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Absorption, Distribution, Metabolic Fate, and Elimination of Pefloxacin Mesylate in Mice, Rats, Dogs, Monkeys, and Humans

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Pefloxacin mesylate is well absorbed by the oral route. The antimicrobial activity in dog, cynomolgus monkey, and human plasma was essentially due to unchanged drug which respectively accounted for 64, 94, and 84% of the total activity (ratios derived from relative area under the curve [AUC] values). Half-lives ranged from 1.9 h in mice to 8.6 h in humans. Protein binding was weak, about 20% in plasma. Except in brain, concentrations in most of the organs and tissues tested in rats and dogs were higher than the plasma levels. Microbiological activity in urine was mainly due to pefloxacin and norfloxacin, the *N*-desmethyl metabolite. The norfloxacin/pefloxacin ratios were 0 in mice, ca. 1 in rats and dogs, 1.6 in cynomolgus monkeys, and 2.3 in humans. The principal urinary compounds were unchanged drug in mice, pefloxacin glucuronide and pefloxacin *N*-oxide in rats and dogs, norfloxacin and pefloxacin in monkeys, and pefloxacin *N*-oxide and norfloxacin in humans. The urinary recovery of identified metabolites was 29.5% of the dose in mice, 37.8% in rats, 36.3% in dogs, 26.5% in monkeys, and 58.9% in humans. Biliary excretion occurred and was extensive in rats and dogs, mainly as a glucuronide conjugate of the drug. In rat and human bile, the main active compound was unchanged pefloxacin.

Pefloxacin [1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(4-methyl-1-piperazinyl)-quinoline-3-carboxylic acid (1589 RB)] has a high order of in vitro activity against gram-positive and gram-negative bacteria (2, 6, 7) and when administered as a single dose has significant activity against infections with *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Serratia marcescens* in mice (unpublished data). This paper concerns the absorption, distribution, metabolism, and elimination of pefloxacin dihydrate mesylate in mice, rats, dogs, cynomolgus monkeys, and humans.

(These results were presented in part at the 13th International Congress of Chemotherapy, Vienna, Austria, 1983.)

MATERIALS AND METHODS

Drug and reagents. Pefloxacin dihydrate mesylate (Fig. 1) and possible metabolites were synthesized in our laboratories; the structures are shown in Fig. 2. All reagents were of analytical grade. Doses and concentrations are expressed with reference to the anhydrous substance except for doses administered to rats and dogs, which are expressed in terms of the dihydrate (the degree of hydration of the salt was unknown when the early experiments were performed); hence, the quoted doses of 10, 25, and 50 mg/kg in these animals correspond to 9.2, 23.1, and 46.2 mg of anhydrous pefloxacin per kg.

In vivo experiments. Male Swiss mice (24 to 28 g), male Wistar rats (200 to 300 g), male and female beagle dogs (13 to 16 kg), and male *Macaca fascicularis* monkeys (3.2 to 4.4 kg) were treated after a 17-h fast. All animal experiments were carried out in our laboratory except for the monkey assays, which were performed at IFM Center (Evreux, France). Pefloxacin mesylate was dissolved in saline for intravenous administration. Oral or intraduodenal doses were given to mice, rats, and monkeys as aqueous solutions and to the dogs as capsules. Healthy human male volunteers (54 to 75 kg, 19 to 29 years old) were given 400 or 800 mg as capsules or tablets. Blood samples collected on heparin were obtained from rodents by orbicular puncture, and those from the other species were obtained by venipuncture. Plasma was separated by centrifugation. For the tissue distribution experiments in rats and dogs, organs were excised, weighed, placed in 10 to 20 times their weight of 0.1 M monobasic potassium phosphate buffer (pH 8), heated at 80°C in a water bath for 15 min, and homogenized (Polytron Kinematica, Lucerne, Switzerland). Urine was collected from 0 to 24 and 24 to 48 h after administration of the drug; an additional collection of 48- to 72-h urine was made in monkeys and humans. Biliary excretion was studied in rats anesthetized with ethyl carbamate (1 g/kg intraperitoneally). Bile was collected from a polyethylene catheter introduced by surgical procedure into the common bile duct. Collections made over the periods indicated after drug administration were pooled. Biliary excretion was also studied in one dog anesthetized with chloralose-ethyl carbamate (0.06 to 0.75 g/kg, intravenously). After ligature of the cystic duct, a polyethylene catheter was introduced into the common bile duct. Biliary excretion was studied in human patients with T-tubes in the common duct. Bile samples were collected 1, 2, 4, 8, 12, and 24 h after administration of the drug.

Pefloxacin assays. (i) Microbiological assay. The cup-diffusion method (1) was used with *Bacillus subtilis* ATCC 6633 as the reference bacterium and pH 8 medium. The assay was sensitive to 0.06 μg of pefloxacin per ml.

(ii) Fluorimetric assay. A sample of plasma, bile, or tissue homogenate at pH 7.4 (0.5 M phosphate buffer) was extracted with 10 ml of chloroform. The chloroform phase was separated and then reextracted with 4 ml of 1 N acetic acid. This extract containing pefloxacin was subjected to fluorimetry with a Jobin Yvon JY 3-D spectrofluorimeter (λ excitation = 331 nm, λ emission = 441 nm, uncorrected values). Calibration curves were prepared over the range 0.5 to 4 μg. Recovery was 85% and precision was 2 to 4%. This assay was used for the determination of drug tissue and plasma levels in rats and dogs and for biliary levels in rats. Comparison of the fluorimetric assay and a specific high-pressure liquid chromatography (HPLC) assay of pefloxacin (3) was

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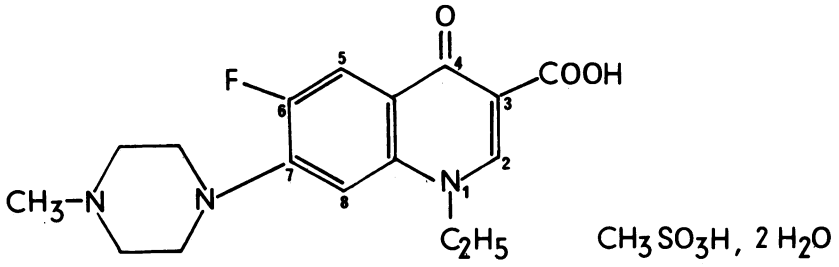

FIG. 1. Structure of pefloxacin mesylate.

made on samples of dog plasma (Fig. 3) and rat bile (see Table 7). Agreement between both methods was good; the specificity for tissue assays was not tested. The sensitivity was 0.1 μg of pefloxacin per ml of plasma or bile and 0.1 μg per g of tissue.

(iii) HPLC. Pefloxacin and metabolites were separated by ion-pair chromatography by using tetrabutylammonium iodide and a C18 silica column (Lichrosorb RP18; E. Merck AG, Darmstadt, Germany). The effluent was monitored at 270 nm. This assay has been described elsewhere (3) and was used to determine pefloxacin plasma levels in mice, dogs, monkeys, and humans. It was also used in the quantitative determination of pefloxacin and active metabolites in urine. In metabolism studies, XAD2 resin or chloroform extracts from urine samples and untreated bile samples were also analyzed by the same HPLC method, except that the acetonitrile concentration gradient was modified (initial concentration of acetonitrile in the eluent was 10%, increasing linearly to 13% at 5 min and then to 37% at 20 min).

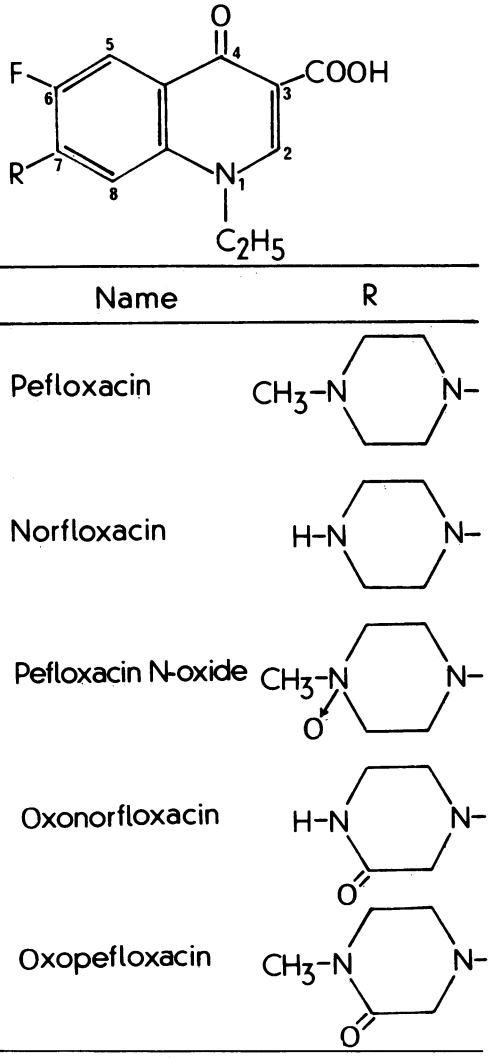

FIG. 2. Chemical structures of pefloxacin and synthesized metabolites.

Protein binding. Protein binding of pefloxacin was examined by equilibrium dialysis (Dianorm apparatus with 2-ml cells and Diachema cellulose membranes; molecular weight cutoff, 5,000). Membranes were washed with dialysis buffer (0.067 M sodium phosphate, 0.05 M NaCl buffer, pH 7.4) for 4 h before use. Pefloxacin base was dissolved in fresh rat, dog, or human plasma to achieve concentrations of 2.5, 5, 10, 15, and 20 μg of drug per ml, and 2-ml portions were dialyzed against 2 ml of buffer for 2 h at 37°C. The drug levels in the media were assayed by fluorimetry.

Metabolism. Metabolites were isolated from plasma, urine, and bile by thin-layer chromatography (TLC) or HPLC and compared with synthesized analogs, except for the conjugate of pefloxacin, whose nature was assessed by β-glucuronidase cleavage or chemical detection.

TLC. Samples of plasma, urine, and bile (pH 6 to 7) were concentrated with Amberlite XAD2 resin. The resin was washed with distilled water and eluted with 80% ethanol–20% water (vol/vol). The eluates were concentrated under reduced pressure, and appropriate amounts were applied to thin-layer plates of silica (Merck F 254) or cellulose (Camag, Muttenz, Switzerland) as described in Table 1, which also shows the R_f values of the reference compounds. The compounds separated by chromatography were revealed by examination of the chromatogram under UV light, fluorescence, spraying with Dragendorff reagent and 7 N

TABLE 1. R_f values of pefloxacin and main metabolites on TLC plates

Compound	R_f in TLC system:[a]			
	AI	AII	AIII	B
Pefloxacin	0.66	0.53	0.42	0.70
Oxopefloxacin			0.38	0.59
Norfloxacin	0.57	0.46	0.32	0.61
Oxonorfloxacin			0.30	0.48
Pefloxacin *N*-oxide	0.35	0.20	0.15	0.40

[a] TLC systems: AI, silica with 3:2 dioxane-ammonia; AII, silica with 4:1:2 acetonitrile-methlethylketone-ammonia; AIII, silica with 6:4:1 chloroform-methanol-ammonia; B, cellulose with 2:1:1:0.4 *n*-butanol-ethanol-water-ammonia. All ratios are by volume.

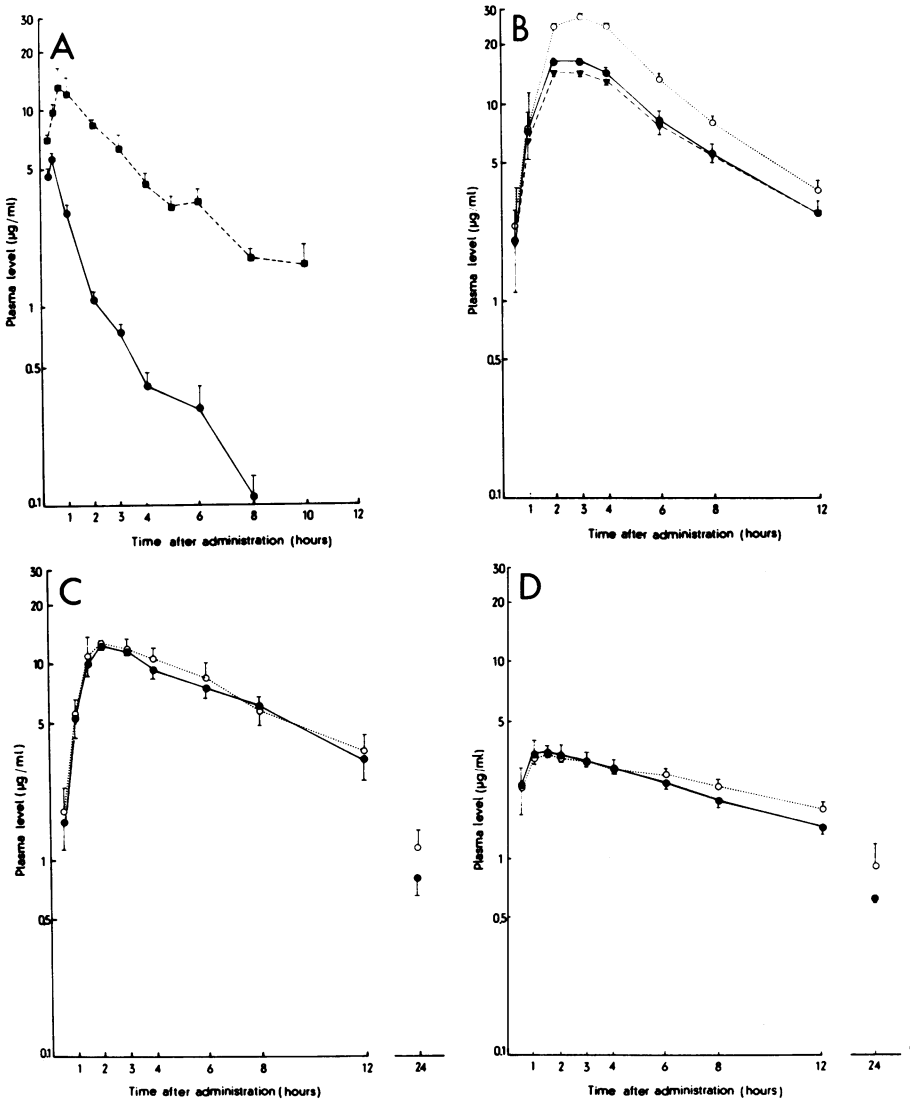

FIG. 3. Concentrations of pefloxacin in plasma of mice, rats, dogs, monkeys, and humans (mean ± standard error). (A) Plasma levels in rats (■) and mice (●). (B) Plasma levels in dogs as measured by biological assay (○), fluorimetric assay (▼), and HPLC assay (●). (C) Plasma levels in monkeys as measured by biological assay (○) and HPLC assay (●). (D) Plasma levels in humans as measured by biological assay (○) and HPLC assay (●). See Table 2 for dosages.

H_2SO_4, or by bioautography with *Escherichia coli* 015ISM as the reference strain and triphenyltetrazolium chloride as the stain. In all systems containing ammonia, pefloxacin glucuronide was converted to an unidentified compound of higher R_f value (presumably pefloxacin amide). Samples were also examined by TLC on silica plates after hydrolysis with β-glucuronidase with a *n*-butanol-acetic acid-water (2:1:1) or methanol-water (8:1) mixture (vol/vol) as the mobile phase and naphthoresorcinol-sulfuric acid spray as the detection reagent.

Quantitative determination of metabolites. Pefloxacin, norfloxacin, oxonorfloxacin, and oxopefloxacin were assayed in urine by the HPLC method described previously. Total pefloxacin in urine and bile was assayed by the same method after hydrolysis of the glucuronide conjugate (heating at 80°C for 1 h in 2.5 N NaOH). This method was faster than enzymatic hydrolysis and did not convert *N*-oxide pefloxacin to parent compound. The concentration of conjugate was taken to be the difference between the total and free pefloxacin levels. The *N*-oxide metabolite in urine was assayed by TLC. Samples of 2 ml of urine (pH 6 to 7) were passed through 2 g of Amberlite XAD2 resin. The column was washed with 20 ml of distilled water and eluted with 10 ml of ethanol-water (80:20 [vol/vol]). The solvent was evaporated and the residue was moistened with 0.5 ml of the above mixture to which ammonia was added to 5% (vol/vol). A 0.04-ml sample was applied to a plate coated with a 25-mm layer of silica gel 60 F 254 (Merck). The chromatogram was developed in dioxane-ammonia (3:2 [vol/vol]) and left in the tank for more than 1 h after the solvent front reached the top of the plate. The area corresponding to the *N*-oxide derivative was scraped off, and the compound was eluted with 4 ml of 1.7% ammonia. Absorption at 270 nm was determined, and the values were corrected against blank urine samples

treated in parallel. The working range was 5 to 40 μg (on plates). Precision reached 6% and recovery reached 90%.

Determinaton of MICs. MICs of pefloxacin and synthesized metabolites were determined by the agar dilution technique (8).

RESULTS

Plasma levels and protein binding. Plasma pefloxacin levels in mice, rats, dogs, monkeys, and humans given a single oral dose are shown in Fig. 3, and Table 2 displays the observed peak levels (C_{max}), the areas under the curve (AUC, calculated from zero to the last time of measurement by the trapezoidal rule) and the apparent elimination half-lives ($t_{1/2\beta}$, calculated by linear regression analysis on the elimination phase). In mice and rats receiving a dose of 50 mg/kg, the mean plasma levels of pefloxacin reached peaks of 5.8 and 13.0 μg/ml, respectively. The respective AUCs were 8.8 and 56 μg · h/ml. In dogs given a 50-mg/kg dose of drug orally, the peak pefloxacin level was 14.9 μg/ml as measured by fluorimetry, 17 μg/ml as measured by HPLC, and 27.5 μg/ml as measured by bioassay. The ratio between the AUC values from HPLC (100.6 μg · h/ml) and bioassay (156.4 μg · h/ml) was 0.64 ± 0.01 (mean ± standard error). In monkeys and humans, activity in plasma was due largely to unchanged drug. The peak levels were 12.4 μg/ml (HPLC assay) and 13.8 μg/ml (bioassay) in monkeys given 25 mg/kg and 3.84 and 3.77 μg/ml, respectively, in humans given 0.4 g of drug. The respective AUCs of pefloxacin were 108 and 48 μg · h/ml in monkeys and humans. The AUC ratio (HPLC to bioassay) was 0.94 ± 0.05 in monkeys and 0.84 ± 0.06 in humans.

Protein binding of pefloxacin was low and virtually independent of drug concentration in the range studied. The values were 20.3 ± 1.8% in rat plasma and 19.1 ± 1.1% in dog plasma (mean of five experiments for concentrations of 2.5, 5, 10, 15, and 20 μg of drug per ml). The value for human plasma from two subjects varied between 20 and 30% (for concentrations between 2 and 15 μg/ml).

Tissue distribution. The concentrations of pefloxacin attained in the organs, tissues, and body fluids of rats and dogs are shown in Table 3. After a single dose of 100 mg/kg, tissue levels in rats were two to three times greater than in plasma except for in the brain, in which concentrations were one-fifth or less than plasma levels, and in the liver, kidney, and spleen, where concentrations were three to six times greater. The tissue/plasma ratios were similar after 15 repeated doses. In dogs, after daily dosing with 100 mg/kg for 6 weeks, tissue levels were generally three to five times greater than the plasma level except in the cerebrospinal fluid and brain, where they were two to three times less, and in the liver, where they were 8 to 10 times greater. The high concentration of conjugated drug in bile reflects the important part played by the liver in the metabolism of pefloxacin.

Metabolism. (i) Principal metabolites in rat, dog, monkey, and human plasma. The major metabolites in rat and dog plasma were pefloxacin glucuronide, pefloxacin *N*-oxide, and norfloxacin. The last two were also found in monkey and human plasma, but no conjugate was detected. Figure 4 shows a typical chromatogram obtained from 0- to 8-h pooled plasma in these species.

(ii) Principal metabolites in urine and bile. Urine and bile of mice contained unchanged drug and two identified metabolites, the glucuronide and *N*-oxide. Urine also contained three unidentified metabolites. In rat and dog urine and bile, the principal metabolites were the glucuronide, the *N*-oxide, and norfloxacin. Substantial amounts of unchanged drug were also present. Typical HPLC chromatograms of an XAD2 extract of rat urine and of dog bile are shown in Fig. 5. The last peak eluted in chromatogram A was doubtless pefloxacin glucuronide since it gave free pefloxacin after β-glucuronidase or alkaline hydrolysis (chromatogram D). Monkey urine contained norfloxacin and pefloxacin and lesser amounts of pefloxacin *N*-oxide, oxopefloxacin, and oxonorfloxacin plus traces of pefloxacin glucuronide. Human urine contained pefloxacin *N*-oxide and norfloxacin plus appreciable amounts of parent compound and oxonorfloxacin together with traces of pefloxacin glucuronide. An HPLC chromatogram of an XAD2 extract of human urine is shown in Fig. 5. Biliary excretion in monkeys and humans was less extensive than in rats and dogs. The most common metabolites were the parent compound, pefloxacin glucuronide, pefloxacin *N*-oxide, and norfloxacin.

(iii) Antibacterial activity of the metabolites. The activities of pefloxacin and the identified metabolites were determined (see Table 4). Norfloxacin was found to be as active in vitro as pefloxacin. The *N*-oxide and glucuronide analogs of pefloxacin showed little activity.

Urinary excretion of pefloxacin and principal metabolites. Urinary HPLC data on pefloxacin and norfloxacin concentrations and the results of the microbiological assay in urine are shown in Table 5. Except in mice, which do not produce the metabolite and have the highest recovery of unchanged drug (16.4%), the antimicrobial activity in urine was essentially due to pefloxacin and norfloxacin. Recoveries of both compounds were similar in rats and dogs (6.9 and 5.9%, respectively, in rats and 4.7% for both compounds in dogs). Norfloxacin predominated in monkeys and humans, the values being 9.3% norfloxacin and 5.9% pefloxacin in the former and 20.2% norfloxacin and 8.7% pefloxacin in the latter. In humans, the two compounds accounted for 29% of the administered dose, this being the highest recovery in any subject. Urinary recovery of pefloxacin and its principal metabolites are shown in Table 6. Total recovery of parent drug and identified metabolites was the highest in humans (about 59% of the dose).

TABLE 2. Pharmacokinetic parameters for pefloxacin

Species and assay	Parameter (mean ± SD)[a]		
	C_{max} (μg/ml)	AUC (μg · h/ml)	$t_{1/2}$ (h)
Rat	13.0 ± 3.4	56	3.3
Mouse	5.8 ± 0.3	8.8	1.9
Dog			
Biological	27.5 ± 1.0	156.4 ± 7.2	3.21 ± 0.14
Fluorimetric	14.9 ± 0.3	91.7 ± 31	4.18 ± 0.11
HPLC	17.1 ± 0.6	100.6 ± 6.2	3.76 ± 0.17
Monkey			
Biological	13.8 ± 0.6	116 ± 16	7.11 ± 0.51
HPLC	12.4 ± 0.3	108 ± 11	5.56 ± 0.25
Human			
Biological	3.77 ± 0.34	46.0 ± 4.1	10.1 ± 0.72
HPLC	3.84 ± 0.33	48.2 ± 4.2	8.6 ± 0.65

[a] The dosages used were: rat (n = 3), 50 mg/kg; mouse (n = 5), 50 mg/kg; dog (n = 4), 50 mg/kg; monkey (n = 3), 25 mg/kg; human (n = 6), 0.4 g per person.

TABLE. 3. Pefloxacin concentrations in organs, tissues, and body fluids of rats and dogs after oral dosing at 100 mg/kg[a]

Specimen	Rat[b]								Pefloxacin concn in dog at 6 wk[c]	
	0.5-h[d] pefloxacin concn after:				3-h[d] pefloxacin concn after:					
	1 dose		15 doses[e]		1 dose		15 doses[e]			
	Fluorimetric assay	Biological assay	Fluorimetric assay	Biological assay	Fluorimetric assay	Biological assay	Fluorimetric assay	Biological assay	Fluorimetric assay	Biological assay
Plasma	29.2 ± 2.3	31.8 ± 2.2	29.6 ± 2.2	38.5 ± 2	9.9 ± 0.5	14.2 ± 0.7	9.1 ± 0.7	10.3 ± 0.7	19.7 (17–22)	20.7 (19–24)
Brain	6.4 ± 0.52	5.6 ± 0.38	3.44 ± 0.54	2.51 ± 0.51	1.92 ± 0.17		1.96 ± 0.09		7.1 (6.1–7.9)	5.7 (5–6.5)
Liver	186 ± 28	168 ± 28	149 ± 16	151 ± 16	43.8 ± 3.1	51.6 ± 4.0	49.6 ± 2.4	40.8 ± 2	194 (174–235)	195 (157–235)
Kidney	135 ± 7	127 ± 11	95.0 ± 5.4	90.4 ± 6.5	39.8 ± 3.4	47.2 ± 6.9	41.8 ± 2.6	41.6 ± 3.7	94 (90–98)	95 (87–104)
Heart	72.4 ± 3.1	79.1 ± 4.1	67.4 ± 5.2	65.6 ± 4.5	24.3 ± 1.7	32.0 ± 2.4	24.8 ± 1.2	22.8 ± 1.2	78 (66–92)	73 (61–84)
Lung	61.8 ± 2.7	61.7 ± 3.1	48.0 ± 4.8	52.4 ± 2.8	18 ± 1.2	22.2 ± 2.1	23.8 ± 1.5	17.5 ± 1.3	55 (45–62)	55 (42–64)
Spleen	98.8 ± 26.6	114 ± 25	107 ± 21	105 ± 17	28.6 ± 3.4	37.0 ± 5.8	35.8 ± 2	32.4 ± 2	93 (72–112)	105 (77–142)
Thymus	60.6 ± 4.4	72.2 ± 5.7	46.6 ± 4.2	47.6 ± 5.5	24.5 ± 1.9	20.2 ± 2	25.4 ± 1.2			
Muscle	70.8 ± 3.8	78.7 ± 9.9	56.6 ± 7.4	56.4 ± 5.3	29.6 ± 3.4	34.6 ± 3	26.8 ± 1.0	28.0 ± 1.5	66 (58–82)	69 (66–71)
Testis	11.2 ± 1.46	12.8 ± 2.18	12.2 ± 1.8	16 ± 2.9	7.2 ± 0.6	13.6 ± 1	8.1 ± 0.5	11.8 ± 0.7	58[f]	61[f]
Spinal fluid									8.8 (7.5–10)	
Pancreas									94 (79–116)	98 (75–143)
Prostate									68[f]	73[f]
Bile in gall bladder										
Before hydrolysis										694 (525–840)
After hydrolysis										8,343 (5,300–12,480)

[a] Concentrations are given in micrograms per gram (organs and tissues) or per milliliter (fluids).
[b] Mean of five male rats ± standard error.
[c] Mean of one male and two female dogs. The range is given in parentheses.
[d] Time of sacrifice after the last dose.
[e] Sacrificed on day 6.
[f] The male dog only.

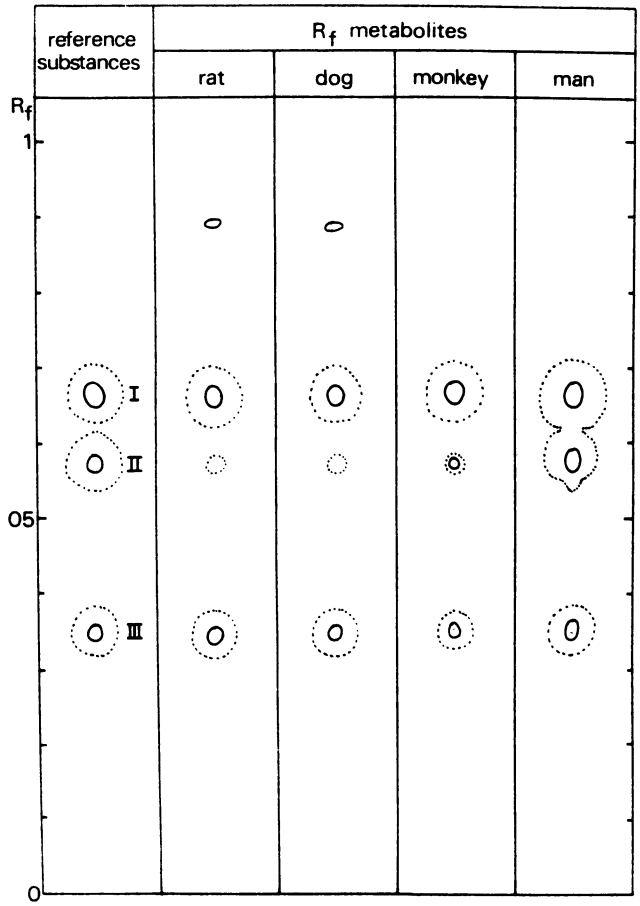

FIG. 4. Thin-layer chromatogram of plasma of rats, dogs, monkeys, and humans receiving pefloxacin. System AI was used (see Table 1). Fluorescence observation (——) and bioautography (....) borders are shown. References: I, pefloxacin; II, norfloxacin; III, pefloxacine *N*-oxide.

Biliary excretion of pefloxacin. The microbiological activity and pefloxacin content of bile were determined before and after alkaline hydrolysis in rats given 10 mg of drug per kg by the intravenous or intraduodenal route (Table 7). Most of the antimicrobial activity detected was due to pefloxacin. The similar concentrations obtained by bioassay and fluorimetric assay after hydrolysis (intraduodenal dosing) suggested that glucuronide conjugate of pefloxacin might be slightly active, since free activity determined by bioassay was higher than free pefloxacin levels. The concentration of pefloxacin glucuronide was five to eight times greater than that of free drug. Absorption of pefloxacin mesylate was extensive since biliary elimination over 0 to 8 h was similar with both dose routes (Fig. 6). The values for free and total (free plus conjugated) pefloxacin were, respectively, 3.94 ± 0.18% and 34.54 ± 2.52% after intravenous dosing and 5.43 ± 0.85% and 34.58 ± 5.79% after intraduodenal dosing. Bile was also an important route of elimination in dogs. In one animal given 5 mg/kg intravenously, levels of free pefloxacin reached 102 to 259 μg/ml (HPLC assay) or 117 to 344 μg/ml (microbiological data) from 0.5 to 10 h after dosing. Total recovery of free drug during the first 12 h accounted for 5.3% of the administered dose and recovery of the glucuronide accounted for 15.1% (Fig. 6). Figure 6 shows the microbiological activity and pefloxacin levels found in humans after a 0.8-g dose. Microbiologically active levels between 5 and 30 μg/ml were found between 2 and 24 h after dosing, the activity mainly being due to the parent compound (AUC

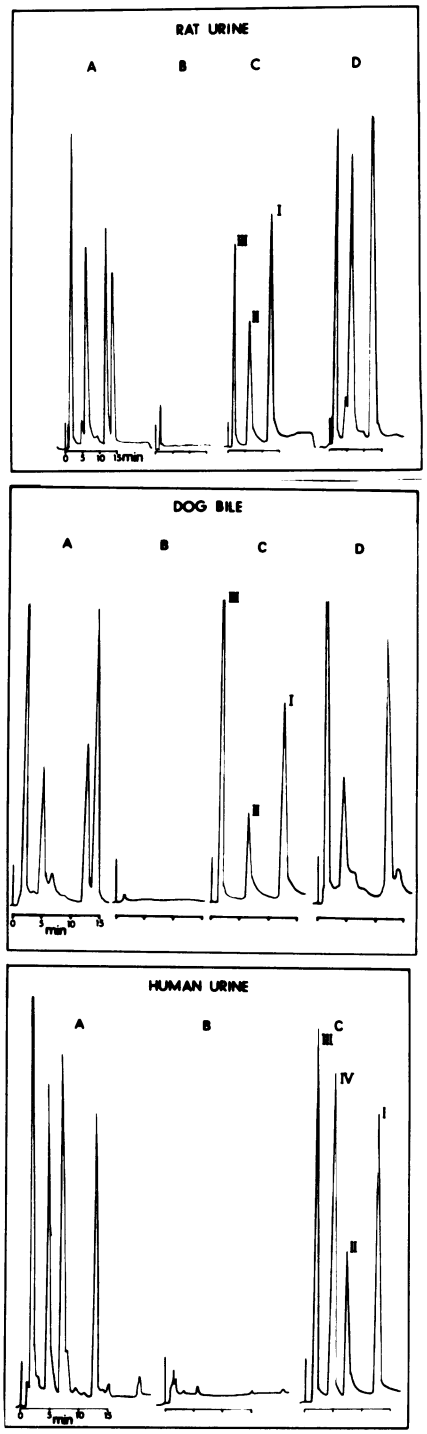

FIG. 5. HPLC chromatograms obtained from rat urine, dog bile, and human urine. Oral doses were 100 mg/kg in rats and dogs and 0.8 g in humans. Abbreviations: A, XAD2 extract from urine or untreated bile; B, blank urine or blank bile; C, reference compounds (I, pefloxacin; II, norfloxacin; III, pefloxacin *N*-oxide; IV, oxonorfloxacin); D, same as A after alkaline hydrolysis.

TABLE 4. In vitro antibacterial activity of pefloxacin and main metabolites

Compound	MIC (μg/ml) for:							
	Staphylococcus sp. 209P	*Streptococcus* sp. DM19	*Bacillus subtilis* ATCC 6633	*Pseudomonas aeruginosa* A22	*Escherichia coli* 54127 CIPH	*Salmonella enteritis* Danysz	*Serratia* sp. Bou	*Proteus mirabilis*
Pefloxacin	0.125	1	0.125	1	0.125	0.25	1	0.25
Norfloxacin	1	1	0.125	0.5	1	0.50	2	0.125
Oxopefloxacin	0.125	4	1	4	1	0.25	16	2
Oxonorfloxacin	1	4	1	16	4	0.8	50	4
Pefloxacin *N*-oxide	128	>128	32	>128	64	64	>128	

TABLE 5. Urinary elimination of pefloxacin and norfloxacin as measured by microbiological and HPLC assays

Species	No.	Dose (mg/kg)	Time (h)	Mean concn (μg/ml)[a]			Mean recovery (% of dose) ± SE	
				Microbiological assay[b]	HPLC assay			
					Pefloxacin	Norfloxacin	Pefloxacin	Norfloxacin
Mouse	3[c]	50	0–24	127 (92–157)	99 (90–115)	ND[d]	14.6 ± 1.2	
			24–48	28 (11–44)	25 (9–42)	ND	1.83 ± 0.45	
Rat	3	10	0–24	39 (36–45)	18 (15–19)	14 (12–16)	7.45 ± 1.18	5.88 ± 0.72
	3	25	0–24	75 (60–102)	38 (31–46)	39 (29–50)	6.18 ± 0.68	6.37 ± 0.91
	3	50	0–24	246 (221–275)	115 (110–125)	91 (65–112)	7.09 ± 0.47	5.47 ± 0.48
Dog	4	10	0–24	17 (10–20)	9 (6.8–11.7)	9 (7.2–11.4)	4.45 ± 0.56	4.45 ± 0.26
	4	25	0–24	56 (35–74)	32 (17–41)	39 (32–45)[e]	4.85 ± 0.70	5 (4.8–5.2)[e]
Monkey	3	25	0–24	100 (74–222)	42 (26–68)	61 (29–100)	5.04 ± 0.37	7.30 ± 0.44
			24–48	31 (19–48)	10 (8.5–13)	22 (14–34)	0.90 ± 0.12	1.95 ± 0.20
			48–72	4.1 (3.6–4.7)				
Human	5	0.8[f]	0–24	111 (53–149)	42 (22–53)	80 (40–120)	5.2 ± 0.96	11.08 ± 1.26
			24–48	85 (50–151)	23 (12–37)	66 (37–119)	2.86 ± 0.61	7.98 ± 1.54
			48–72	12 (3.7–27)	3.6 (1.2–6)		0.60 ± 0.15	1.16 ± 0.32[g]

[a] The range of values is shown in parentheses.
[b] Expressed as microgram equivalents of pefloxacin.
[c] Three groups of 10 mice.
[d] ND, Not detected.
[e] $n = 2$.
[f] Dose given was 0.8 g per person.
[g] Calculated as microbiological recovery − pefloxacin recovery.

TABLE 6. Urinary recovery of pefloxacin and main metabolites after oral administration[a]

Compound	Mean recovery (% of dose) ± SE				
	Mouse (50-mg/kg dose, $n = 3 \times 10$)	Rat (25-mg/kg dose, $n = 3$)	Dog (10-mg/kg dose, $n = 4$)	Monkey (25-mg/kg dose, $n = 3$)	Human (0.8 g per person, $n = 5$)
Pefloxacin	16.5 ± 1	6.6 ± 0.8	4.9 ± 0.6	6.2 ± 0.5	9.3 ± 1
Norfloxacin	ND[b]	6.4 ± 0.9	4.5 ± 0.3	9.8 ± 0.8	20.2 ± 1
Pefloxacin glucuronide	8.8 ± 1.2	14.7 ± 0.9	14.9 ± 1.4	2.5 ± 1.4	tr
Pefloxacin *N*-oxide	4.3 ± 0.6	10.1 ± 0.8	12.3 ± 0.8	4.8 ± 0.5	23.2 ± 1.6
Oxonorfloxacin	ND	tr[c]	tr[c]	2.2 ± 0.5	5.4 ± 0.5
Oxopefloxacin	tr	tr[c]	tr[c]	0.92 ± 0.16	0.75 ± 0.05
Total	29.5 ± 1.9	37.8	36.3 ± 1.9	26.5 ± 3.1	58.9 ± 3.1

[a] The 0 to 48 h urine was used for mice, rats, and dogs, and the 0 to 72 h urine was used for monkeys and humans. The recovery percentages (± standard error) as determined by microbiological assay were as follows: mouse, 20.4 ± 0.9; rat, 12.0 ± 1.3; dog, 8.7 ± 1.3; monkey, 14.6 ± 0.2; human, 27.2 ± 1.8.

[b] ND, Not detected.

[c] Oxonorfloxacin and oxopefloxacin were not assayed by HPLC in rats and dogs but were estimated by TLC.

TABLE 7. Biliary excretion of pefloxacin in rats

Bile sample (h)	Mean pefloxacin concn (μg/ml) in bile ± SE ($n = 4$)									
	10-mg/kg intravenous dose						10-mg/kg intraduodenal dose			
	Microbiological assay		Fluorimetry		HPLC		Microbiological assay		Fluorimetry	
	Free	Conjugated[a]	Free	Conjugated[a]	Free	Conjugated[a]	Free	Conjugated[a]	Free	Conjugated[a]
0–2	37 ± 4	202 ± 19	25 ± 2	195 ± 8	21 ± 2	188 ± 13	62 ± 12	218 ± 47	43 ± 7	213 ± 48
2–4	34 ± 6	160 ± 23	19.5 ± 2	144 ± 24	21 ± 3	148 ± 14	40 ± 7	136 ± 25	28 ± 4	145 ± 26
4–6	19.1 ± 2.8	80 ± 10	9.8 ± 0.8	74 ± 9	10.2 ± 1.5	79 ± 8	16.7 ± 2.7	62 ± 9	10.1 ± 1.6	68 ± 9
6–8	9.7 ± 2.4	43 ± 8	3.8 ± 0.5	41 ± 6			14.0 ± 1.9	50 ± 4	7 ± 1	57 ± 6

[a] Total level after hydrolysis − free level.

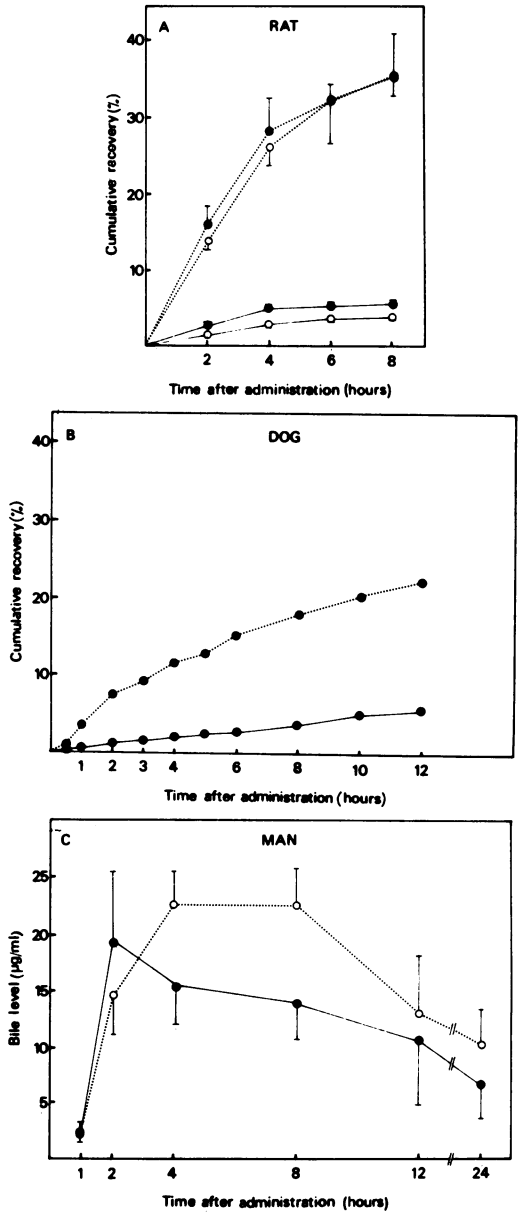

FIG. 6. Recovery of free (——) and total (....) pefloxacin in rat and dog bile and active bile levels in humans as determined by HPLC (——) and bioassay (....). Single administrations were intravenous (○) and intraduodenal (●) 10-mg/kg doses for four rats, an intravenous 5-mg/kg dose for one dog, and a 0.8-g oral dose for three humans. Each point represents a mean ± standard error.

ratio, 0.70). Biliary pefloxacin levels between 2 and 12 h after dosing were two to five times greater than the plasma levels (data not shown). The conjugated/free pefloxacin ratio was only 0.9 (range, 0.2 to 2.8).

DISCUSSION

Norfloxacin (AM 715), enoxacin (AT-2266), and pefloxacin are closely related fluoropyridonecarboxylic acids for which there is ample evidence of potent and equivalent in vitro antibacterial activity (2, 5, 7). Enoxacin seems to have better oral availability, and the tissue levels obtained are greater than those obtained with norfloxacin (4). The results of the present study indicate extensive pefloxacin mesylate absorption after oral administration; antibacterial activity in plasma is largely due to unchanged drug. The AUCs of pefloxacin in plasma of mice and dogs (8.8 and 100.6 µg · h/ml at a dose of 50 mg/kg) are in the same range as those for enoxacin (7.45 and 52.2 µg · h/ml at doses of 50 and 25 mg/kg) (4). The AUC of pefloxacin in rat plasma (56 µg · h/ml at 50 mg/kg) and AUC of active compounds in dog plasma (156 µg · h/ml at 50 mg/kg) appear to be greater than those of enoxacin (7.08 µg · h/ml in rats given 50 mg/kg, 52.2 µg · h/ml in dogs given 25 mg/kg). Pefloxacin exhibits little protein binding and is widely distributed, even penetrating into dog cerebrospinal fluid. After a single oral dose of 50 mg/kg to rats, mean peak levels of enoxacin in plasma, lung, muscle, and kidney are 2.47, 4.60, 5.35, and 33.9 µg/ml or g (4), whereas those of pefloxacin after a twofold dose are 31.8, 71.7, 78.7, and 127 µg/ml or g. After repeated dosages in dogs, the concentrations of pefloxacin in heart, lung, liver, kidney, spleen, and muscle are from 2.7 to 9.4 times the plasma levels, whereas those of enoxacin are from 1.5 to 5 times the plasma levels. Pefloxacin is excreted in urine and bile mainly as metabolites, except in mice where the parent compound predominates. In urine, the *N*-desmethyl metabolite (norfloxacin) and unchanged drug are the main compounds responsible for the activity encountered in all species tested except mouse. The antibacterial activity in urine of mice, rats, and dogs to which pefloxacin mesylate was given orally represented recoveries of 20.4, 12, and 8.7% of the dose, values which are 1.6 to 4.6 times higher than those obtained with norfloxacin and 3.4 to 7.4 times lower than those reached with enoxacin (4). Total recoveries of pefloxacin and metabolites from rat and dog urine (38 and 36%) closely agree with recent experiments performed with ^{14}C-labeled drug (unpublished data): radioactivity recoveries of 34% in rats and 38% in dogs were obtained in urine after an oral dose of 0.5 mg/kg, whereas fecal recoveries were 60% in rats and 51% in dogs. Biliary excretion is an important way of elimination since recoveries of 35 and 20% as free plus conjugated pefloxacin are achieved in rat and dog bile. In humans, urinary recovery of drug and metabolites (59% of the dose) indicates that urinary elimination is superior to biliary excretion. These overall pharmacokinetic properties of pefloxacin mesylate are what makes it different from norfloxacin and enoxacin. Recently, the wide distribution of the drug was demonstrated in human tissues. This explains why pefloxacin is not only successful in urinary tract infections but also in respiratory infections, mediastinitis, meningitis, and endocarditis (results presented by B. Pangon, C. Morel, Y. Benard, and M. Wolff at the 13th International Congress of Chemotherapy, Vienna, Austria, 1983). Clinical studies of pefloxacin activity in treatment of systemic and local infections are under way.

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