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Pharmacokinetics of Trovafloxacin (CP-99,219), a New Quinolone, in Rats, Dogs, and Monkeys

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The pharmacokinetics of trovafloxacin [CP-99,219; 7-(3-azabicyclo[3.1.0]hexyl)-naphthyridone] were studied in rats, dogs, and monkeys following oral and intravenous administration. After intravenous dosing, the systemic clearances of trovafloxacin in rats, dogs, and monkeys were 12.5, 11.1, and 7.2 ml/min/kg of body weight, respectively, and the respective volumes of distribution were 0.9, 1.7, and 4.3 liters/kg, with corresponding elimination half-lives of 0.7, 1.8, and 7.0 h. After the administration of oral doses of 50, 20, and 20 mg/kg to rats, dogs, and monkeys serum trovafloxacin concentrations reached a maximum at 0.6, 2.3, and 2.3 h, respectively, with respective maximum concentrations of trovafloxacin in serum of 11.5, 3.5, and 5.2 μg/ml; the corresponding elimination half-lives were 2.2, 2.5, and 7.5 h. The oral bioavailability of trovafloxacin was 68, 58, and 85% in rats, dogs, and monkeys, respectively. The binding of trovafloxacin to serum proteins was concentration independent, averaging 92, 75, and 66% for rats, dogs, and monkeys, respectively. Trovafloxacin penetrated well into tissues in dogs. The urinary recoveries of unchanged drug were less than 5% in dogs and monkeys, with or without incubation with alkali or Glusulase (β-glucuronidase and sulfatase). In rats, 99.8% of the orally administered radioactivity was recovered in feces, while 20.6, 3.4, and 67.1% of the radioactive dose in bile duct-cannulated rats were recovered in feces, urine, and bile, respectively. These results suggest that the elimination of trovafloxacin from rats, and possibly from dogs and monkeys, is primarily through biliary excretion.

Trovafloxacin, 7-(3-azabicyclo[3.1.0]hexyl)-naphthyridone (CP-99,219), is a new synthetic fluoroquinolone antibiotic agent with a broad spectrum of activity against gram-positive and gram-negative bacteria. It has shown a number of favorable characteristics in preclinical in vitro and in vivo testing. In broth susceptibility studies, trovafloxacin demonstrated a broad spectrum of in vitro activity against gram-positive and gram-negative bacteria and could be differentiated from ciprofloxacin, ofloxacin, and other marketed fluoroquinolones by its greater activity against many clinically significant gram-positive organisms, most notably, streptococci such as *Streptococcus pneumoniae* (2, 4, 10, 11). Trovafloxacin administered orally was able to control systemic gram-positive and gram-negative infections in mice; it was appreciably more potent than temafloxacin, ciprofloxacin, and ofloxacin in protecting mice against lethal infections with *S. pneumoniae* or *Streptococcus pyogenes* (8).

The antimicrobial spectrum and potency of this compound justified further studies of its pharmacokinetics, metabolism, and pharmacodynamics. In the present study, the pharmacokinetics of trovafloxacin in the rat, dog, and monkey were characterized following oral and intravenous administration. In addition, the binding to serum proteins and blood/plasma distribution ratios were determined. (Preliminary results from the study have been presented previously [9, 16]).

MATERIALS AND METHODS

Chemicals. The mesylate salts of trovafloxacin, an analytical internal standard [7-(2-methyl-3-azabicyclo[3.1.0]hexyl)-naphthyridone], and [^{14}C]trovafloxacin (47.7 μCi/mg, 99% radiochemical purity) were synthesized at Pfizer Central Research. Methylcellulose and potassium phosphate monobasic-sodium hydroxide buffer (pH 9.0) were purchased from Fisher Scientific Co. Glycerol formal solution (75%) and tetrabutyl ammonium hydroxide were obtained commercially from Sigma Chemical Co. Dibutyl amine phosphate (D-4) reagent was bought from Waters Associates. Glusulase (β-glucuronidase and sulfatase) was purchased from Dupont. All other reagents were reagent grade and were used as received without further purification. All doses and concentrations of trovafloxacin provided in this report are expressed as free base equivalents.

Animals. Male and female Sprague-Dawley rats (body weight, approximately 230 g), purebred beagle dogs (body weight, 11 kg), and cynomolgus monkeys (body weight, 3 kg) were obtained as stock animals from the Pfizer Animal Resources Department.

Drug administration. Trovafloxacin was administered orally and intravenously to fasting animals. A suspension for oral administration was prepared by homogenizing the drug powder in 0.5% methylcellulose aqueous solution. Solutions for intravenous administration were prepared by dissolving trovafloxacin in 37.5% glycerol formal aqueous solution. All oral doses were administered via gavage. Intravenous doses were administered by venipuncture of the cephalic vein in dogs and the saphenous vein in monkeys and through a polyethylene catheter inserted into the femoral vein in rats.

Sprague-Dawley rats were prepared by the surgical implantation of a polyethylene catheter into the femoral vein. Following a recovery period of 13 to 14 h, rats (four per sex per dosing route) were administered trovafloxacin either as an intravenous dose of 10 mg/kg of body weight or as an oral dose of 50 mg/kg. In addition, the serum concentration-time profiles of trovafloxacin and total radioactivity in another four male rats were determined following oral administration of a 50-mg/kg (20 μCi) oral dose of [^{14}C]trovafloxacin. Blood samples were collected for up to 8 h postdosing, and fecal samples were obtained for up to 96 h postdosing.

Trovafloxacin was administered intravenously to six beagle dogs (three males and three females) at a dose of 5 mg/kg by a 5-min infusion. Following a washout period of 1 week, the same animals were administered an oral dose of 20 mg/kg. Blood samples were collected for up to 12 h postdosing. For determination of levels in tissues, trovafloxacin was administered orally to another two male dogs at 10 mg/kg/day for 5 days. At 2 h postdosing on day 5, the dogs were euthanized with pentobarbital, and their tissues were harvested for determination of trovafloxacin concentrations.

Three cynomolgus monkeys received a 20-mg/kg dose of trovafloxacin administered as a 5-min infusion, and a parallel group of three monkeys was administered an oral dose of 20 mg/kg. Blood samples were serially collected for up to 30 h postdosing.

In an experiment of urinary, fecal, and biliary excretion in rats, five male rats were prepared by the surgical implantation of a cannula into the bile duct. Following a recovery period of 24 h, a 50-mg/kg oral dose of [^{14}C]trovafloxacin (20 μCi) was administered. The animals were placed separately into metabolic cages for urine and feces collections. Samples of urine, bile, and feces were

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collected for up to 96 h postdosing, and the total radioactivity excreted in these matrices was determined by liquid scintillation counting. In addition, four dogs and four monkeys were orally administered trovafloxacin at a dose of 10 mg/kg. Serial urine samples were collected from 0 to 48 h postdosing. Aliquots (0.5 ml) of each urine sample from dogs and monkeys were treated prior to assay by using each of the following three treatments: (i) buffering samples with a pH 3 phosphate buffer (0.025 M), (ii) incubation with 500 μl of 0.1 N sodium hydroxide at 37°C for 1 h and then neutralization and buffering with the pH 3 buffer, and (iii) incubation with 10 μl of Glusulase and 500 μl of pH 4.5 buffer (0.13 M phosphate) at 37°C for 1 h and then a treatment with the pH 3 buffer. The treated samples were analyzed for trovafloxacin.

Determination of trovafloxacin in serum. The concentrations of trovafloxacin in serum and urine were determined by a reverse-phase high-performance liquid chromatography (HPLC) method with UV detection, as reported previously (13). Following solid-phase extraction, chromatographic separation was accomplished by using a C_{18} column and a phosphate mobile phase (0.04 M H_3PO_4–acetonitrile–tetrabutyl ammonium hydroxide–0.005 M D-4 reagent [83:16.85:0.05:0.1; vol/vol/vol/vol; pH 3]). Trovafloxacin and the internal standard (a methyl derivative of trovafloxacin) were detected by UV at A_{275}. The calibration curves were linear over a concentration range of 0.1 to 20.0 μg/ml ($R^2 = 0.999$). The average recoveries were greater than 70% for both compounds. The intraday and interday coefficients of variation in both urine and serum were less than 10%.

Determination of trovafloxacin in tissue and body fluids. Tissue or body fluid trovafloxacin concentrations were determined by HPLC. Tissue samples (0.5 g or 0.5 ml) were homogenized with 5 ml of extraction mixture (0.15 M H_3ClO_4 and 0.15 M H_3PO_4 in a 50% CH_3OH aqueous solution) and the internal standard, and the mixture was centrifuged at 1,000 × *g*. The supernatant was decanted and evaporated to dryness. The residue was redissolved in 2 ml of phosphate solution (0.025 M KH_2PO_4 [pH 3.0]), which was extracted twice with 5 ml of ethyl acetate. Following evaporation of the ethyl acetate to dryness, the residue was redissolved in 0.5 ml of mobile phase, which was then washed with 1 ml of hexane. After the removal of hexane layer, the sample was introduced onto the HPLC column. The calibration curves were linear over a concentration range of 0.2 to 30.0 μg/g ($R^2 > 0.998$). The average recovery was 60 to 70% for both trovafloxacin and the internal standard. Intraday and interday coefficients of variation were less than 5%.

Serum protein binding and blood/plasma ratios. Serum protein binding of trovafloxacin in rats, dogs, and monkeys was examined by an equilibrium dialysis method at nominal starting concentrations in serum of 1 and 5 μg/ml. Dialysis was performed in 1.36-ml dialysis cell blocks (Equilibrium Dialyzer; Spectra/Por, Houston, Tex.) separated with Spectra/Por membranes (molecular weight cutoff, 12,000 to 14,000). The membranes were prepared by sequentially soaking them in HPLC-grade water, 30% ethanol, and 0.1 M phosphate buffer (pH 7.4) for 30, 60, and 30 min, respectively. The dialysis cells were immersed in a water bath at 37°C and were rotated on a dialysis wheel at 20 rpm. The time to reach equilibrium was previously determined to be 6 h. At the end of dialysis, aliquots from both the serum and the buffer sides were collected for determination of trovafloxacin content by the HPLC-UV method. Each serum sample was dialyzed in duplicate, with postdialysis buffer and serum samples assayed in triplicate. The percentage of free trovafloxacin in serum was estimated, with adjustment for osmotically induced volume shifts across the membrane (1).

For determination of blood/plasma ratios, fresh blood was collected from rats, dogs, and monkeys ($n = 3$ each) in clear glass test tubes containing 20 IU of heparin per ml of blood. The pooled blood was cooled on ice until it was used, which was within 30 min of collection. To 20-ml scintillation vials containing 2 ml of blood, 0.01 μCi of [^{14}C]trovafloxacin, and different amounts of nonlabeled trovafloxacin were added to make final trovafloxacin concentrations of 0.5, 5, and 20 μg/ml. The trovafloxacin-spiked blood aliquots were incubated at 37°C for 20 min, after which time the plasma was separated by centrifugation. Aliquots of the plasma were then assayed for radioactivity by liquid scintillation counting. Blood/plasma ratios were calculated as the ratio of the radioactivity in the whole blood to that in plasma.

Pharmacokinetic analysis. Pharmacokinetic parameters were calculated by noncompartmental analyses (7). The terminal phase rate constant (λ_z) was estimated by least-squares regression analysis of the concentration-time data obtained over the terminal log-linear phase. The corresponding half-life ($t_{1/2}$) was calculated from the equation $t_{1/2} = \ln 2/\lambda_z$. The area under the concentration-time curves from time zero to infinity ($AUC_{0-\infty}$) was calculated by the linear trapezoidal rule with extrapolation to infinity. The maximum concentration of drug in serum (C_{max}) was obtained directly from the concentration in serum data, with T_{max} defined as the time of the first occurrence of C_{max}. The systemic clearance (CL) was obtained as the ratio of intravenous dose/$AUC_{0-\infty}$, and the volume of distribution (V_{area}) was estimated as CL/λ_z. The oral bioavailability (F) was calculated as the ratio of the dose-corrected $AUC_{0-\infty}$ following oral and intravenous administration. Results are expressed as the mean and the standard deviation. The significance of the data was evaluated by Student's t test. Values of P of <0.05 were considered significant.

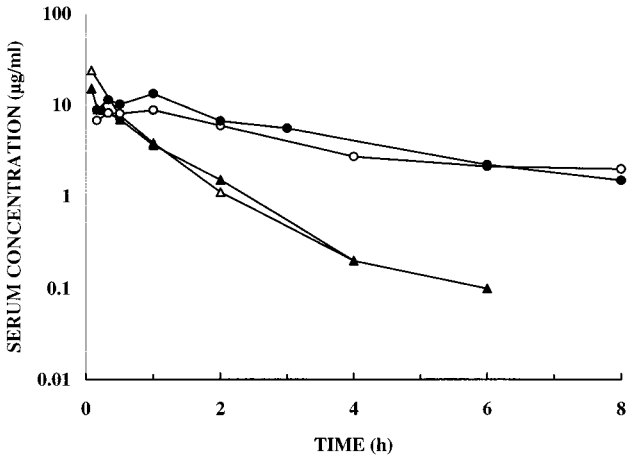

FIG. 1. Mean concentrations of trovafloxacin in serum following administration of an intravenous dose (10 mg/kg; triangles) and an oral dose (50 mg/kg; circles) to male (solid symbols) and female (open symbols) rats.

RESULTS

Concentrations in serum and tissues. The serum concentration-time profiles of trovafloxacin following intravenous administration to rats, dogs, and monkeys are illustrated in Fig. 1 to 3, respectively. A relatively wide difference in elimination rates among these three animal species was shown, with mean terminal phase $t_{1/2}$s of 0.7 ± 0.1 h in rats, 1.8 ± 0.3 h in dogs, and 7.0 ± 2.2 h in monkeys (Table 1). On other hand, rats and dogs had similar CL values: for rats, 12.5 ± 2.9 ml/min/kg; for dogs, 11.1 ± 3.0 ml/min/kg. Monkeys, on the other hand, exhibited a lower value of CL, i.e., 7.2 ± 1.1 ml/min/kg. V_{area}s for rats, dogs, and monkeys were 0.9 ± 0.2, 1.7 ± 0.5, and 4.3 ± 2.3 liters/kg, respectively. In rats, after administration of a 50-mg/kg oral dose, the peak concentrations in serum were achieved at approximately 0.6 ± 0.3 h postdose, with a mean C_{max} of 11.5 ± 3.3 μg/ml and a mean $t_{1/2}$ of 2.2 ± 0.9 h, in contrast to the $t_{1/2}$ of 0.7 ± 0.1 h following intravenous administration. After oral dosing of a 20-mg/kg dose to dogs, five of six dogs experienced emesis at approximately 1 h postdosing. Serum trovafloxacin concentrations reached a maximum at 2.3 ± 1.2 h, with a mean C_{max} of 3.5 ± 1.5 μg/ml and $t_{1/2}$ of 2.5 ± 0.7 h. In monkeys receiving a single oral dose of 20 mg/kg, the mean T_{max} was 2.3 ± 1.5 h and C_{max} was 5.2 ± 2.5 μg/ml; the concentrations of trovafloxacin in serum declined in a biexpo-

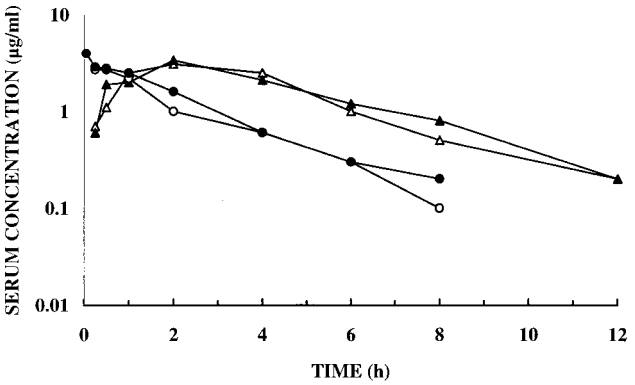

FIG. 2. Mean concentrations of trovafloxacin in serum following administration of an intravenous dose (5 mg/kg; circles) and oral dose (20 mg/kg; triangles) to male (solid symbols) and female (open symbols) dogs.

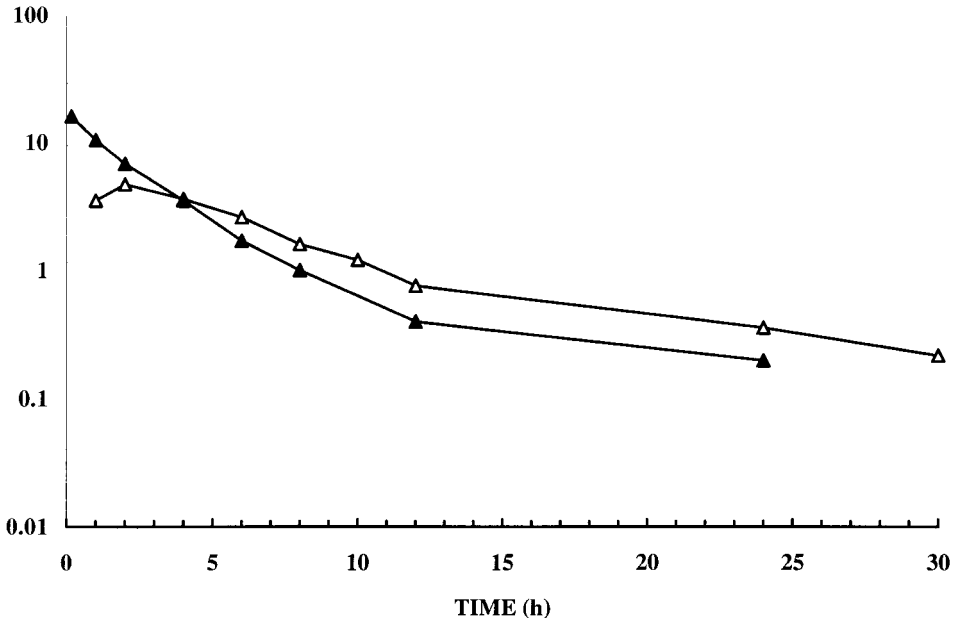

FIG. 3. Mean concentrations of trovafloxacin in serum following administration of intravenous (solid triangles) and oral (open triangles) administration of a single dose of 20 mg/kg to monkeys.

nential manner, with an additional distribution phase starting at approximately 12 h postdosing and a mean terminal phase elimination $t_{1/2}$ of 7.5 ± 0.5 h (Fig. 3). Comparison of the mean $AUC_{0-\infty}$ after oral administration with that following intravenous dosing indicated that the absolute bioavailability of trovafloxacin in rats, dogs, and monkeys was 68, 58, and 85%, respectively.

The mean pharmacokinetic parameters observed in male and female rats following intravenous and oral administration of trovafloxacin were not appreciably different between sexes (Table 1). Similarly, the profiles of the mean concentration of drug in serum following intravenous and oral administration of trovafloxacin were not significantly different between male and female dogs.

Following oral administration to two dogs at 10 mg/kg/day for 5 days, trovafloxacin concentrations in brain, lung, liver, kidney, muscle, fat, heart, bile, and cerebrospinal fluid were determined at 2 h postdosing. The mean concentration of trovafloxacin in serum was 3.9 μg/ml. Tissue/serum ratios were high in bile (ratio, 63), moderate in liver (ratio, 4.5), kidney

TABLE 1. Pharmacokinetic parameters of trovafloxacin in serum following intravenous and oral administration in rats, dogs, and monkeys[a]

Species	Dose (mg/kg)	Route[b]	Sex (no. of animals)[c]	T_{max} (h)	C_{max} (μg/ml)	CL (ml/min/kg)	V_{area} (liters/kg)	$AUC_{0-\infty}$ (μg · h/ml)	$t_{1/2}$ (h)	F (%)
Rats	10	i.v.	M (4)			13.9 ± 3.5	0.7 ± 0.2	14.1 ± 3.4	0.7 ± 0.1	
			F (4)			11.0 ± 1.2	1.0 ± 0.1	11.2 ± 1.2	0.8 ± 0.1	
			M and F (8)			12.5 ± 2.9	0.9 ± 0.2	12.6 ± 2.8	0.7 ± 0.1	
	50	p.o.	M (4)	0.7 ± 0.4	13.3 ± 2.0			47.0 ± 7.9	2.4 ± 1.2	67
			F (4)	0.5 ± 0.3	9.8 ± 3.8			38.0 ± 13.2	2.0 ± 0.2	68
			M and F (8)	0.6 ± 0.3	11.5 ± 3.3			42.5 ± 11.2	2.2 ± 0.9	68
Dogs	5	i.v.	F (3)			12.4 ± 3.8	1.9 ± 0.7	7.1 ± 1.8	1.8 ± 0.3	
			M (3)			9.7 ± 1.7	1.5 ± 0.4	8.8 ± 1.7	1.7 ± 0.3	
			M and F (6)			11.1 ± 3.0	1.7 ± 0.5	8.0 ± 1.8	1.8 ± 0.3	
	20	p.o.	M (3)	2.5 ± 1.0	3.2 ± 1.7			16.7 ± 7.5	2.4 ± 0.8	60
			F (3)	2.1 ± 1.4	3.9 ± 1.3			18.7 ± 11.5	2.7 ± 0.6	57
			M and F (6)	2.3 ± 1.2	3.5 ± 1.5			17.7 ± 9.0	2.5 ± 0.7	58
Monkeys	20	i.v.	M and F (3)			7.2 ± 1.1	4.3 ± 2.3	47.1 ± 7.2	7.0 ± 2.2	
	20	p.o.	M and F (3)	2.3 ± 1.5	5.2 ± 2.5			40.2 ± 4.7	7.5 ± 0.5	85

[a] Values represent means ± standard deviations.
[b] p.o., oral administration; i.v., intravenous administration.
[c] M, male; F, female.

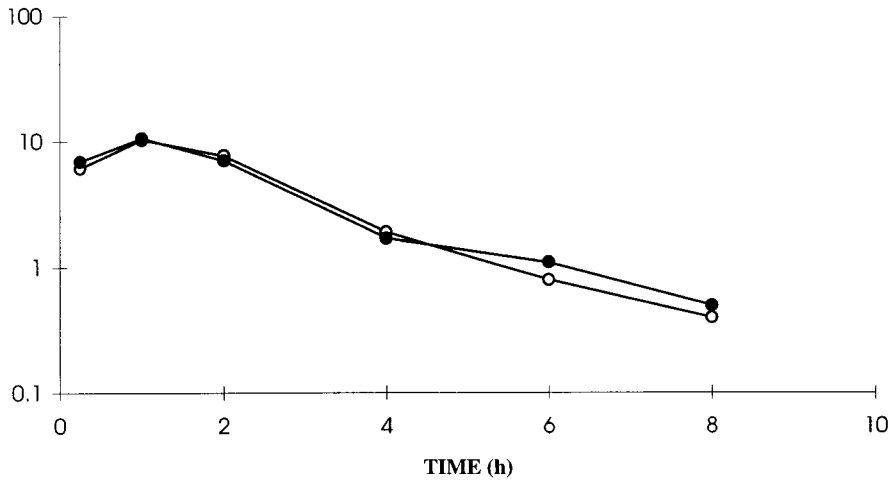

FIG. 4. Mean concentrations of trovafloxacin (open circles) and radioactivity (solid circles) in serum expressed as trovafloxacin equivalents following administration of a 50-mg/kg oral dose of [^{14}C]trovafloxacin to rats.

(ratio, 2), heart (ratio, 1.9), muscle (ratio, 1.6), and lung (ratio, 0.9), and low in brain (ratio, 0.4), fat (ratio, 0.2), and cerebrospinal fluid (ratio, 0.2).

Excretion. After oral administration of [^{14}C]trovafloxacin to rats, the profiles of radioactivity in serum were close to the corresponding profiles of the trovafloxacin concentration in serum (Fig. 4). Approximately 99.8% ± 8.1% of the administered radioactivity was recovered in feces (Table 2). Furthermore, when [^{14}C]trovafloxacin was given orally to the bile duct-cannulated rats, 20.6% ± 9.7%, 3.4% ± 2.4%, and 67.1% ± 4.6% of the administered radioactivity were recovered in feces, urine, and bile, respectively. Nearly 80% of the administered radioactivity was recovered within 24 h postdosing; approximately 45% of the radioactivity excreted in bile was recovered within 8 h.

Recovery of nonconjugated trovafloxacin was generally low in the urine of dogs (<3% of the administered dose). The mean urinary concentration from 0 to 4 h postdosing was 23.0 ± 22.9 μg/ml following the administration of a single 10-mg/kg dose (Table 3). Most of the drug in urine was excreted within 4 h. The total recovery (0 to 48 h) of trovafloxacin in urine was approximately 2.3%. Treatment of urine samples with alkali or Glusulase released a small additional amount of trovafloxacin (0.4% of the dose). Control fortified urine samples incubated with alkali confirmed the stability of trovafloxacin during the treatment procedure. In monkeys, recovery of nonconjugated trovafloxacin was low (2.1%), with most of the unchanged drug excreted between 8 and 24 h. Incubation with alkali or Glusulase resulted in a three- to fourfold increase in trovafloxacin recovery in samples obtained through 8 h, but only a slight increase in samples obtained between 8 and 24 h. A slightly greater recovery of trovafloxacin was obtained following alkaline treatment (4.9%) than following the treatment with Glusulase (4.3%).

TABLE 2. Excretion of [^{14}C]trovafloxacin in urine, feces, and bile after administration of a single oral dose of 50 mg/kg to rats

Time (h)	% Radioactivity in:			
	Feces of intact rats[a]	Bile duct-cannulated rats[b]		
		Feces	Urine	Bile
0–8	0.6 ± 0.5		1.7 ± 2.4	45.5 ± 11.0
8–24	87.3 ± 13.7	13.9 ± 9.5	0.7 ± 0.2	16.8 ± 8.3
24–48	9.8 ± 9.1	4.9 ± 3.5	0.6 ± 0.5	4.6 ± 7.0
48–72	1.7 ± 1.7	1.5 ± 1.2	0.3 ± 0.4	0.2 ± 0.2
72–96	0.5 ± 0.4	0.3 ± 0.3		
Total	99.8 ± 8.1	20.6 ± 9.7	3.4 ± 2.4	67.1 ± 4.6

[a] Values are means ± standard deviations for four rats.
[b] Values are means ± standard deviations for five rats.

Serum protein binding and blood/plasma ratios. The in vitro binding of trovafloxacin to serum proteins was determined by equilibrium dialysis. The binding of trovafloxacin to serum proteins was 89 to 96, 75, and 65 to 67% in rats, dogs, and monkeys, respectively (Table 4). The binding was similar at the two concentrations examined in these species.

The blood/plasma distribution ratios of trovafloxacin were determined at 37°C in heparinized blood obtained from rats, dogs, and monkeys. No degradation of trovafloxacin was observed during the 20-min incubation time. The ratios were approximately unity at the concentrations examined in all three species (Table 4). Thus, concentrations in whole blood can be expected to be about the same as those in plasma in these species with normal hematocrits.

DISCUSSION

The present investigation demonstrated that trovafloxacin is rapidly and well absorbed from the gastrointestinal tract, is moderately bound to plasma proteins, is well distributed into various tissues in rats, dogs, and monkeys, and is excreted primarily in bile in rats. After oral administration, trovafloxacin was well absorbed and exhibited moderate to high bioavailabilities in rats (68%), dogs (58%), and monkeys (85%). The high variability and lower mean value for bioavailability in dogs may largely be due to emesis in five of six dogs. In rats and dogs, similar concentration profiles were observed in male and female animals following both intravenous and oral administrations, suggesting no gender difference for trovafloxacin pharmacokinetics in the two species (Fig. 1 and 2). The drug had mean $t_{1/2}$s in dogs of 1.8 and 2.5 h after oral and intravenous administration, respectively. A short $t_{1/2}$ of trovafloxacin was observed in rats following intravenous administration (0.7 h), while a longer $t_{1/2}$ of 2.2 h was observed after oral dosing. The apparently longer $t_{1/2}$ observed after oral administration in rats and dogs may have been the result of prolonged absorp-

TABLE 3. Excretion of trovafloxacin in urine after administration of a single oral dose of 10 mg/kg in dogs and monkeys

Species	Time (h)	Excretion of trovafloxacin after the following treatments[a]:					
		A		B		C	
		Level (μg/ml)	Recovery (% of dose)	Level (μg/ml)	Recovery (% of dose)	Level (μg/ml)	Recovery (% of dose)
Dogs	0–4	23.0 ± 22.9	1.7 ± 1.4	27.8 ± 28.1	2.0 ± 1.8	28.0 ± 26.0	2.1 ± 1.6
	4–8	6.3 ± 2.1	0.3 ± 0.1	7.7 ± 2.7	0.4 ± 0.1	7.6 ± 2.1	0.3 ± 0.1
	8–24	3.1 ± 1.8	0.2 ± 0.1	3.2 ± 1.8	0.2 ± 0.1	3.4 ± 1.7	0.2 ± 0.1
	24–48	0.3 ± 0.1	0.0 ± 0.0	0.4 ± 0.1	0.1 ± 0.0	0.4 ± 0.1	0.1 ± 0.0
	0–48		2.3 ± 1.4		2.7 ± 1.8		2.7 ± 1.6
Monkeys	0–4	10.3 ± 6.2	0.4 ± 0.1	45.3 ± 27.6	1.8 ± 0.4	37.6 ± 26.4	1.5 ± 0.5
	4–8	8.2 ± 7.2	0.3 ± 0.2	28.6 ± 26.7	1.1 ± 0.7	27.0 ± 25.6	1.0 ± 0.7
	8–24	3.3 ± 2.3	1.2 ± 0.8	4.8 ± 3.4	1.7 ± 1.0	4.2 ± 3.0	1.5 ± 0.9
	24–48	0.4 ± 0.3	0.2 ± 0.1	0.5 ± 0.4	0.2 ± 0.1	0.4 ± 0.3	0.2 ± 0.1
	0–48		2.1 ± 0.8		4.9 ± 1.8		4.3 ± 1.9

[a] Values represent means ± standard deviations for four animals. A, normal assay; B, assay after alkaline incubation; C, assay after Glusulase incubation.

tion, probably because of the low aqueous solubility of trovafloxacin (3). In monkeys following intravenous administration, trovafloxacin concentrations declined biexponentially. The additional dispositional phase emerged after 8 to 12 h postdosing, and this phase had an elimination $t_{1/2}$ (7.0 h) that was similar to that after oral administration (7.5 h). The long $t_{1/2}$ in monkeys suggested that trovafloxacin may have a long $t_{1/2}$ in humans as well. This was confirmed in clinical trials in which the $t_{1/2}$ estimate in healthy volunteers was approximately 10 h, which may allow for once-daily dosing (14, 15).

Serum protein binding of trovafloxacin in all three species examined was moderate to high and independent of the concentration in the concentration range studied, averaging from 65 to 96%. The serum protein binding of trovafloxacin in humans was previously determined to be 70% (15). In contrast, the level of serum protein binding of other fluoroquinolones reported in the literature ranges from 15 to 40% (6). Trovafloxacin was also found to be distributed nearly evenly between whole blood and plasma in all three species. This indicated that trovafloxacin is unlikely to distribute preferentially into erythrocytes in these species. The volumes of distribution of trovafloxacin were greater than their total body water in rats (0.9 liters/kg), dogs (1.7 liters/kg), and monkeys (4.3 liters/kg), suggesting that trovafloxacin penetrates extensively into tissues. This was confirmed in dogs by the findings of good penetration into various tissues except brain, cerebrospinal fluid, and fat. Good tissue penetration plus its improved potency against many clinically relevant microorganisms suggests that trovafloxacin may be useful in the treatment of various systemic infections.

Following the administration of a 50-mg/kg oral dose of [^{14}C]trovafloxacin to rats, the profiles of radioactivity in serum were essentially superimposed on the corresponding profiles of the concentration of trovafloxacin in serum, suggesting that there were no or few circulating metabolites in serum samples. In the bile duct-cannulated rats, 20.6, 3.4, and 67.1% of the administered radioactivity were recovered in feces, urine, and bile, respectively, indicating that the elimination of trovafloxacin is primarily through biliary excretion, unlike most other fluoroquinolone antimicrobial agents, for which renal elimination is a major route (6).

In dogs, treatment of urine with alkali or Glusulase produced only minor increases in the apparent concentrations of trovafloxacin, suggesting that glucuronide conjugates play a minor role in the urinary excretion of this compound in dogs. In contrast, treatment of monkey urine with alkali or Glusulase resulted in three- to eightfold increases in apparent trovafloxacin concentrations, suggesting that glucuronide conjugation may play a significant role in the urinary excretion of this compound in monkeys. However, urinary excretion of both unchanged drug and glucuronide conjugates is a minor pathway for elimination of trovafloxacin in both dogs and monkeys. Conjugation of trovafloxacin, presumably β-glucuronidation, accounted for a slightly greater portion of drug excretion in urine from monkeys than in urine from dogs. Higher drug concentrations in alkali-treated urine than in Glusulase-incubated urine in monkeys suggest a possible intramolecular transacylation involving migration of the aglycone from position 1 to positions 2, 3, and 4 of glucuronic acid. These isomers are susceptible to alkali-treated hydrolysis but not to β-glucuronidase (5, 12). Although the overall urinary excretion of unchanged trovafloxacin was less than 3% of the administered dose in both dogs and monkeys, the concentrations of trovafloxacin in urine remained at or above the MICs for most clinically relevant pathogens for 24 h after administration of

TABLE 4. Serum protein binding and blood/plasma distribution ratios of trovafloxacin in rats, dogs, and monkeys

Species	Nominal concn (μg/ml)	% Bound fraction	Blood/plasma (mean ± SD)
Rats	0.5		0.98 ± 0.26
	1.0	89	
	5.0	96	0.81 ± 0.08
	20.0		0.74 ± 0.12
Dogs	0.5		0.85 ± 0.04
	1.0	75	
	5.0	75	0.96 ± 0.10
	20.0		1.00 ± 0.05
Monkeys	0.5		1.02 ± 0.14
	1.0	65	
	5.0	67	0.94 ± 0.05
	20.0		1.16 ± 0.03

the dose (10, 11). In contrast, in a parallel study the urinary excretion of ciprofloxacin in dogs and monkeys accounted for 12 and 18% of the administered dose, respectively (data not shown), while the urinary excretion of ciprofloxacin and other fluoroquinolones reported in the literature ranges from 30 to 80% (6). These data suggest that although trovafloxacin may have a disposition substantially different from those of other fluoroquinolones, it still may have sufficient concentrations in urine to inhibit most gram-negative bacteria responsible for urinary tract infections (10, 11). This contention was confirmed in phase I clinical trials in which the concentrations in urine following the administration of oral doses of 100 mg or above were well above the MICs for organisms that cause urinary tract infections for at least 24 h (14).

The pharmacokinetic properties of trovafloxacin, including the long $t_{1/2}$ in humans and good tissue distribution in rats, dogs, and monkeys, combined with its reported antibacterial spectrum, may allow for once-daily dosing in humans for most indications, including infections of the upper and lower respiratory tracts, skin or related soft tissue, and urinary tract. Clinical trials are in progress to confirm the expected clinical utility of trovafloxacin.

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