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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF 6,8-DIFLUORO-1-(2-FLUOROETHYL)-1,4-DIHYDRO-7-(4-METHYL-1-PIPERAZINYL)-4-OXO-3-QUINOLINECARBOXYLIC ACID AND ITS METABOLITES IN LABORATORY ANIMALS

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SUMMARY

A simple, sensitive and specific high-performance liquid chromatographic method for a new quinolone antimicrobial agent, 6,8-difluoro-1-(2-fluoroethyl)-1,4-dihydro-7-(4-methyl-1-piperazinyl)-4-oxo-3-quinolinecarboxylic acid (AM-833, I), and its metabolites in serum and urine has been developed for their simultaneous determination. This method is based on ion-pair extraction and separation by ion-pair reversed-phase chromatography with ultraviolet or fluorescence detection. The major metabolites in the serum and urine of mice, rats, dogs and monkeys were N-desmethyl I (compound II) and I N-oxide (compound III). Rabbit serum and urine contained N-desmethyl-3-oxo I (compound IV), 3-oxo I (compound V) and N-desmethyl-4-formyl I (compound VI) in addition to compounds I, II and III. Unchanged drug accounted for 80—90% of total serum concentrations in mice and more than 90% in rats, dogs and monkeys up to 6 h after dosing, whereas the fraction of compound I in rabbits was 34—67%. Unchanged drug was the most predominant in the urine of mice, rats, dogs and monkeys, whereas compound II was the most abundant in rabbit urine. Although rabbits and monkeys excreted 70—80% of dose in three-day urine, the total urinary excretion of mice, rats and dogs was relatively low, 40—50% of oral dose. The fraction of compound I in total urinary excretion was 63, 73, 27, 55 and 78% in mice, rats, rabbits, dogs and monkeys, respectively. These results suggest that there is a species difference in the metabolism and excretion pathway of compound I.

INTRODUCTION

6,8-Difluoro-1-(2-fluoroethyl)-1,4-dihydro-7-(4-methyl-1-piperazinyl)-4-oxo-3-quinolinecarboxylic acid (AM-833) is a new quinolonecarboxylic acid derivative (I, Fig. 1). It has potent antibacterial activity against various pathogens

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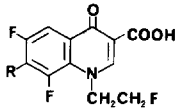


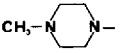
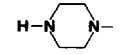
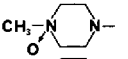
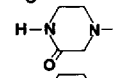
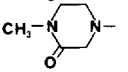
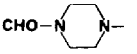
	Compound	R
I	AM-833	
II	N-Desmethyl AM-833	
III	AM-833 N-oxide	
IV	N-Desmethyl-3-oxo AM-833	
V	3-Oxo AM-833	
VI	N-Desmethyl-4-formyl AM-833	

Fig. 1. Chemical structures of compound I (AM-833) and its metabolites (II—VI).

covering Gram-positive and Gram-negative bacteria, and also a great oral efficacy against experimental infections in mice and rats [1].

Animal pharmacokinetic studies [2] disclosed that compound I was orally absorbed completely, well distributed to various tissues and excreted from the body without accumulation. However, a determination of urinary and biliary recovery of compound I could not explain good absorption of the drug. The identification study of urinary metabolites demonstrated that N-desmethyl I (compound II), I N-oxide (compound III), N-desmethyl-3-oxo I (compound IV) and N-desmethyl-4-formyl I (compound VI) are the major metabolites [3]. The present work was undertaken to develop a specific and sensitive analytical procedure to separate the metabolites in biological specimens using high-performance liquid chromatography (HPLC), and to investigate the metabolic fate of compound I in mice, rats, rabbits, dogs and cynomolgus monkeys.

EXPERIMENTAL

Chemicals

Compound I, authentic metabolites and internal standards were synthesized in our laboratory. Methanol was of HPLC grade (Kanto Chemical) and other reagents were of analytical grade. The reagents were used without further purification. Tetra-*n*-butylammonium hydrogen sulphate and sodium dodecyl sulphate were purchased from Tokyo Kasei Kogyo.

Preparation of standard solutions

For calibration of the chromatographic system, compound I and authentic metabolites were dissolved in 0.002 *M* sodium hydroxide solution as stock solution, and the aliquots were added to either 0.5 ml of serum or 0.5 ml of urine. Duplicate standards were prepared at seven concentrations up to 10

μg/ml in serum, and at eight concentrations up to 200 μg/ml in urine. Stock solutions were freshly prepared just before analysis.

Extraction procedures

Aliquots of serum sample (0.5 ml) were mixed with 100 μl of internal standard solution, 0.5 ml of 1 *M* acetic acid and 0.5 ml of 25 m*M* sodium dodecyl sulphate solution and extracted with 7 ml of a chloroform—isopropanol (7:3) mixture. The organic layer (5 ml) was separated and evaporated. The residue was dissolved in 0.1 ml of a mixture of acetonitrile—0.04 *M* phosphoric acid (1:1) and a portion of the resulting solution (10 μl) was subjected to HPLC. The 25 m*M* sodium dodecyl sulphate solution was replaced by a 10 m*M* solution for mouse and rat serum (0.1 ml). Urine samples (0.5 ml) were treated with the same procedure as serum samples, except that the ion-pairing reagent concentration was reduced to 5 m*M*, and the residue after evaporation was dissolved in 0.2 ml of the mixture described above. Internal standard solution were made by dissolving pipemidic acid (compound VII), for biological samples of rabbits and dogs, or 6,8-difluoro-1-ethyl-1,4-dihydro-7-(4-dimethylamino-1-piperazinyl)-4-oxo-3-quinolinecarboxylic acid (compound VIII), for those of other species, in 0.002 *M* sodium hydroxide solution.

Chromatography

The HPLC system used here consisted of a Shimadzu Model LC-6A pump, a Model SPD-6AV UV—VIS spectrophotometric detector, a Model SCL-6A system controller, a Model SIL-6A autoinjector, a Model C-R3A Chromatopac as integrator and a Hitachi Model 650-10LC fluorescence spectrophotometer. Compound I and its metabolites were separated by ion-pair chromatography using tetra-*n*-butylammonium hydrogen sulphate as the ion-pairing reagent and a C_{18} silica column (TSK gel ODS-120T, particle size 5 μm; Toyo Soda). A 250 mm × 4.6 mm I.D. stainless-steel column packed with TSK gel ODS-120T was connected to a 10 mm × 4.0 mm I.D. guard column packed with the same gel. Biological samples of animals except rabbits were separated by an isocratic mobile phase consisting of methanol (28%) and 5 m*M* tetra-*n*-butylammonium hydrogen sulphate (72%) at a flow-rate of 0.8 ml/min. Biological samples of rabbits were analysed by a linear gradient of a mixture consisting of methanol (25%) and 5 m*M* tetra-*n*-butylammonium hydrogen sulphate (75%), and pure methanol. The fraction of pure methanol was linearly increased from zero to 40% for 30 min, and then the former mixture alone was allowed to flow for a subsequent 15 min at a flow-rate of 1.0 ml/min. The effluent was monitored at 340 nm for rabbits, and 320 nm for mice, rats and monkeys, using a UV detector. The effluent of dog samples was monitored by a fluorescence spectrophotometer (excitation: 290 nm; emission: 450 nm; slit: 10 nm). The concentrations of compound I and its metabolites were determined from a linear regression of peak-area ratios in rabbits and peak-height ratios in other species as a function of their concentrations. The overall recovery value was based on unextracted standards. The reproducibility of this method was validated by replicating all the procedures five times on each standard.

Animal experiments

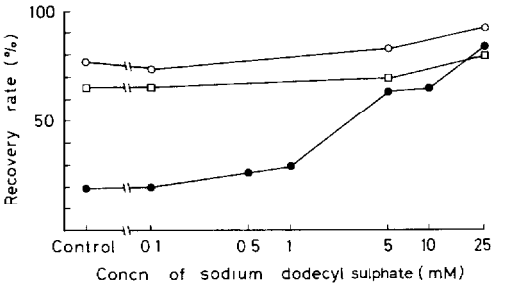
Male ICR mice (30—37 g), male Wistar rats (270—300 g), male Japanese

White rabbits (2.7—2.8 kg), male cynomolgus monkeys (4.0—6.0 kg) and male beagle dogs (9.8—10.5 kg) were treated after overnight fasting. All experiments except those on mice were performed on three animals at a dose of 10 mg/kg. The oral dose of compound I was given to mice as a 0.1% aqueous solution, and to rats, monkeys and dogs as a 0.5% aqueous solution. The intravenous dose of compound I was given to rabbits as a 2% aqueous solution. The aqueous solution was prepared by dissolving an aliquot of compound I in 0.1 *M* sodium hydroxide solution and adjusting the pH to 9 with 2 *M* hydrochloric acid and the osmolarity to physiological pressure with sodium chloride. Blood samples were serially obtained from rats by cardiac puncture, and those from rabbits, dogs and monkeys by venipuncture, at 0.5, 2, 4, 6 and 24 h after dosing. Mice blood samples were taken from three animals by decapitation at each sampling time and the individual samples were used for assay. Serum was separated by centrifugation (2000 *g*, 10 min) after standing for 1 h at 37°C. Urine was collected every day for three days after dosing in an individual metabolism cage. Serum and urine samples were stored at −20°C until analysed.

RESULTS

Assay procedure

A simple, sensitive and specific HPLC method was developed for quantitative determination of compound I and its metabolites in biological specimens. It has been known that rabbits metabolize compound I to various metabolites, as shown in Fig. 1, and excrete them in urine together with compound I [3]. Amphoteric II and water-soluble III were hardly extractable when non-polar solvent was used, as generally accepted [4—10]. Ion-pair extraction was employed to overcome this difficulty and proved its utility. It has been generally known that the kind and amount of ion-pairing reagent, the presence of salts and proteins, the kind of organic solvent and pH can influence the extractability of a drug from the aqueous phase to the organic phase [11]. Sodium dodecyl sulphate was appropriate as an ion-pairing reagent for this analysis. A 0.5-ml aliquot of 1 *M* acetic acid was added before extraction in this procedure since the resulting pH (2—3) was the most suitable for better extraction when chloroform—isopropanol (7:3) was employed as the organic



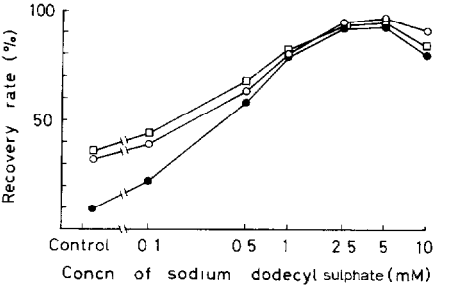


Fig. 2. The effect of initial concentration of sodium dodecyl sulphate on the overall recovery rate of compounds I (○), II (●) and III (□) from serum.

Fig. 3. The effect of initial concentration of sodium dodecyl sulphate on the overall recovery rate of compounds I (○), II (●) and III (□) from urine.

phase. The effect of sodium dodecyl sulphate concentration on extraction of compounds I, II and III from serum and urine are given in Figs. 2 and 3, respectively. Sodium dodecyl sulphate solution at 25 m*M* was found to be suitable for the analysis of serum I and its two metabolites, whereas 5 m*M* solution was the best for urine analysis. The mean overall recovery values of serum I, II and III under the present conditions were 90.1 ± 1.6, 85.5 ± 1.5 and 84.0 ± 1.0%, respectively (Table I). The respective recovery values from urine were 96.7 ± 0.1, 93.7 ± 1.2 and 95.0 ± 0.4% (Table I).

Utilizing the reversed-phase chromatographic conditions described for this assay, compound I was satisfactorily resolved from each of its metabolites and the internal standard, as shown in Fig. 4 (rabbit serum) and Fig. 5 (rabbit urine). The chromatograms obtained by injection of control serum and urine exhibited no interfering peaks at the retention times of compound I, its metabolites and internal standard. The retention times under these assay conditions were approximately as follows: VII, 7.7 min; I, 9.5 min; II, 11.0 min; III, 13.5 min, IV, 27.6 min; V, 31.1 min; VI, 33.4 min. The overall recovery values from serum and urine are summarized in Table I. All recovery values were greater than 80%. The calibration curves of compound I and its metabolites were rectilinear and passed through the origin over a range of 0.05—10 μg/ml of serum, and 0.5—200 μg/ml of urine ($r > 0.999$). The coefficients of variation in serum and urine ($n = 5$) are summarized in Table II.

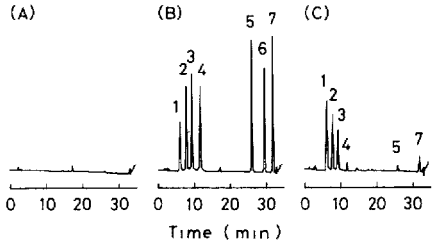


Fig. 4. Representative chromatograms of rabbit serum. (A) Control serum; (B) serum spiked with internal standard and 5.0 μg/ml each of compound I and its metabolites; (C) serum 1 h after intravenous dosing. Peaks: 1 = internal standard VII; 2 = I; 3 = II; 4 = III; 5 = IV, 6 = V; 7 = VI.

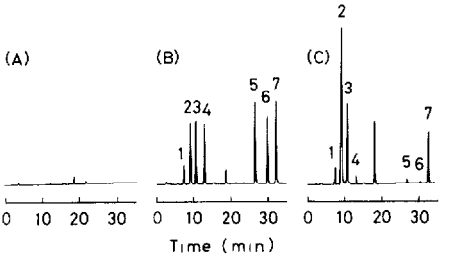


Fig. 5. Representative chromatograms of rabbit urine. (A) Control urine; (B) urine spiked with internal standard and 50 μg/ml each of compound I and its metabolites; (C) urine from 2 to 4 h after intravenous dosing. Peaks: 1 = internal standard VII, 2 = I; 3 = II, 4 = III, 5 = IV; 6 = V; 7 = VI.

Although a linear gradient elution was used for separation of five metabolites from compound I in biological samples of rabbits as described above, an isocratic elution was enough for analysis of biological samples of mice, rats, dogs and monkeys since compounds I, II and III were detected in their samples but other metabolites were not found. Figs. 6 and 7 show the typical chromatograms of serum and urine samples of dogs given 10 mg/kg I, respectively, where isocratic elution was employed. Isocratic elution can save analytical time and maintain analytical accuracy. Under this condition, good separation of peaks was achieved, as shown in Figs. 6 and 7. No interfering peaks owing to endogenous substances were seen. The approximate retention times under the

TABLE I

OVERALL RECOVERY FOR THE SERUM AND URINE ASSAY

Each value represents mean ± S.D. of five determinations.

Compound	Overall recovery (%)	
	Serum	Urine
I	90.1 ± 1.6	96.7 ± 0.1
II	85.5 ± 1.5	93.7 ± 1.2
III	84.0 ± 1.0	95.0 ± 0.4
IV	92.3 ± 3.9	104.6 ± 1.2
V	90.4 ± 3 5	98.7 ± 1.2
VI	97.2 ± 2.5	90.6 ± 2.1

TABLE II

CALIBRATION CURVE REPLICATE ANALYSIS FOR THE RABBIT SERUM AND URINE ASSAY

Compound	Coefficient of variation (n = 5) (%)					
	Serum			Urine		
	0.5 μg/ml	5.0 μg/ml	10 μg/ml	10 μg/ml	50 μg/ml	100 μg/ml
I	7.0	2.1	2.5	0.9	1.5	1.4
II	7.1	3.5	3.4	4.3	1.5	4.8
III	2.8	3.4	2.9	6.2	5.0	2.1
IV	12.5	5 0	3.7	1.2	6.1	1.0
V	26.9	4.7	2.9	1.2	2.2	2.2
VI	2.8	13.2	4.8	2.6	9 6	1.2

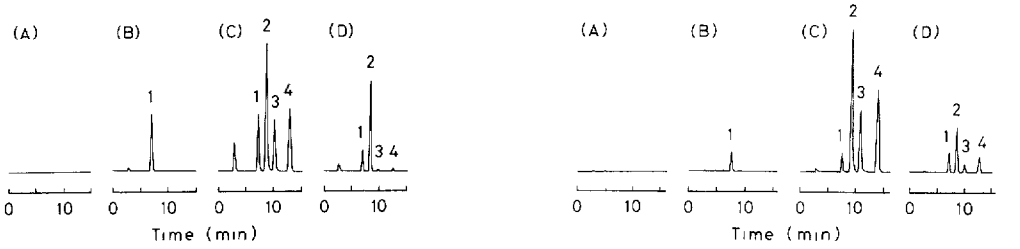


Fig 6. Representative chromatograms of dog serum. (A) Control serum; (B) serum spiked with internal standard, (C) serum spiked with internal standard and 5.0 μg/ml each of compound I and its metabolites; (D) serum 2 h after oral dosing. Peaks: 1 = internal standard VII, 2 = I, 3 = II; 4 = III.

Fig. 7. Representative chromatograms of dog urine. (A) Control urine; (B) urine spiked with internal standard; (C) urine spiked with internal standard and 50 μg/ml each of compound I and its metabolites; (D) urine for 24 h after oral dosing. Peaks: 1 = internal standard VII, 2 = I, 3 = II; 4 = III.

TABLE III

CALIBRATION CURVE REPLICATE ANALYSIS FOR THE DOG SERUM AND URINE ASSAY

Compound	Coefficient of variation (n = 5) (%)					
	Serum			Urine		
	0.5 μg/ml	5.0 μg/ml	10 μg/ml	1 μg/ml	10 μg/ml	100 μg/ml
I	1.4	2.3	2.3	4.1	1.1	1.0
II	3.9	2.3	1.8	10.2	2.5	1.1
III	3 7	1.9	2.4	1.7	1.5	1.6

isocratic elution were as follows: VII, 7.2 min; I, 8.6 min; II, 10.1 min; III, 12.7 min. The calibration curves obtained in the 0.01—10 μg/ml range in 0.5 ml of serum and in the 0.1—200 μg/ml range in 0.5 ml of urine were fairly rectilinear and passed through the origin ($r > 0.999$). The reproducibility of this method in serum and urine is summarized in Table III. Compound VIII was used as internal standard instead of VII for analysis of biological specimens of mice, rats and monkeys. This internal standard showed a retention time of ca. 18.0 min with a peak well resolved from those of compound I, its metabolites and endogenous substances. Compound III was decomposed to compound I at ambient temperature and 4°C, but was stable at −20°C for four weeks in urine. Degradation of compound III in one week was 80 and 10% at room temperature and 4°C in urine, respectively.

Metabolites in serum

Mean serum concentrations of compound I and its metabolites after oral administration at a dose of 10 mg/kg are presented in Table IV. The major metabolites in mouse, rat, dog and monkey serum were compounds II and III. Rabbit serum contained compounds IV, V and VI in addition to compounds I, II and III. Less than 10% of compound I was found in the form of each metabolite in mouse, rat, dog and monkey serum. Compound I was more extensively metabolized in rabbits so that ca. 30% of the total concentration was accounted for by metabolites at 30 min after dosing. Compound I constituted 80—90% of total serum concentrations in mice, and more than 90% in rats, dogs and monkeys up to 6 h after dosing, whereas the fraction of compound I in rabbits was 34—67%. Compound II was more predominant than compound III in mice and rabbits, and the reverse relationship held in rats and dogs. Monkeys showed almost the same levels of both metabolites. As described above, rabbits demonstrated specific and extensive metabolism of compound I, being I > II > VI > III > IV ≈ V in serum.

Serum peak levels of compound I were 3.22, 4.64, 7.63 and 6.62 μg/ml at an oral dose of 10 mg/kg in mice, rats, dogs and monkeys, respectively, which were observed 0.5 h after dosing in mice, rats and dogs and 2 h after dosing in monkeys.

TABLE IV

SERUM CONCENTRATIONS OF COMPOUND I AND ITS METABOLITES IN MICE, RATS, RABBITS, DOGS AND MONKEYS

Aqueous solution was given at a dose of 10 mg/kg. Rabbits were dosed intravenously and other species orally. Each value represents mean ± standard error of three animals. The values in parentheses show the per cent of total concentrations of compound I and its metabolites.

Species	Compound	Serum concentration (μg/ml)				
		0.5 h	2 h	4 h	6 h	24 h
Mice	I	3.22 ± 0.31 (85)	1.26 ± 0.16 (89)	0.72 ± 0 11 (86)	0.58 ± 0.17 (79)	0.05 ± 0.05 (100)
	II	0.35 ± 0.05 (10)	0.09 ± 0.01 (6)	0.12 ± 0.09 (14)	0.09 ± 0.05 (12)	0.00 ± 0.00 (0)
	III	0.18 ± 0.09 (5)	0.06 ± 0.06 (4)	0.00 ± 0.00 (0)	0.06 ± 0.06 (8)	0.00 ± 0.00 (0)
Rats	I	4.64 ± 0.24 (90)	3.75 ± 0.10 (92)	2.53 ± 0.20 (93)	1.69 ± 0.10 (92)	0.18 ± 0.05 (95)
	II	0.19 ± 0.03 (4)	0.13 ± 0.02 (3)	0.07 ± 0.03 (3)	0.05 ± 0.03 (3)	0.01 ± 0.01 (5)
	III	0.30 ± 0.07 (6)	0.19 ± 0.03 (5)	0.12 ± 0.06 (5)	0.09 ± 0.05 (5)	N.D.*
Rabbits	I	5.67 ± 0.14 (67)	3.73 ± 0.45 (61)	1.77 ± 0.17 (52)	0.53 ± 0.06 (34)	0.20 ± 0.04 (34)
	II	1.37 ± 0.18 (17)	1.27 ± 0.20 (22)	0.93 ± 0.13 (29)	0.48 ± 0.09 (32)	0.32 ± 0.07 (57)
	III	0.38 ± 0.06 (5)	0.33 ± 0.01 (6)	0.22 ± 0.02 (7)	N.D.	N.D.
	IV	0.14 ± 0.07 (2)	0.07 ± 0.07 (1)	0.05 ± 0.05 (1)	N.D	N.D.
	V	0.17 ± 0.08 (2)	0.08 ± 0.08 (1)	0.08 ± 0.08 (2)	0.09 ± 0.09 (6)	N.D.
	VI	0.68 ± 0.19 (8)	0.57 ± 0.12 (10)	0.27 ± 0.10 (8)	0.40 ± 0.14 (27)	0.05 ± 0.05 (9)
Dogs	I	7.63 ± 1.17 (99)	7.57 ± 0.28 (95)	6.89 ± 0.13 (93)	5.73 ± 0.27 (92)	3.05 ± 0.39 (93)
	II	0.00 ± 0.00 (0)	0.07 ± 0.07 (1)	0.20 ± 0.10 (3)	0.20 ± 0.10 (3)	0.03 ± 0.03 (1)
	III	0.07 ± 0.03 (1)	0.30 ± 0.00 (4)	0.33 ± 0.03 (5)	0.27 ± 0.03 (5)	0.20 ± 0.00 (6)
Monkeys	I	4.78 ± 0.41 (91)	6.62 ± 1.29 (94)	5.20 ± 1.00 (92)	3.71 ± 0.82 (90)	0.27 ± 0.09 (84)
	II	0.15 ± 0.01 (3)	0.23 ± 0.06 (3)	0.29 ± 0.03 (5)	0.29 ± 0.08 (7)	0.02 ± 0.01 (6)
	III	0.29 ± 0.12 (6)	0.21 ± 0.06 (3)	0.17 ± 0.02 (3)	0.11 ± 0.03 (3)	0.03 ± 0.03 (9)

*N.D. = Not detected.

Metabolites in urine

Mean urine concentrations and urinary recoveries of compound I and its metabolites are presented in Table V. All animals except rabbits were orally treated at a dose of 10 mg/kg and their urine was collected every day for three days. Rabbits were dosed intravenously at the same dose. Compound I was the most predominant in all animals except rabbits, in which compound II was the most abundant. Mice excreted 46.8% of the oral dose over three days, with compound I amounting to 29.4%. Mice excreted compound II as the main urinary metabolite, with a trace amount of compound III. Rats excreted 42.3% of the dose over three days and 73% of it was as compound I. Rabbit urine contained more abundant metabolites: 36.1% II, 18.9% I, 8.2% VI, 3.7% III, 2.9% IV and 0.6% V of intravenous dose, totalling 70.5% over three days. Dogs excreted 48.8% over three days as 26.6% I, 12.2% II and 10.0% III after oral

TABLE V

URINARY EXCRETION OF COMPOUND I AND ITS METABOLITES IN MICE, RATS, RABBITS, DOGS AND MONKEYS

Aqueous solution was given at a dose of 10 mg/kg Rabbits were dosed intravenously and other species orally Each value represents mean ± standard error of three animals

Species	Compound	Urinary level (μg/ml)			Cumulative recovery* (% of dose)	Total urinary excretion (% of dose)
		0—24 h	24—48 h	48—72 h		
Mice	I	41 8 ± 8 7	3 1 ± 1 2	0 6 ± 0 6	29 4 ± 2 8 (63)	
	II	24 2 ± 5 4	1.9 ± 0 7	Trace	17.4 ± 2 3 (37)	46.8 ± 5 1
	III	Trace	Trace	N D **	0	
Rats	I	36 1 ± 5 6	10.3 ± 2.4	2.0 ± 0 8	31.0 ± 5 5 (73)	
	II	7 0 ± 2 2	2.5 ± 0 5	0 3 ± 0 3	5.9 ± 0 3 (14)	42 3 ± 5 4
	III	7 2 ± 2 9	2.2 ± 0 5	0 6 ± 0 6	5.3 ± 0 4 (13)	
Rabbits	I	32 8 ± 1.5***		0 2 ± 0.2	18.9 ± 3 7 (27)	
	II	70 0 ± 25.5		0 4 ± 0 2	36.1 ± 4 8 (51)	
	III	6 7 ± 0.4		0 1 ± 0 1	3.7 ± 0 7 (5)	70.5 ± 1 2
	IV	5 9 ± 2.2		N D	2.9 ± 0 4 (4)	
	V	1 0 ± 0.6		0 3 ± 0 3	0.6 ± 0 5 (1)	
	VI	16 7 ± 4 6		N D	8.2 ± 0 4 (12)	
Dogs	I	36 7 ± 5 2	97.8 ± 17 2	25 4 ± 4 9	26.6 ± 2 4 (55)	
	II	18 2 ± 6 8	42.3 ± 18 9	6 7 ± 1 6	12.2 ± 4 6 (25)	48.8 ± 8 3
	III	15 3 ± 3 0	39.5 ± 10.9	7 1 ± 0 6	10.0 ± 2 0 (20)	
Monkeys	I	282 0 ± 35 5	17.1 ± 4 2	5 5 ± 1 8	60.1 ± 4 6 (78)	
	II	53 1 ± 6 9	1.4 ± 0 9	N D	11 0 ± 0 8 (14)	77.1 ± 4 7
	III	26 2 ± 5 3	2.4 ± 0.2	1 6 ± 0 8	6.0 ± 0 8 (8)	

*The values in parentheses show the per cent of total recoveries of compound I and its metabolites.
**N D. = Not detected.
***Urine was collected for 48 h

dose. Compound I predominated in monkey urine, the values being 60.1% I, 11.0% II and 6.0% III, totalling 77.1% of the administered dose.

DISCUSSION

Compound I has a 4-methyl-1-piperazinyl moiety at the 7-position of the quinoline nucleus like pefloxacin, ofloxacin and amifloxacin, whereas

norfloxacin, enoxacin and ciprofloxacin do not have a 4-methyl group on the 7-position piperazinyl moiety. 4-Methyl-1-piperazinyl quinolinecarboxylic acid derivatives, such as compound I, pefloxacin [6], amifloxacin [12] and ofloxacin [13], are metabolized to the corresponding N-desmethyl metabolites, probably via the corresponding N-oxides that are detected as metabolites in all compounds. These relatively polar metabolites are much harder to extract from biological specimens than the parent drugs. Recovery of pefloxacin is 94%, but its N-desmethyl derivative, norfloxacin, is extracted at the 65% level when the neutralized urine is extracted with chloroform—isopentanol [4]. Enoxacin is also not extractable with non-polar solvent [5]. Of enoxacin, 47% is extracted at pH 7.0—8.0 when equal volumes of chloroform and aqueous phases are used. It is necessary to improve the extractability of these polar metabolites for simultaneous determination of compound I and its metabolites. Better recovery of these polar metabolites is achieved by ion-pair extraction using sodium dodecyl sulphate as an ion-pairing reagent. Compounds IV, V and VI are recovered well in the presence of sodium dodecyl sulphate under the acidic conditions employed here, but their extraction is not associated with the presence of sodium dodecyl sulphate because of the weak basicity of the amide bond. The combination of the ion-pair extraction technique and ion-pair reversed-phase HPLC enables us to determine compound I and its metabolites simultaneously. As described above, the analytical procedure developed here gives excellent extractability, satisfactory separation of chromatographic peaks, no perturbation owing to endogenous substances, linearity over a wide concentration range, good reproducibility and high sensitivity. Therefore, this analytical method may be valuable for rapid and accurate determination of compound I and its metabolites in biological specimens.

This procedure was applied to discover the species differences in the metabolism of compound I. Compounds II and III are detected as metabolites in serum and urine of all animals. Compound I accounts for greater than 80% of the total serum concentration in all animals except rabbits. In particular, the values are greater than 90% in rats, dogs and monkeys. The distribution of compound I and its metabolites in serum of rats, dogs and monkeys is close to that of ofloxacin, which is metabolized to the same type of metabolites [13]. In rabbit serum, compounds IV, V and VI are also detected in addition to the above two metabolites. Serum concentration of compound VI in rabbits is greater than that of compound III, and both oxo metabolites are relatively minor. Compound I occupies about two thirds of the total serum concentration 30 min after dosing, and its fraction decreases to one third at 6 h in rabbits. Compound I is the most predominant in urine of mice, rats, dogs and monkeys, but compound II is the most abundant in rabbit urine. The fraction of compound I in the total urinary excretion is 63, 73, 27, 55 and 78% in mice, rats, rabbits, dogs and monkeys, respectively. These data indicate that compound I is extensively metabolized in rabbits, but is relatively stable in other animals. Pefloxacin is extensively metabolized in all animals including mice, rats, rabbits, dogs, monkeys and humans [6, 14]. The fraction of unchanged pefloxacin in total urinary excretion is 56% in mice, 17% in rats, 9% in rabbits, 13% in dogs and 23% in monkeys. The fraction of unmetabolized amifloxacin is 47% in rats and 68% in monkeys [12]. Ofloxacin is excreted in

urine of rats, dogs and monkeys as unchanged drug at 85.2, 78.8 and 87.9% of total excretion, respectively [13]. This evidence indicates that compound I is a little less stable than ofloxacin, but more stable than pefloxacin and amifloxacin from the standpoint of metabolism in experimental animals. Two metabolic pathways are proposed for N-dealkylation of alkylamine compounds [15]. One possibility is a decomposition to amine and aldehyde through unstable carbinolamine. The another pathway is an oxidative N-dealkylation via N-oxide. The latter mechanism is predominant in mice, rats, dogs and monkeys, whereas both mechanisms are operating in rabbits in the metabolism of compound I.

Although rabbits and monkeys excrete 70—80% of the dose in three-day urine, the total urinary excretion of mice, rats and dogs is relatively low, 40—50% of oral dose. On the other hand, Takagi et al. [2] report that the systemic availability of compound I in mice, rats and dogs is perfect after oral administration. These results suggest the possibility that compound I is also excreted into bile, probably with its metabolites and further conjugated forms.

The elimination of compound I from serum is extremely fast in rabbits, although the animal species smaller in body weight have a tendency to show a shorter elimination half-life [2]. The present study also demonstrates the relatively rapid elimination of compound I in rabbits, as shown in Table IV. The exceptional phenomena in rabbits might be associated with species characteristics such as metabolism, protein binding and excretion mechanism. This study shows that the extensive metabolism of compound I in rabbits might be one reason for the fast serum elimination. The excretion mechanism must also be taken into consideration, since a structurally similar compound, norfloxacin, has been known to be renal-handled in different ways in rabbits and dogs [16].

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