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Pharmacokinetics of New Broad-Spectrum Cephamycin, YM09330, Parenterally Administered to Various Experimental Animals

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The pharmacokinetics of YM09330, a new semisynthetic cephamycin, were determined after intravenous and intramuscular administration to experimental animals. Mean plasma levels of YM09330 at 30 min after intravenous administration of 20 mg/kg were 5.5 μg/ml for mice, 17 μg/ml for rats, 24 μg/ml for rabbits, 42 μg/ml for dogs, and 76 μg/ml for monkeys; plasma half-lives were 13.0, 15.9, 30.5, 55.5, and 75.6 min, respectively. The half-lives of YM09330 were longer than those of cefmetazole in all species tested. In monkeys, plasma levels of YM09330 were higher and more prolonged than those of cefazolin. In rats and dogs, the concentrations of YM09330 were highest in the kidneys, followed by the liver, plasma, lung, spleen, and heart in that order; they were similar to those of cefazolin in rats. Urinary excretion of YM09330 within 48 h of intravenous administration was 67% of the dose in mice, 52% in rats, 74% in rabbits, 53% in dogs, and 60% in monkeys. In rats, 48% of the dose of YM09330 was recovered in the bile within 24 h. Approximately 100% of the dose was recovered in the urine and bile combined. No active metabolite of YM09330 was detected in the plasma, urine, or bile. However, small amounts of an antibacterially active tautomer of YM09330 were recovered in the urine of mice, rats, and dogs, whereas large amounts of the tautomer were recovered in the urine of rabbits and monkeys. Serum protein binding of YM09330 was 30% for rats, 51% for rabbits, 39% for dogs, 87% for monkeys, and 91% for humans.

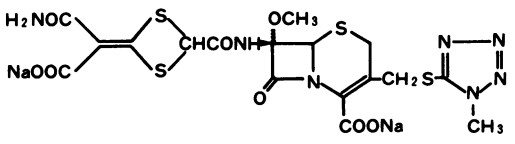
YM09330, disodium(6*R*,7*S*)-7-[[4-(carbamoylcarboxylatomethylene)-1,3-dithietan-2-yl]carboxamido]-7-methoxy-3-[[(1-methyl-1*H*-tetrazol-5-yl)thio]-methyl]-8-oxo-5-thia-1-azabicyclo]4.2.0]oct-2-ene-2-carboxylate, is a new semisynthetic parenteral cephamycin antibiotic with a broad spectrum of antibacterial activity (6; K. Yano, K. Suzaki, M. Saito, M. Toda, T. Saito, and S. Mitsuhashi, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 19th, Boston, Mass., abstr. no. 564, 1979). The chemical structure is shown in Fig. 1. The activities of YM09330 against gram-negative bacteria, including β-lactamase-producing resistant strains, were much higher than those of cefoxitin, cefmetazole, and cefazolin. In addition, YM09330 was not hydrolyzed by any of the β-lactomases obtained from cephalosporinase-producing bacteria, whereas cefamandole and cefuroxime were hydrolyzed by *Proteus vulgaris*-type and *Pseudomonas cepacia*-type β-lactamases (17). Tachibana et al. reported the pharmacokinetics of YM09330 in animals (16; A. Tachibana, M. Komiya, Y. Kikuchi, K. Yano, and K. Mashimo, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 19th, Boston, Mass., abstr. no. 563, 1979). The present communication compares the pharmacological properties of YM09330 with those of cefmetazole and cefazolin in mice, rats, rabbits, dogs, and rhesus monkeys.

MATERIALS AND METHODS

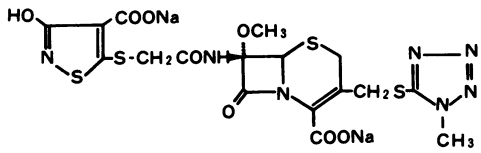
Antibiotics. The antibiotics used in the study were YM09330 (Yamanouchi, Japan), cefmetazole (CS-1170, Sankyo, Japan), and cefazolin (CEZ, Fujisawa, Japan). All were obtained as lyophilized sodium salts.

Animals. Male ICR mice, aged 5 to 8 weeks, weighing 25 to 33 g; Sprague-Dawley rats, aged 6 to 8 weeks, weighing 200 to 350 g; male albino rabbits weighing 2.5 to 3.3 kg; female beagle dogs weighing 8 to 16 kg; and rhesus monkeys of both sexes weighing 3 to 6 kg were used.

Administration of the drugs. The antibiotics were dissolved in sterile physiological saline for intravenous administration and in sterile distilled water for intramuscular administration. The injection volumes were 10 ml/kg of the body weight by the intravenous route for mice and rats, 1 ml/kg of the body weight by the intravenous route for rabbits, dogs, and monkeys, and 0.5 ml/kg of the body weight by the intramuscular route for rats and dogs.



YM09330



Tautomer

FIG. 1. *Chemical structure of YM09330. Disodium(6R,7S)-7-[[4-(carbamoylcarboxylatomethylene)-1,3-dithietan-2-yl]carboxamido]-7-methoxy-3-[[(1-methyl-1H-tetrazol-5-yl)thio]-methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate.*

Plasma, urine, and bile specimens. Blood samples were obtained periodically after administration and were immediately heparinized, then centrifuged at 1,000 × *g* for 15 min. Urine specimens were collected during several consecutive periods after dosing. Mice, rats, rabbits, and monkeys were housed in metabolism cages during the collection of urine samples. Dog urine was collected periodically by bladder catheterization. Bile specimens were obtained from rats, rabbits, and dogs. These animals were anesthetized with ether or pentobarbital, and a polyethylene cannula was inserted into the bile duct and the gallbladders of rabbits and dogs were isolated by ligatures. The rats were housed in a restraining cage, and rabbits and dogs were fixed in a supine position during bile collection. Plasma, urine, and bile specimens were kept in a frozen state until assayed for the drug concentration.

Bioassay. Concentrations of the antibiotics in plasma, urine, and bile were determined by the paper disk method on nutrient agar or sensitivity test agar with *Escherichia coli* NIHJ as the test organism for YM09330 and *Bacillus subtilis* ATCC 6633 as the test organism for cefmetazole and cefazolin. Standard solutions were prepared in pooled plasma for plasma assays and in 0.1 M phosphate buffer (pH 7.0) for assays of urine and bile. For assays of antibiotic concentrations in tissues, organs were removed and immediately homogenized in 3 volumes of ice-cold 0.1 M phosphate buffer (pH 7.0); the homogenates were centrifuged at 1,000 × *g* for 30 min. The antibiotic concentrations in the supernatants were determined by the paper disk method. The samples were diluted when necessary with the corresponding pooled animal plasma for plasma samples or with 0.1 M phosphate buffer (pH 7.0) for urine and bile samples until the concentrations were in the range of the standard curves. Assay results were expressed as equivalent concentration of free antibiotic acid.

Bioautography. Specimens of urine obtained from mice, rats, rabbits, dogs, and monkeys during the first 3 h after dosage were studied. Thin-layer chromatography was performed on commercially available silica gel plates containing a fluorescent indicator (silica gel F_{254}, E. Merck), with a solvent system of ethyl acetate-acetic acid-water (10:7:3 [vol/vol]). Two microliters of each urine sample was spotted on the plates, which were then subjected to ascending development in the solvent system until the solvent front reached 10 cm from the origin. The chromatogram was air dried, and spots were visualized with short-wave ultraviolet light and placed on an agar plate inoculated with *E. coli* NIHJ for 15 min. The chromatogram was removed, and the agar plate was incubated overnight at 37°C. In the above system, YM09330 produced a growth inhibition zone at a R_f value of 0.75, and the tautomer of YM09330 (Fig. 1) produced a growth inhibition zone at a low R_f value of 0.2 to 0.3.

Serum protein binding. YM09330, cefmetazole, or cefazolin was added to fresh pooled sera of rats, rabbits, dogs, monkeys, and humans at concentrations of 100 μg/ml and incubated at 37°C for 1 h with gentle shaking. These solutions were subjected to centrifugal ultrafiltration with CF50 centriflo membrane cones (Amicon). The protein-free ultrafiltrates containing the unbound antibiotics of each solution were assayed to determine the antibiotic contents with the paper disk method with *E. coli* NIHJ as the test organism for YM09330 and *B. subtilis* ATCC 6633 as the test organism for cefmetazole and cefazolin. Binding to the ultrafiltration membrane cones did not occur.

Pharmacokinetic study. Drug concentrations in plasma after intravenous administration of the antibiotics were analyzed by the two-compartment open model for rats, rabbits, dogs, and monkeys, and by the one-compartment open model for mice (5). In the two-compartment open model, an exponential equation, $C_p = Ae^{-\alpha t} + Be^{-\beta t}$, is used to express the plasma concentration-time curve, where C_p represents the drug concentration in plasma at time t after dosing, α and β are the hybrid rate constants for the distribution and elimination phases, respectively, and A and B are the zero-time intercepts of the two components of the biexponential curves. Specific first-order rate constants (K_{12}, K_{21}, and K_{el}), as well as the volumes of central compartment (V_c), peripheral tissue compartment (V_t), and steady state (V_{dss}) during elimination (β) phase, were calculated from the usual equations (5, 13). The area under the plasma concentration-time curve (AUC) was derived from the expression AUC $= A/\alpha + B/\beta$ and extrapolated to infinity. The half-lives of antibiotics in plasma were calculated by the following equations: $t_{1/2\alpha} = \ln 2/\beta$ and $t_{1/2\beta} = \ln 2/\beta$. The computer program NONLIN was used to fit the data to obtain the above pharmacokinetic parameters (10).

RESULTS

Concentrations in plasma. YM09330 was administered intravenously in a dose of 20 mg/kg to mice, rats, rabbits, dogs, and monkeys. The plasma concentration curves of YM09330 in the animal species are shown in Fig. 2. The plasma levels of YM09330 after intravenous injection

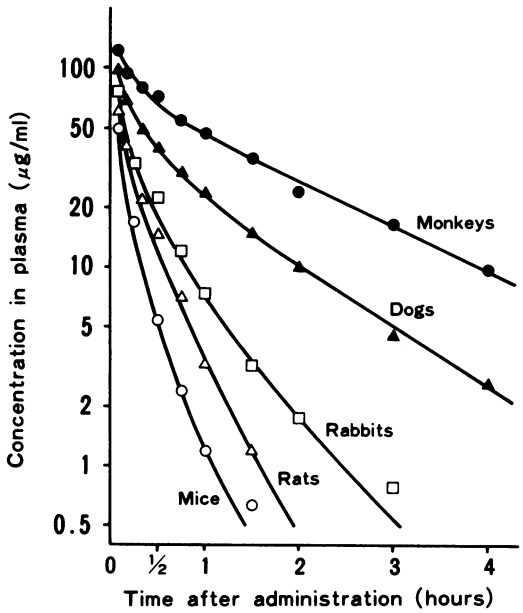


FIG. 2. *Plasma levels of YM09330 after intravenous injection of 20 mg/kg into mice* (○), *rats* (△), *rabbits* (□), *dogs* (▲), *and monkeys* (●).

were observed to decline biphasically. The average drug concentrations at 30 min after dosing were 5.5 μg/ml for mice, 16.1 μg/ml for rats, 23.9 μg/ml for rabbits, 42.6 μg/ml for dogs, and 75.7 μg/ml for monkeys. In dogs and monkeys, which were given YM09330 intravenously, the plasma levels 4 h after dosing were 3.0 μg/ml and 12.0 μg/ml, respectively. Persistence of the drug in plasma was the highest in monkeys and decreased progressively in dogs, rabbits, rats, and mice. The pharmacokinetic parameters of YM09330 concentration curves in plasma, calculated by using the one-compartment open model for mice and by the two-compartment open model for the other animal species with first-order rate constant, are summarized in Table 1. The concentration in the central compartment at time zero (C_p^0) for YM90330 was highest in monkeys, followed by dogs and rabbits. However, the zero-time intercepts (B) obtained by extrapolation of the eliminative linear phase in the animal species tested were about the same, ranging from 41 to 77 μg/ml. The biological half-life times of β-phase ($t_{1/2\beta}$) of YM09330 after intravenous administration of 20 mg/kg were 13.0 min in mice, 15.9 min in rats, 30.5 min in rabbits, and 55.5 min in dogs; they were the highest, 75.6 min, in monkeys. The height and persistence of the YM09330 concentration in plasma of these species were consistent with the area under the plasma concentration curves (AUC). The elimination rate constant (K_{el}) was 0.90 for monkeys, compared with 1.97 for dogs, 3.19 for rabbits, and 4.26 for rats. Although the volumes of distribution at steady state (V_{dss}) per kilogram of body weight were comparable among the animal species, the volumes of central compartment (V_c) per kilogram of body weight were low in monkeys and high in rats. Total clearance of YM09330 was the highest in mice (1,451 ml/h per kg) and the lowest in monkeys (102 ml/h per kg).

The plasma levels of YM09330, cefmetazole, and cefazolin in rats, rabbits, and dogs at various times after intravenous administration of 20 mg/kg are shown in Fig. 3 and 4. Levels of YM09330 were lower than those of cefazolin but higher than those of cefmetazole. The respective half-lives of β-phase ($t_{1/2\beta}$) for YM09330, cefmetazole, and cefazolin were 15.9, 11.0, and 18.7 min in rats; and 30.5, 26.7, and 39.5 min in rabbits (Table 2). The concentration curves of the three antibiotics in plasma of dogs were nevertheless closely related, and the calculated β-phase half-life time of YM09330 was 55.5 min in dogs, longer than that of cefmetazole (44.6 min) and comparable to that of cefazolin (52.3 min). In monkeys, on the other hand, plasma levels of YM09330 were higher and more prolonged than those of cefazolin. The β-phase half-life times in monkeys were 75.6 min for YM09330 and 42.3 min for cefazolin. Compared with cefazolin, the elimination of YM09330 from plasma was rapid in rats and rabbits, and slow in monkeys.

Concentrations in tissue. The concentrations of YM09330, cefmetazole, and cefazolin in tissues were determined in rats at 5, 10, 20, 30, 45, 60, 90, and 120 min after an intravenous injection of 20 mg/kg with the results shown in Fig. 5. The concentrations of YM09330 were highest in the kidneys, followed by the plasma, liver, lung, heart, and spleen in that order. At 60 min after intravenous drug administration, the concentrations of YM09330 and cefazolin in the rat were still high in the kidneys, plasma, liver, and lung, whereas those of cefmetazole were not detectable in any organ. Of the three antibiotics examined, cefmetazole seemed to be the fastest in the elimination from the various tissues, as well as from the plasma of rats. In addition, the concentration of cefmetazole in the liver was the highest of all the tissues tested, followed by the kidneys and plasma in rats, which was in good agreement with the high recovery rate of cefmetazole in rat bile (15). Similar experiments were performed in dogs (Table 3). Distribution of YM09330 in various tissues of dogs after an intravenous injection of 20 mg/kg was similar to that in rats. At 2 h after dosage, the concentra-

TABLE 1. *Pharmacokinetic parameters of YM09330 after intravenous injection of 20 mg/kg to experimental animals*

Animal	Pharmacokinetic parameter[a]													
	A (μg/ml)	B (μg/ml)	C_p^0 (μg/ml)	α (h^{-1})	β (h^{-1})	$t_{1/2\alpha}$ (min)	$t_{1/2\beta}$ (min)	AUC (μg·h/ml)	V_c (ml/kg)	V_{dss} (ml/kg)	K_{12} (h^{-1})	K_{21} (h^{-1})	K_{el} (h^{-1})	Cl (tot) (ml/h per kg)
Mouse[b]			66		3.20		13.0	20.6		241			2.58	1,451
Rat	56	54	111	10.8	2.62	3.9	15.9	26.0	180	250	2.51	6.60	4.26	767
Rabbit	93	41	134	7.8	1.36	5.3	30.5	42.0	150	237	10.0	3.66	3.19	479
Dog	102	62	164	8.5	0.75	4.9	55.5	83.3	122	249	4.10	3.17	1.97	240
Monkey	100	77	177	5.1	0.55	8.2	75.6	168.0	113	167	1.56	3.18	0.90	102

[a] C_p^0, Drug concentration in plasma immediately after administration; AUC, area under plasma concentration-time curve; V_c, volume of distribution for central compartment; V_{dss}, volume of distribution at steady state; Cl (tot), total clearance of the drug.

[b] Values of mice were based on a one-compartment model.

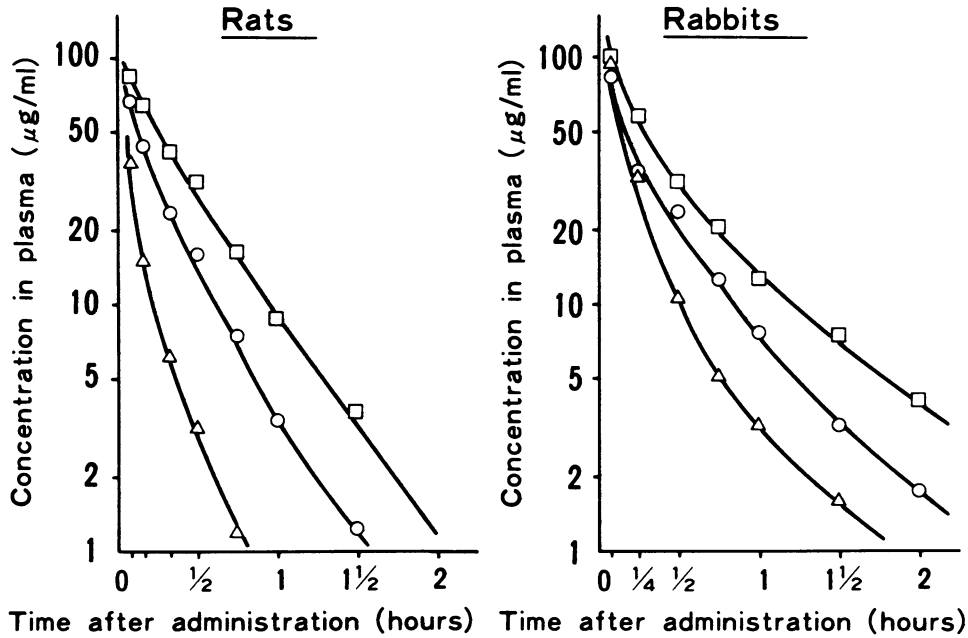


FIG. 3. *Comparative plasma concentration curves of YM09330* (○), *cefmetazole* (△), *and cefazolin* (□) *after intravenous injection of 20 mg/kg into rats and rabbits. Each observed point represents mean of three animals.*

tion of YM09330 was highest in the kidneys (12.6 μg/g), followed by the liver (9.7 μg/g), and the plasma (8.4 μg/ml). The ratios of concentrations in tissue to those in plasma were 1.5 for the kidneys, 0.9 to 1.3 for the liver, 0.3 for the lung, and 0.1 for the heart and spleen regardless of the time after dosage.

Urinary and biliary excretion. The excretion of YM09330 in urine and bile after intravenous and intramuscular administration of 20 mg/kg is summarized in Table 4. Recovery of YM09330 in urine over a 48-h period was 67.2% of the dose in mice, 52% of the dose in rats and dogs, 74.2% of the dose in rabbits, and 59.7% of the dose in monkeys. The major excretion occurred within 6 h of drug administration. After that, only 1 to 2% of the dose was recovered, and none was detected in the 24- to 48-h urine collections. Results similar to those of the intravenous schedule were obtained after intramuscular administration of YM09330 to rats and dogs.

From 48.1 and 51.9% of the dose of YM09330 was recovered in the bile of the rat over a period of 24 h after intravenous and intramuscular administration, the greatest part being excreted within the first 3 h. Altogether 100% of the

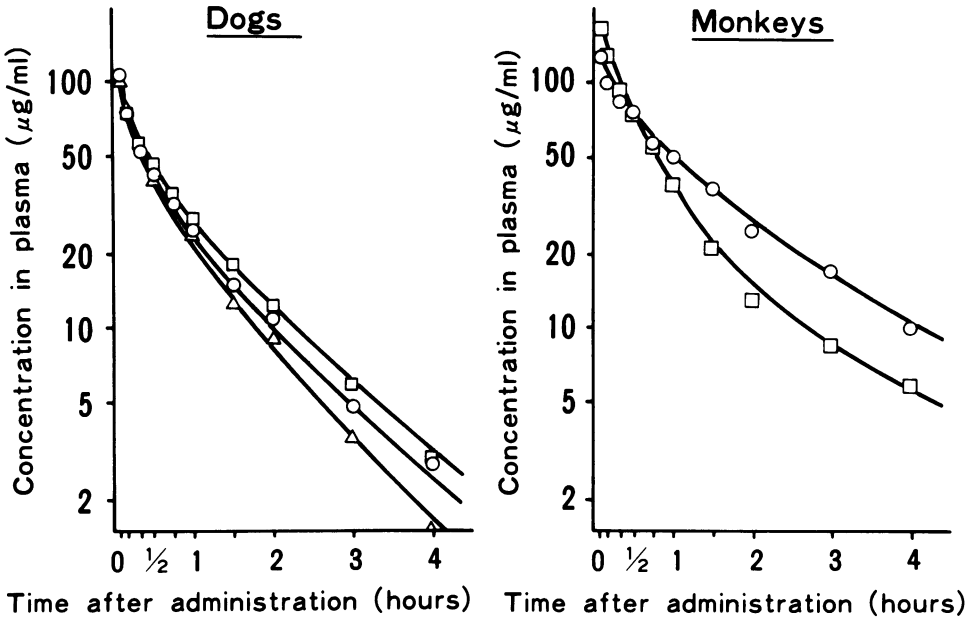


FIG. 4. *Comparative plasma concentration curves of YM09330 (○), cefmetazole (△), and cefazolin (□) after intravenous injection of 20 mg/kg into dogs and monkeys. Each observed point represents mean of three animals.*

TABLE 2. *Comparative half-life time of YM09330, cefmetazole, and cefazolin in plasma after intravenous injection of 20 mg/kg to experimental animals*

Antibiotic	Half-life time (min) for:				
	Mouse[a]	Rat	Rabbit	Dog	Monkey
YM09330	13.0	15.9	30.5	55.5	75.6
Cefmetazole	9.5	11.0	26.7	44.6	NT[b]
Cefazolin	13.5	18.7	39.5	52.3	42.3

[a] Values were based on one-compartment model.
[b] NT, Not tested.

intravenous dose and 94.5% of the intramuscular dose were recovered from urine and bile of the rat combined. In contrast, biliary excretion of YM09330 in rabbits and dogs was comparatively low, 4.42 and 17.4% of the dose, respectively, at 24 h. The total recoveries of YM09330 from bile and urine were 78.4% in rabbits and 70.2% in dogs.

Metabolite and tautomer in urine. Metabolites of YM09330 were sought by thin-layer chromatography-bioautography and high-performance liquid chromatography. No antimicrobially active metabolite was detected in urine or bile obtained after the administration of YM09330 to experimental animals. However, it was found that a small part of YM09330 was excreted in rat urine as a biologically active tautomer. The bioautograms of urine obtained from animals receiving YM09330 are shown in Fig. 6. The growth inhibition zones for YM09330 had a high R_f value in the solvent system, whereas those for the tautomer had a low R_f value. Very little, if any, of the tautomer of YM09330 was excreted in the urine of mice, rats, and dogs. In contrast, a large amount of the tautomer was recovered in the urine of rabbits and monkeys.

Binding to serum protein. As indicated in Table 5, YM09330 was highly bound to the serum protein of humans and monkeys, whereas binding to the protein of dogs, rabbits, and rats was relatively low. The extent of binding of YM09330 to human and animal serum protein was similar to that of cefmetazole. Binding of both YM09330 and cefazolin to serum protein was high in humans and monkeys, but low in dogs. There was a marked difference, however, between the binding of YM09330 and cefazolin in rats and rabbits. Thus, the respective bindings were 29.7 and 50.6% for YM09330 and 92.7 and 90.8% for cefazolin.

DISCUSSION

YM09330 is a new broad-spectrum cephamycin-type antibiotic which differs from the cephalosporin type in the marked stability against β-lactamases (17). The minimum inhibitory concentrations for 70% of the clinical isolates of *E.*

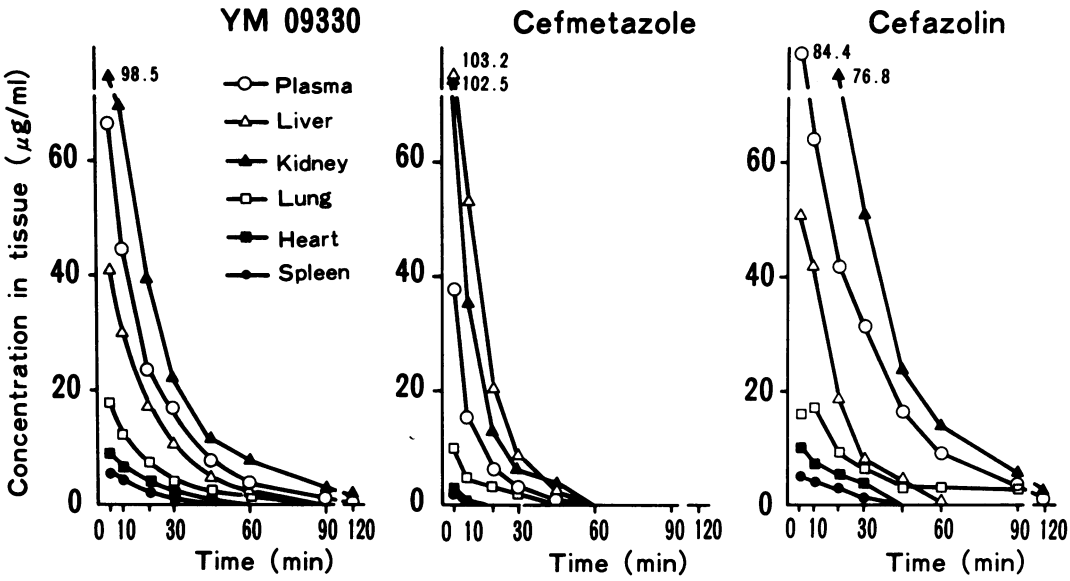


FIG. 5. *Drug concentrations in plasma and tissues after intravenous injection of 20 mg/kg of YM09330, cefmetazole, and cefazolin into rats. Each point represents mean of three animals.*

TABLE 3. *Concentrations of YM09330 in tissues after intravenous injection of 20 mg/kg to beagle dogs*

Tissue	Concn in tissue (μg/g or ml)[a] at:		
	½ h	1 h	2 h
Plasma	33.3 ± 4.8	20.6 ± 3.3	8.4 ± 1.2
Lung	14.9 ± 1.7	6.4 ± 0.20	2.34 ± 0.37
Heart	3.4 ± 0.10	1.84 ± 0.06	(0.58)
Spleen	4.5 ± 0.15	2.22 ± 0.24	0.87 ± 0.04
Liver	43.3 ± 9.8	18.9 ± 2.4	9.7 ± 1.5
Kidney	60.3 ± 11.5	31.2 ± 1.7	12.6 ± 3.2

[a] Each value represents mean of three dogs ± standard error.

coli, Klebsiella pneumoniae, Haemophilus influenzae, Proteus mirabilis, indole-positive *Proteus* species, and *Serratia marcescens* ranged from 0.17 to 4.5 μg of YM09330 per ml, indicating that YM09330 is one of the most active cephamycin derivatives thus far reported (17).

The pharmacokinetic profile of YM09330 after intravenous and intramuscular administration of 20 mg/kg to experimental animal species was examined in this study. The concentrations of YM09330 in plasma were much higher than those of cefmetazole (cephamycin antibiotic) in every animal species tested, whereas compared with those of cefazolin, they were lower in mice, rats, and rabbits but higher in monkeys. In general, the plasma levels of the cephalosporins such as cefazolin, cefoxitin, cefamandole, cefmetazole, and cefotaxime are higher in dogs than in monkeys (1, 11). It should be pointed out that the concentrations of YM09330 in plasma were higher and more prolonged in monkeys than in dogs. Although the monkeys used in the study showed approximately one-fourth of the body surface area as compared with the dogs, the half-lives in the β-phase and the AUC of YM09330 after an intravenous dose of 20 mg/kg were 75.6 min and 168 μg.h/ml for monkeys, compared with 55.5 min and 83.3 μg.h/ml for dogs, respectively. Of the animal species examined in the study, the values of the total drug clearance per body weight and of the elimination rate constant for YM09330 observed in monkeys were the lowest. It is of interest that the pharmacokinetic profiles of YM09330 in the rhesus monkeys and humans are similar (unpublished data).

The tissue levels of YM09330 in rats were similar to those of cefazolin and were much higher than those of cefmetazole. As is the case with various parenteral β-lactam antibiotics, including the cephalosporins, which are metabolized in the liver to the less active compounds (18), YM09330 showed higher levels in the kidneys than in the liver and plasma. However, the concentrations of cefmetazole in the tissues of rats were highest in the liver, followed by the kidneys and plasma. This is believed to be related to the high excretion of cefmetazole into rat bile (15).

Cephalosporins, such as cephalothin (3, 7, 19), cephapirin (2, 9), cephacetrile (8, 12), cefotaxime (4, 20), with an acetoxymethyl substituent at the 3-position of β-lactam skeleton are metabolized to less active metabolites. YM09330 differs from

TABLE 4. *Excretion of YM09330 in urine and bile after intravenous and intramuscular injection of 20 mg/kg to mice, rats, rabbits, dogs, and monkeys*

Animal	Route of administration	Dose (mg/kg)	Recovery (% of the dose)[a]					
			0–6 h	6–24 h	24–48 h	0–48 h[b] (A)	0–24 h[c] (B)	Total (A + B)
Mouse	i.v.	20	64.6	2.51	ND[d]	67.2 ± 5.36[e]		
Rat	i.v.	20	50.1	1.82	ND	52.0 ± 2.24	48.1 ± 2.04	100.1
Rat	i.m.	20	41.8	0.84	ND	42.6 ± 1.21	51.9 ± 2.34	94.5
Rabbit	i.v.	20	73.7	0.61	ND	74.3 ± 5.92	4.42	78.7
Dog	i.v.	20	51.9	0.94	ND	52.8 ± 2.27	17.4 ± 1.56[f]	70.2
Dog	i.m.	20	50.8	1.56	0.05	52.4 ± 1.41		
Monkey	i.v.	20	58.1	1.66	ND	59.7 ± 0.49		

[a] The values were obtained by bioassay with *E. coli* NIHJ as the test organism.
[b] In urine.
[c] In bile.
[d] ND, Not detectable.
[e] Mean ± standard error.
[f] Anesthetized dog.

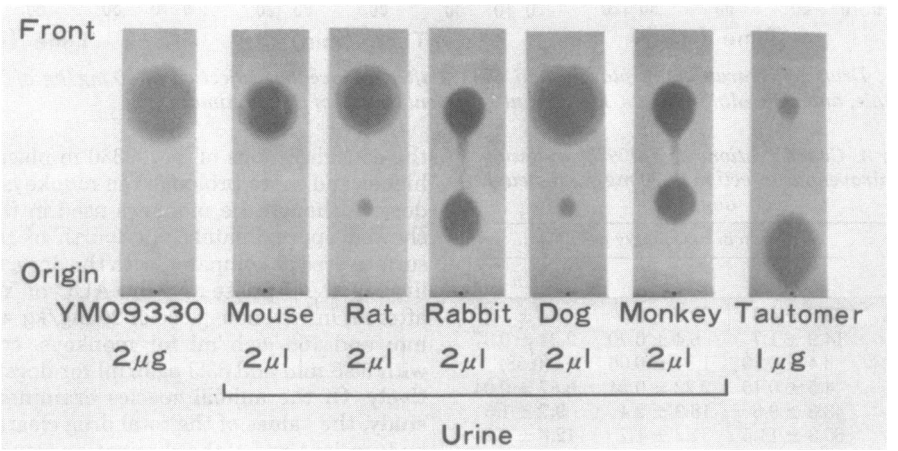


FIG. 6. *Bioautograms of urine samples obtained 3 h after intravenous injection of 20 mg/kg of YM09330 into mice, rats, rabbits, dogs, and monkeys. Solvent system: ethyl acetate-acetic Acid-water (10:7:3). E. coli NIHJ was used as the test organism.*

TABLE 5. *Binding of YM09330, cefmatazole, and cefazolin to experimental animal and human serum*[a]

Antibiotic	Concn (μg/ml)	Protein binding (%) to:				
		Human	Monkey	Dog	Rabbit	Rat
YM09330	100	90.9	87.2	38.8	50.6	29.7
Cefmetazole	100	84.8	80.8	25.0	59.0	44.0
Cefazolin	100	87.0	92.1	27.9	90.8	92.7

[a] The protein-free ultrafiltrates obtained by centrifugal ultrafiltration were measured by bioassay with *E. coli* NIHJ for YM09330 and *B. subtilis* ATCC 6633 for cefmetazole and cefazolin as the test organisms.

the above cephalosporins with respect to a substituent at the 3-position. No metabolite resulting from enzymatic degradation was detected in the plasma, urine, or bile of experimental animals by thin-layer chromatography or high-performance liquid chromatography. These findings are consistent with the total recovery (approximately 100% of the dose) from the urine and bile of rats. As is shown in Fig. 6, it was found that a small amount of YM09330 was converted to its tautomer in the urine of mice, rats, and dogs and that a large amount of the tautomer was detected in the urine of rabbits and monkeys. The tautomer has a 3-hydroxy-4-carboxy isothiazole ring at the 7-β-position of β-lactam skeleton, showed a lower R_f value and a longer retention time on thin-layer chromatography and high-performance liquid chromatography, respectively, and possessed an antibacterial activity quite similar to that of YM09330 (6, 16). A further investigation of the equilibrium reaction between YM09330 and the tautomer showed that YM09330 was easily converted to the tautomer by a high alkaline pH (over pH 9)

and a high concentration of Mg^{2+} (16). Because of the high concentration of Mg^{2+} (about 20 mM), the high pH value (about pH 8.5) in rabbit urine, and the high pH value (about pH 9) of monkey urine, a considerable part of YM09330 in those specimens was present as the tautomer. On the other hand, a very small amount of the tautomer was detected in plasma of rats and rabbits and tissues of rats. These findings indicate that YM09330 could be converted to the tautomer after excretion into urine and bile. Since the antibacterial activities of the tautomer were the same as those of YM09330 in various pH media, the urinary and biliary recoveries determined by bioassay were in good agreement with those of YM09330 and the tautomer determined by high-performance liquid chromatography, in spite of differences in the extent of the tautomer formation in urine and bile (16). In humans, less than 5% of the dose was found in the form of the tautomer in urine after intravenous administration of YM09330 to normal volunteers in a preclinical study, and a very small amount of the tautomer was detected in human plasma (unpublished data). The equilibrium reaction in body fluids between YM09330 and the tautomer will be reported separately.

Serum protein binding of YM09330 was similar to that of cefmetazole in animal species tested and was higher than that of cefotaxime (11, 20) and ceftizoxime (11) in humans. Although YM09330, like cefazolin (14), was highly bound to the serum protein of humans, there was no apparent correlation between plasma half-life and serum protein binding in different species. In addition, no significant differences in the minimal inhibitory concentrations of YM09330 against various bacteria were observed when serum was added to culture media (17).

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