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Pharmacokinetics of the Cephalosporin SM-1652 in Mice, Rats, Rabbits, Dogs, and Rhesus Monkeys

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The pharmacokinetics of SM-1652 were studied in mice, rats, rabbits, dogs, and rhesus monkeys. The plasma half-lives of SM-1652, administered intravenously at a dose of 20 mg/kg, were 11.0 min in mice, 26.0 min in rats, 65.8 min in rabbits, 72.6 min in dogs, and 150.9 min in monkeys. The 24-h urinary excretion of SM-1652 was 30 to 35% of the dose in mice and rats, 70 to 75% in rabbits and dogs, and 45% in monkeys. Biliary excretion of the antibiotic over a 24-h period was 60 and 19% in rats and rabbits, respectively; it was 19% in dogs over a 9-h period after SM-1652 administration. Approximately 95% of the intravenous dose of SM-1652 was recovered as the unchanged form in the urine and bile of rats and rabbits. The binding of SM-1652 to serum protein was 44.0% in mice, 46.0% in rats, 90.4% in rabbits, 93.2% in monkeys, 30.0% in dogs, and 96.3% in humans.

As has been reported previously, the semisynthetic cephalosporin SM-1652, sodium 7-[D(−)-α-(4-hydroxy-6-methylpyridine-3-carboxamido)-α-(4-hydroxyphenyl)acetamido]-3-[(1-methyl-1*H*-tetrazol-5-yl)thiomethyl]-3-cephem-4-carboxylate (Fig. 1), has exhibited a broad spectrum of activity against both gram-positive and gram-negative bacteria in vitro and in vivo (M. Kato, M. Fukasawa, H. Noguchi, T. Okuda, T. Komatsu, K. Yano, and S. Mitsuhashi, Program Abstr. Int. Congr. Chemother. 12th, Florence, Italy, abstr. no. 300, 1981). It is as active as cefoperazone and cefotaxime against gram-positive cocci in vitro and significantly more active than these compounds and cefmenoxime against clinical isolates of *Pseudomonas aeruginosa*. The present report is concerned with the pharmacokinetics of SM-1652 as displayed in mice, rats, rabbits, dogs, and rhesus monkeys. A preliminary report of the results of these studies has been presented elsewhere (8).

MATERIALS AND METHODS

Antibiotic. SM-1652 was synthesized in the laboratories of Yamanouchi Pharmaceutical Co., Ltd., Tokyo, Japan.

Experimental animals. Included in this study were male ddY mice (weight, about 30 g), male Sprague-Dawley rats (200 to 250 g for measurement of the antibiotic levels in plasma and 300 to 350 g for examination of urinary and biliary excretion), male albino rabbits (2.5 to 3.5 kg), female beagle dogs (9.0 to 13.0 kg), and male rhesus monkeys (4.5 to 5.1 kg).

Administration of antibiotic. SM-1652, dissolved in sterile physiological saline just before use, was administered intravenously at a dose of 20 mg/kg within 10 s to mice, rats, rabbits, dogs, and monkeys.

Plasma samples. In the experiments with mice and rats, three animals were sacrificed with ether at 5, 10, 20, 30, 45, 60, 90, and 120 min after dose administration. Blood was withdrawn from the inferior vena cava and heparinized. At each bleeding time, samples from three mice were combined in one test tube. Blood from each of three rats was processed separately. In the tests with rabbits and dogs, blood samples were taken from each animal at 5, 10, 20, 30, 45, 60, 90, 120, 150, and 180 min after administration. Blood specimens from each monkey were obtained at 5, 10, 20, 30, and 45 min and 1, 2, 3, 4, 5, and 6 h after administration. Plasma was separated from these heparinized blood samples by centrifuging at 1,400 × *g* at 4°C, removed by aspiration, and stored at −20°C until assayed, usually within 3 days.

Urine and bile specimens. Urine specimens were collected 0 to 3, 3 to 6, and 6 to 24 h after dose administration. All samples of urine from mice, rats, and monkeys were obtained in metabolism cages. Urine specimens from rabbits and dogs were taken in metabolism cages and by bladder catheterization at the end of each period.

Collection of bile from rats, rabbits, and dogs was performed as follows. Rats were anesthetized with ether; rabbits and dogs were anesthetized by intravenous administration of 27 mg of sodium pentobarbital per kg. A midline incision was made, and the gallbladders of rabbits and dogs were ligated. A polyethylene cannula was inserted into the common bile duct and fastened securely with silk suture, and the free end was brought through the abdominal wall. Rats were maintained in restraining cages with free access to water; rabbits and dogs were fixed in a supine position. SM-1652 was administered intravenously to rats and rabbits after they had awakened from anesthesia. Bile samples were collected 0 to 3, 3 to 6, and 6 to 24 h after dose administration. Dogs were anesthetized by

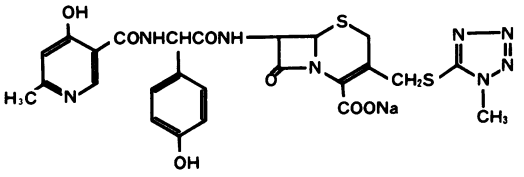

FIG. 1. Chemical structure of SM-1652.

the periodic intramuscular injection of sodium pentobarbital throughout the experiments. Bile specimens were collected 0 to 3, 3 to 6, and 6 to 9 h after administration of SM-1652. Urine and bile were stored at −20°C until assayed, usually within 3 days.

Microbiological assay. SM-1652 concentrations in biological materials were determined by a disk-plate diffusion method on sensitivity test agar (Eiken, Tokyo) inoculated with *Escherichia coli* NIHJ as the test organism. Calibration curves from spiked plasma of each animal species were used for assays of plasma samples. Standard solutions of SM-1652 for analyses of urine and bile collected from all species were prepared with a phosphate buffer (0.067 M; pH 7.0). Urine and bile specimens were diluted, when necessary, with the phosphate buffer so that the antibiotic concentrations would drop into the concentration range of the standard curves. The 95% confidence limits for triplicate assays of plasma, urine, and bile were less than 14.3%

Bioautographic analysis for microbiologically active urinary and biliary metabolites of SM-1652. Urine and bile specimens (2 to 10 μl each) collected for 0 to 3 h from all the animal species were applied to a silica gel plate containing a fluorescent indicator (Silica Gel 60 F_{254}; E. Merck, Darmstadt, Germany). The plate was then subjected to ascending development in a solvent system of acetone-ethyl acetate-acetic acid-distilled water (160:8:15:30 [vol/vol]) until the solvent front reached 10 cm from the origin. The plate was air dried, and optical spots were detected under UV light at 254 nm. The plate was then placed on an agar plate inoculated with *Bacillus subtilis* ATCC 6633 for 15 min. The silica gel plate was removed, and the agar plate was incubated overnight at 30°C. In the solvent system described above, SM-1652 produced a growth inhibition zone at an R_f value of 0.81.

Serum protein binding. One volume of an aqueous solution of SM-1652 was added to 9 volumes of fresh pooled sera of mice, rats, rabbits, monkeys, dogs, and humans to obtain an antibiotic concentration of 100 μg/ml. The reaction mixture was incubated at 37°C for 30 min with gentle shaking and then subjected to centrifugal ultrafiltration with a CF-50 Centriflo membrane cone (Amicon Corp., Lexington, Mass.). The protein-free ultrafiltrate, containing the unbound antibiotic, was microbiologically assayed as mentioned above. Although the binding of the antibiotic to the membrane cones was less than a few percent, it was corrected by adding 1 volume of an aqueous solution of the antibiotic to 9 volumes of an isotonic buffer solution (pH 7.4) as a substitute for serum and subjecting this protein-free reaction mixture to the same procedures.

Pharmacokinetic analysis. For mice and rats, a single plasma concentration-time curve per species was formed, because blood samples from three mice at each bleeding time were assembled in one test tube, and the mean antibiotic level in the plasma of three rats at each sampling time was regarded as a single point. For rabbits, dogs, and monkeys, each plasma concentration-time curve from each animal was analyzed individually. Pharmacokinetic analyses on plasma concentration-time curves were performed by using a two-compartment open model, with the exception of the curve for mice, which was computed by using a one-compartment model. The antibiotic concentration in plasma (C_1) at time t after dose administration can be expressed by either of the following exponential equations: $C_1 = C_1^0 e^{-k_{el} t}$ (one-compartment open model) or $C_1 = Ae^{-\alpha t} + Be^{-\beta t}$ (two-compartment open model), where C_1^0 is the concentration at time zero, k_{el} is the elimination rate constant, A and B are the zero time intercepts of the two components of the biexponential curves, and α and β are the hybrid rate constants for the distribution and elimination phases, respectively. Pharmacokinetic parameters were calculated by standard equations based on one- and two-compartment open models (4). The area under the plasma concentration-time curve (AUC) was obtained by the integration of curves from zero to infinite time, giving the following equations: $AUC = C_1^0/k_{el}$ and $AUC = A/\alpha + B/\beta$ in the one- and two-compartment open models, respectively. The nonlinear least-square program NONLIN (9) was employed for regression analyses of the curves.

RESULTS

Plasma levels of SM-1652. The plasma concentration-time curves of SM-1652, administered intravenously at a dose of 20 mg/kg to mice, rats, rabbits, dogs, and monkeys, are shown in

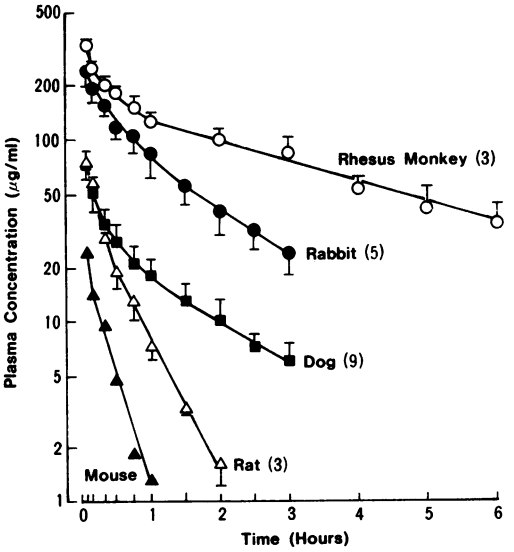

FIG. 2. Plasma concentrations of SM-1652 administered intravenously at a dose of 20 mg/kg to mice, rats, rabbits, dogs, and rhesus monkeys. The figures in parentheses indicate the number of animals. Three rats were sacrificed at each bleeding time.

TABLE 1. Pharmacokinetic parameters of SM-1652 administered intravenously at a dose of 20 mg/kg to experimental animals

Parameter (units)	Mouse[a]	Rat	Rabbit (5)[b]	Dog (9)	Monkey (3)
C_1^0 (μg/ml)	30.3	114.9	307.7 ± 79.1	118.2 ± 41.4	428.4 ± 64.3
A (μg/ml)		77.9	152.2 ± 44.1	85.6 ± 41.4	251.7 ± 74.0
B (μg/ml)		36.9	155.5 ± 37.6	32.6 ± 6.8	176.7 ± 14.3
α (h^{-1})		6.48	6.60 ± 2.83	7.90 ± 3.81	6.02 ± 2.25
β (h^{-1})		1.60	0.64 ± 0.10	0.58 ± 0.08	0.28 ± 0.05
k_{12} (h^{-1})		1.66	2.42 ± 1.32	4.13 ± 2.68	3.05 ± 1.56
k_{21} (h^{-1})		3.18	3.68 ± 1.47	2.50 ± 0.41	2.59 ± 0.63
k_{el} (h^{-1})	3.77	3.28	1.15 ± 0.23	1.86 ± 1.00	0.66 ± 0.23
$t_{1/2(\alpha)}$ (min)		6.42	7.39 ± 3.26	6.07 ± 2.11	7.69 ± 3.16
$t_{1/2(\beta)}$ (min)	11.0	26.0	65.8 ± 10.6	72.6 ± 9.4	150.9 ± 28.9
V_1 (ml/kg)		174.1	68.0 ± 14.8	184.3 ± 52.2	47.3 ± 6.7
V_2 (ml/kg)		90.8	41.3 ± 5.8	269.8 ± 98.7	52.4 ± 11.2
V_d (ml/kg)	660.2	264.9	109.3 ± 18.5	454.0 ± 120.3	99.8 ± 5.7
AUC (μg·h/ml)[c]	8.1	35.1	268.6 ± 49.0	67.4 ± 13.2	684.5 ± 138.5
Cl (body) (ml/h per kg)	2,489	571.0	78.2 ± 23.1	307.6 ± 67.6	31.2 ± 11.7

[a] Based on one-compartment model.
[b] The figures in parentheses indicate the number of animals. The values for rabbits, dogs, and monkeys are mean ± standard deviation.
[c] A single plasma concentration-time curve per species was formed for mice and rats.

Fig. 2. The antibiotic concentrations in plasma were observed to decline monophasically in mice and biphasically in the other species. The average drug concentrations in plasma at 30 min after dose administration were 4.7 μg/ml in mice, 19 μg/ml in rats, 118 μg/ml in rabbits, 28 μg/ml in dogs, and 180 μg/ml in monkeys. In mice and rats, the minimum antibiotic concentrations were 1.3 and 1.6 μg/ml at 1 and 2 h, respectively. The SM-1652 concentrations in the plasma of rabbits and dogs at 3 h post-administration were 24 and 6.0 μg/ml, respectively. The mean antibiotic concentration in monkey plasma at 6 h was 35 μg/ml.

The pharmacokinetic parameters obtained by analyzing the plasma concentration-time curves according to one- and two-compartment open models are listed in Table 1. The calculated plasma concentrations of SM-1652 at time zero (C_1^0) were 30.3 μg/ml in mice, 114.9 μg/ml in rats, 307.7 μg/ml in rabbits, 118.2 μg/ml in dogs, and 428.4 μg/ml in monkeys. The plasma half-lives [$t_{1/2(\beta)}$] were 11.0 min in mice, 26.0 min in rats, 65.8 min in rabbits, 72.6 min in dogs, and 150.9 min in monkeys. These $t_{1/2(\beta)}$ values were inversely proportional to the elimination rate constants (k_{el}), except for dogs. Although the k_{el} value was greater in dogs than in rabbits, the $t_{1/2(\beta)}$ was longer in dogs than in rabbits. This results from the high ratio of k_{12}/k_{21} in dogs. Since a large amount of the antibiotic distributed to the peripheral compartment of dogs would be transferred to the central (plasma) compartment in response to a decrease in the SM-1652 concentration in the central compartment, the $t_{1/2(\beta)}$ in dogs become long despite the relatively high k_{el}. This was reflected in the large volume of distribution per kilogram of body weight (V_d) at steady state in dogs. Therefore, it appears that the distribution of SM-1652 in dogs was considerably different from the distribution in rabbits and monkeys. The AUC values (μg · h/ml) were 8.1 for mice, 35.1 for rats, 268.6 for rabbits, 67.4 for dogs, and 684.5 for monkeys. The body clearance per kilogram of body weight (Cl_{body}; milliliters per hour per kilogram) was 2,489 in mice, 571.0 in rats, 78.2 in rabbits, 307.6 in dogs, and 31.2 in monkeys.

Urinary and biliary excretion. When SM-1652 was given intravenously at a dose of 20 mg/kg, the urinary excretion of the cephalosporin for 24

TABLE 2. Urinary excretion of SM-1652 administered intravenously at a dose of 20 mg/kg to mice, rats, rabbits, dogs, and monkeys

Species (no.)	Cumulative % of the dose excreted into urine (mean ± SD)		
	0–3 h	0–6 h	0–24 h
Mouse (10)	30.8 ± 4.4	31.6 ± 4.1	31.7 ± 4.0
Rat (6)	30.8 ± 4.5	34.0 ± 5.4	35.2 ± 5.4
Rabbit (9)	68.0 ± 17.5	73.1 ± 17.0	74.1 ± 16.6
Dog (14)	49.5 ± 5.1	60.4 ± 6.2	70.6 ± 6.6
Monkey (3)	32.6 ± 5.3	43.0 ± 1.6	44.6 ± 1.2

TABLE 3. Biliary excretion of SM-1652 administered intravenously at a dose of 20 mg/kg to rats, rabbits, and dogs

Species (no.)	Cumulative % of the dose excreted into bile (mean ± SD)			
	0–3 h	0–6 h	0–9 h	0–24 h
Rat (9)	52.7 ± 14.5	57.5 ± 15.5		59.6 ± 16.5
Rabbit (3)	16.6 ± 8.1	18.1 ± 8.2		19.1 ± 7.8
Dog[a] (4)	13.6 ± 2.5	17.4 ± 2.9	19.1 ± 2.9	

[a] The dogs were anesthetized throughout the experiments.

h after administration was 31.7% of the dose in mice, 35.2% in rats, 74.1% in rabbits, 70.6% in dogs, and 44.6% in monkeys (Table 2). The biliary excretion of the antibiotic was 59.6% of the dose in rats and 19.1% in rabbits for 24 h, and 19.1% in anesthetized dogs for 9 h post-administration (Table 3). Therefore, the total urinary and biliary excretion of the cephalosporin was 94.8% in rats, 93.2% in rabbits, and 89.7% in dogs. The value for dogs appears somewhat lower than those for rats and rabbits. However, considering that the dogs were anesthetized throughout the experiment and the collection of dog bile was finished at 9 h post-administration, the recovery of 89.7% of the SM-1652 dose in the urine and bile of dogs is not so much lower than the recoveries in rats and rabbits.

Binding of SM-1652 to serum protein. The percentages of SM-1652 that bound to the serum protein of mice, rats, and dogs were relatively low: 44.0% in mice, 46.0% in rats, and 30.0% in dogs. In contrast, a high proportion of SM-1652 bound to the serum protein of rabbits, monkeys, and humans: 90.4, 93.2, and 96.3%, respectively. In these experiments, the SM-1652 concentration in reaction mixtures was 100 μg/ml. The unbound antibiotic was separated by a centrifugal ultrafiltration technique.

DISCUSSION

The pharmacokinetic profile of SM-1652 administered intravenously to mice, rats, rabbits, dogs, and rhesus monkeys was described. It was shown that the antibiotic was significantly long acting. The plasma half-lives of SM-1652 at the β phase were 11.0 min in mice, 26.0 min in rats, 65.8 min in rabbits, 72.6 min in dogs, and 150.9 min in monkeys. The results of studies performed by us, in which cefazolin and cefoperazone were given intravenously at a dose of 20 mg/kg to the same animal species, showed the following plasma half-lives: cefazolin (8), 11.2 min in mice, 21.6 min in rats, 24.7 min in rabbits, 43.9 min in dogs, and 42.3 min in monkeys; cefoperazone (unpublished data), 3.3 min in mice, 7.9 min in rats, 36.0 min in rabbits, and 36.8 min in dogs. Therefore, in mice and rats, the plasma half-lives of SM-1652 were comparable to those of cefazolin and more than three times as long as those of cefoperazone. In rabbits and dogs, the $t_{1/2(\beta)}$ values of SM-1652 were found to be 1.7 to 2.7 times as long as those of cefazolin and cefoperazone. In monkeys, the $t_{1/2(\beta)}$ value of SM-1652 exceeded that of cefazolin by fourfold. According to Actor and co-workers (1), the plasma half-lives of SK&F 75073 (cefonicid) were 124 min in mice, 38 min in dogs, and 97 min in squirrel monkeys. Lee and co-workers (7) reported that the plasma half-lives of ceforanide were 1.1 h in rats, 5.0 h in rabbits, and 1.0 h in dogs. The $t_{1/2(\beta)}$ values of YM09330, presented by Komiya and colleagues (5), were 13.0 min in mice, 15.9 min in rats, 30.5 min in rabbits, 55.5 min in dogs, and 75.6 min in rhesus monkeys. Murakawa and associates (10) reported that the plasma half-lives of ceftizoxime, cefotiam, cefamandole, cefotaxime, and cefmetazole, administered intramuscularly to rhesus monkeys, were 47, 41, 34, 49, and 33 min, respectively. Thus, cefonicid showed the longest plasma half-life in mice, and ceforanide was the longest lasting cephalosporin in rats and rabbits. In contrast, in dogs and monkeys, the plasma half-lives of SM-1652 were much longer than those of these recently developed cephem antibiotics.

From 90 to 95% of SM-1652 administered intravenously to rats, rabbits, and dogs was recovered as a microbiologically active form in urine and bile. By bioautographic analyses, no antibiotically active metabolites were detected in urine and bile samples of the five animal species receiving SM-1652. These facts suggest that SM-1652 is biotransformed to active and inactive metabolites very little in animal bodies. The metabolism, if any, of the antibiotic would be less than a few percent. SM-1652, as well as cefamandole, cefoperazone, cefmenoxime, YM09330, cefmetazole, and moxalactam, possesses a (1-methyl-1*H*-tetrazol-5-yl)thiomethyl substituent at the 3 position of the cephem ring. This substituent appears to be much more stable against enzymatic hydrolysis than the acetoxymethyl group, which is the 3-position moiety of cephalothin (6, 12), cephapirin (2), cephacetrile (11), and cefotaxime (3).

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