danofloxacin	%	demethyl metabolite	%
6	0.602	0.37	61	0.102	17
12	0.277	0.17	61	0.056	20
24	0.102	0.053	52	0.014	14
48	0.075	0.035	47	<0.010	<13

This assessment was confirmed when danofloxacin was administered under proposed commercial-use condition to chickens in drinking water at 5 mg/kg of body weight per day for 3 days: the highest mean level of unchanged danofloxacin was found in liver. Mean concentrations of unchanged danofloxacin in liver depleted 5-fold from 0.221 μg/g at 6 h to 0.041 μg/g at 36 h after withdrawal (Table 9). Lower 6-h levels of unchanged drug were seen in skin/fat (0.134 μg/g) and muscle (0.063 μg/g), and these decreased to <0.025–0.030 μg/g by 36 h after withdrawal. At 6 h, the *N*-demethyl metabolite of danofloxacin was observed at a mean concentration of 0.068 μg/g in liver and below 0.010 μg/g by 36 h. The metabolite was not detected in muscle or skin/fat at any withdrawal time.

DISCUSSION

On the basis of a comparison of iv and po plasma concentration profiles, 102 percent of an oral dose of danofloxacin is absorbed into the systemic circulation and is distributed into tissues. The excellent bioavailability, potency against a range of field isolated pathogens (McGuirk et al., 1989a), favorable pharmacokinetic profile, and tendency to concentrate in lung at multiples of plasma concentrations (Frame et al., 1989) make danofloxacin mesylate effective in treating respiratory diseases.

In parallel with an assessment of the efficacy of danofloxacin, potential concerns relating to the distribution, depletion, and excretion of total unchanged danofloxacin and *N*-demethyldanofloxacin concentrations were addressed in the radiotracer and chemical assay studies described in this paper.

Incorporation of the radiolabel as tritium into the cyclopropyl substituent (Silverman and Hoffman, 1981) seemed to be advantageous since radioactivity was rarely released by oxidative and metabolic degradation of an *N*-ethyl substituent introduced there in a number of other nalidixic acid analogs (Edelson et al., 1977; Nagatsu et al., 1981a,b). Metabolism studies with [U-^{14}C]ciprofloxacin confirmed metabolic stability of an *N*-cyclopropyl substituent (Siefert et al., 1986); no tritium water was found in distillates of water derived from rat urine containing 44% of an intraperitoneal dose of [^{3}H]danofloxacin mesylate (F. R. Mosher, unpublished data). A mass balance assessment based on HPLC analysis of the radioactivity in excreta of poultry administered labeled compound also confirmed its stability; unchanged drug and demethyl metabolite comprised 90% of the residues.

These tissue residue studies demonstrated danofloxacin residues, consisting of total, unchanged, and demethyl metabolite levels, are eliminated rapidly from edible tissues of the chicken. Interestingly, the unchanged drug was found in each tissue, and was 3–4 times higher in concentration than the *N*-demethyl metabolite in liver. Although also found in bile and excreta, *N*-demethyl danofloxacin was not detected in muscle or fat/skin, indicating that it is an excretory metabolite. The formation of *N*-demethyldanofloxacin is not unexpected given the results of numerous metabolism studies of quinolones (Sekine et al., 1976; Edelson et al., 1977; Nagatsu et al., 1981a,b; Sudo et al., 1986; Gau et al., 1986; Borner and Lode, 1986; Outman and Nightingale, 1989).

Table 9. Mean (±1 SD) Danofloxacin and Demethyl Metabolite Residues (Micrograms per Gram) in Edible Tissues after Administration of Danofloxacin at 5 mg/kg in Drinking Water to 21-Day-Old Chickens for 3 Days

withdrawal (h)	danofloxacin			demethyl metabolite		
	liver	muscle	skin/fat	liver	muscle	skin/fat
6	0.221 (0.065)	0.063 (0.022)	0.134 (0.067)	0.068 (0.061)	<0.024	<0.025
12	0.150 (0.048)	0.031 (0.016)	0.085 (0.025)	0.022 (0.014)	<0.025	<0.025
18	0.098 (0.025)	<0.025	0.056 (0.036)	0.028 (0.018)	<0.025	<0.025
24	0.098 (0.050)	<0.025	<0.030	0.034 (0.020)	<0.025	<0.025
36	0.041 (0.017)	<0.025	<0.030	<0.010	<0.025	<0.025

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