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In Vitro and In Vivo Antibacterial Activity of T-1982, a New Semisynthetic Cephamycin Antibiotic

M. TAI,[1] Y. FUKUOKA,[1] A. YOTSUJI,[1] K. KUMANO,[2] M. TAKAHATA,[1] H. MIKAMI,[2] T. YASUDA,[1]* I. SAIKAWA,[1] AND S. MITSUHASHI[2]

Research Laboratory, Toyama Chemical Co., Ltd., Toyama,[1] and Department of Microbiology, School of Medicine, Gunma University, Maebashi, Gunma,[2] Japan

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The activities of T-1982 (sodium 7β-[(2R,3S)-2-(4-ethyl-2,3-dioxo-1-piperazinecarboxamido)-3-hydroxybutanamido]-7α-methoxy-3-[(1-methyl-1H-tetrazol-5-yl)thiomethyl]-3-cephem-4-carboxylate) against various gram-positive and gram-negative bacteria were compared with those of cefmetazole, cefoxitin, cefazolin, and cefoperazone. T-1982 was active against both gram-positive and gram-negative bacteria, including genera resistant to the other cephalosporins. T-1982 exhibited greater activity than did cefmetazole, cefoxitin, cefazolin, or cefoperazone against *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis*, and *Serratia marcescens* and was also highly active against *Bacteroides fragilis*. T-1982 was as stable to various β-lactamases as were cefmetazole and cefoxitin. The therapeutic activities of T-1982 in mice experimentally infected with various gram-negative bacteria were superior to those of cefmetazole, cefoxitin, cefazolin, and cefoperazone.

Research performed at our laboratory demonstrated that the introduction of a 4-ethyl-2,3-dioxo-1-piperazine-carbonyl moiety into the side chains of penicillins and cephalosporins enhanced antibacterial activity. This demonstration led to development of piperacillin (10) and cefoperazone (4), both of which are highly active against gram-positive and gram-negative bacteria. The 4-ethyl-2,3-dioxo-1-piperazinecarbonyl moiety was also introduced into cephamycins, resulting in preparation of T-1982 (sodium 7β-[(2R,3S)-2-(4-ethyl-2,3-dioxo-1-piperazinecarboxamido)-3-hydroxybutanamido]-7α-methoxy-3-[(1-methyl-1H-tetrazol-5-yl)thiomethyl]-3-cephem-4-carboxylate) (Fig. 1).

In this paper, the in vitro and in vivo antibacterial activities of T-1982 and its resistance to destruction by β-lactamases are discussed, and a comparison is made of these qualities with those of other cephalosporins and cephamycins.

MATERIALS AND METHODS

Antibiotics. T-1982 and cefoperazone were synthesized in the Research Laboratory of Toyama Chemical Co., Ltd., Toyama, Japan. All other antibiotics were obtained as commercial preparations. Cefoxitin was from Merck Sharp & Dohme, West Point, Pa., cefmetazole was from Sankyo Co., Ltd., Osaka, Japan, cefazolin was from Fujisawa Pharmaceutical Co., Ltd., Tokyo, Japan, cephaloridine was from Eli Lilly & Co., Indianapolis, Ind., and penicillin G was from Meiji Seika Kaisha Ltd., Tokyo, Japan.

Organisms. Recent clinical isolates were obtained from various hospitals in Japan.

Media. Heart infusion agar and broth and brain heart infusion broth were the products of Eiken Chemical Co., Ltd. GAM agar and broth were the products of Nissui Pharmaceutical Co., Ltd. Other media prepared locally included peptone broth (10 g of polypeptone plus 5 g of NaCl in 1,000 ml of distilled water) and medium B (2 g of yeast extract, 10 g of polypeptone, 8 g of $Na_2HPO_4 \cdot 12H_2O$, 2 g of KH_2PO_4, 1.2 g of $(NH_4)_2SO_4$, 2 g of glucose, 0.4 g of $MgSO_4 \cdot 7H_2O$ in 1,000 ml of distilled water).

In vitro antibacterial activity. The minimum inhibitory concentrations (MICs) were determined by the agar dilution method with a multi-loop inoculating device (Planter; Sakuma, Tokyo, Japan) which delivered about 5 μl of cell suspension to the agar plates. For nonfastidious organisms, the medium employed was heart infusion agar. For tests with *Haemophilus influenzae*, heart infusion agar supplemented with 5% digested horse blood was used. Inocula were prepared from overnight cultures at 37°C in peptone broth, *H. influenzae* excepted. The latter organism was grown in brain heart infusion broth supplemented with 2 μg of β-NAD (Sigma Chemical Co., St. Louis, Mo.) per ml

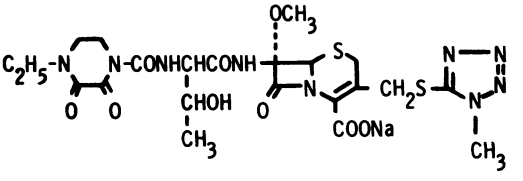

FIG. 1. Chemical structure of T-1982.

and 10 μg of hemin (Sigma) per ml. A final inoculum of about 10^4 colony-forming units (CFU), prepared by dilution of an overnight culture, was applied to agar plates. Final inocula of about 10^6, 10^4, and 10^2 CFU were prepared and used for studies involving inoculum size. MICs were determined after incubation at 37°C for 18 h. For testing *Bacteroides fragilis*, incubation was carried out by the GasPak method (BBL Microbiology Systems, Cockeysville, Md.). GAM broth and agar were used for the preculture and test culture, respectively.

Minimal bactericidal concentrations (MBCs) were determined by the broth dilution method, using heart infusion broth and inocula of 10^6 and 10^4 CFU/ml. The MIC was defined as the lowest concentration of antibiotic which suppressed visible growth during 18 h of incubation at 37°C. Tubes showing no growth were subcultured (one 5-μl loopful) onto heart infusion agar and incubated at 37°C for 18 h. The MBC was defined as the lowest concentration which prevented visible growth on the subculture plate.

Effects of human serum on antibacterial activity were examined by the heart infusion broth dilution method, using an inoculum of approximately 10^4 CFU/ml.

Serum protein binding. The centrifugal ultrafiltration technique was used to determine binding rates. A solution of the antibiotic in 1/15 M phosphate buffer (pH 7.0) was diluted with an appropriate volume of sterile human serum to give a concentration of 25 μg/ml. This solution was poured into a cellulose tube (size 8/32; Visking Union Carbide Co., Chicago, Ill.), which was suspended in a 10-ml glass tube and centrifuged at 1,000 × *g* for 30 min. The concentration of antibiotic in the ultrafiltrate was measured by high-pressure liquid chromatography with a column (25 cm by 4 mm inner diameter) of LiChrosorb RP-18 (E. Merck Co., Darmstadt, Germany). The mobile phase of high-pressure liquid chromatography consisted of 13% CH_3CN, 1.4% 1 M CH_3COOH, and 2.7% 1 M $CH_3COOH \cdot N(C_2H_5)_3$ in water. The flow rate was 1.0 ml/min at ambient temperature. The eluant was monitored by UV detection at 254 nm.

Stability to β-lactamase. Cultures of each strain were prepared in brain heart infusion broth, 20-ml samples of which were inoculated into 200 ml of medium B and shaken for 3 h at 37°C. For induction purposes, cephalosporinase-producing strains were supplemented with penicillin G in amounts ranging from one-eighth to one-half of its MIC and incubated for a further 2 h at 37°C with shaking. Cells were harvested by centrifugation, washed once with 0.1 M phosphate buffer (pH 7.0), and suspended in 5 ml of the same buffer. Cell suspensions were treated by an ultrasonic disrupter for 5 min at 0°C and centrifuged at 4°C for 30 min at 12,000 rpm. The resulting supernatants were used as the crude enzymes. β-Lactamase activity was determined by the iodometric assay of Perret (8). The specific activity of β-lactamase was expressed as micromoles of substrate hydrolyzed per minute per milligram of protein. Protein contents of the crude enzyme preparations were determined by the method of Lowry et al. (3). Substrate specificity was expressed as the relative rate of hydrolysis of substrate, taking the absolute rates of hydrolysis of penicillin G and cephaloridine by penicillinase and cephalosporinase, respectively, as 100.

In vivo antibacterial activity. Fifteen ICR strain male mice, aged 4 weeks and weighing 18 to 20 g each, were used at each dose level. Mice were challenged intraperitoneally with 3- to 310-fold 50% lethal doses of the respective organisms. These inocula, prepared from overnight cultures on heart infusion agar at 37°C, were suspended in physiological saline solution containing 5% mucin (hog gastric mucin, LP-K type; Wako Chemical Co., Tokyo, Japan), unless otherwise specified. In all cases, a series of doses of the antibiotics, increasing by twofold increments, were administered subcutaneously once at 1 h after infection. The total number of surviving mice was recorded, usually at 1 week after infection, and the dose that gave protection to 50% of the infected mice was estimated by the method of Litchfield and Wilcoxon (2).

RESULTS

Antimicrobial activity against clinical isolates. The in vitro antimicrobial activity of T-1982 against gram-positive and gram-negative bacteria, including *B. fragilis*, was compared with those of cefmetazole, cefoxitin, cefazolin, and cefoperazone (Table 1). T-1982 was less active than other antibiotics against *Staphylococcus* spp. but inhibited the growth of more than 90% of the strains at a concentration of 12.5 μg/ml. However, T-1982 was at least four times as potent as the other antibiotics against gram-negative bacteria, including *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Proteus vulgaris, Proteus rettgeri*, and *Serratia marcescens*. Against *Proteus morganii, Citrobacter freundii*, and *Enterobacter* spp. T-1982 was as active as or slightly less active than cefoperazone but more active than cefmetazole, cefoxitin, or cefazolin. T-1982 inhibited the growth of all of the test strains of *H. influenzae* at a concentration of 3.13 μg/ml but was less active than cefoperazone. T-1982 also displayed the greatest activity against *B. fragilis*, followed by cefmetazole, cefoxitin, cefoperazone, and cefazolin, in that order.

The effect of inoculum size on the activity of T-1982 against gram-positive and gram-negative bacteria is shown in Table 2. Only slight or no inoculum effects were observed with T-1982 against all strains, including β-lactamase-producing strains. Cefazolin and cefoperazone showed great variations in activity against β-lactamase-producing strains.

Serum protein binding and effect of serum on antibacterial activity. The binding rates of T-1982, cefmetazole, and cefoxitin to human serum were 54.9, 88.4, and 69.0%, respectively. When human serum was added at 10 to 50%, the MICs of T-1982 for *Staphylococcus aureus, E. coli*, and *Serratia marcescens* were scarcely altered, which was also true for cefmetazole and cefoxitin.

Bactericidal activity. The correlation of MICs and MBCs of T-1982 is shown in Table 3. With

TABLE 1. Comparative in vitro activities of T-1982 and other β-lactam antibiotics

Species (no. of isolates)	Antibiotic	MIC (μg/ml)		
		Range	50%	90%
Staphylococcus sp. (25)	T-1982	6.25–25	12.5	12.5
	Cefmetazole	0.78–1.56	0.78	1.56
	Cefoxitin	1.56–3.13	3.13	3.13
	Cefazolin	0.2–0.78	0.2	0.39
	Cefoperazone	0.39–3.13	0.78	1.56
E. coli (25)	T-1982	≤0.1–0.39	0.2	0.39
	Cefmetazole	0.78–1.56	0.78	1.56
	Cefoxitin	1.56–25	6.25	12.5
	Cefazolin	1.56–25	3.13	6.25
	Cefoperazone	≤0.1–25	0.39	6.25
K. pneumoniae (15)	T-1982	≤0.1–0.78	0.2	0.39
	Cefmetazole	0.78–3.13	1.56	3.13
	Cefoxitin	1.56–25	6.25	25
	Cefazolin	1.56–>100	6.25	>100
	Cefoperazone	0.2–>100	1.56	>100
P. mirabilis (25)	T-1982	0.2–6.25	1.56	3.13
	Cefmetazole	1.56–12.5	3.13	12.5
	Cefoxitin	3.13–25	12.5	25
	Cefazolin	3.13–25	12.5	12.5
	Cefoperazone	0.39–6.25	3.13	6.25
P. vulgaris (20)	T-1982	0.39–6.25	1.56	3.13
	Cefmetazole	1.56–6.25	3.13	6.25
	Cefoxitin	1.56–12.5	6.25	6.25
	Cefazolin	6.25–>100	>100	>100
	Cefoperazone	0.78–>100	6.25	100
P. rettgeri (25)	T-1982	0.2–50	1.56	12.5
	Cefmetazole	0.78–100	1.56	50
	Cefoxitin	1.56–>100	3.13	100
	Cefazolin	0.2–>100	50	>100
	Cefoperazone	≤0.1–100	3.13	25
P. morganii (25)	T-1982	0.78–100	3.13	25
	Cefmetazole	6.25–>100	6.25	50
	Cefoxitin	6.25–>100	12.5	100
	Cefazolin	12.5–>100	>100	>100
	Cefoperazone	0.78–50	1.56	12.5
S. marcescens (20)	T-1982	0.39–100	6.25	100
	Cefmetazole	3.13–>100	100	>100
	Cefoxitin	12.5–>100	100	>100
	Cefazolin	>100	>100	>100
	Cefoperazone	3.13–>100	50	>100
Enterobacter sp. (14)	T-1982	0.2–100	0.39	100
	Cefmetazole	1.56–>100	>100	>100
	Cefoxitin	6.25–>100	>100	>100
	Cefazolin	6.25–>100	>100	>100
	Cefoperazone	≤0.1–>100	0.39	50
C. freundii (20)	T-1982	≤0.1–>100	6.25	50
	Cefmetazole	0.78–>100	50	>100
	Cefoxitin	3.13–>100	>100	>100
	Cefazolin	0.78–>100	>100	>100
	Cefoperazone	≤0.1–>100	12.5	>100
H. influenzae (25)	T-1982	0.78–3.13	1.56	1.56
	Cefmetazole	3.13	3.13	3.13

TABLE 1—*Continued*

Species (no. of isolates)	Antibiotic	MIC (μg/ml)		
		Range	50%	90%
	Cefoxitin	3.13–12.5	6.25	12.5
	Cefazolin	12.5–50	25	25
	Cefoperazone	≤0.1–0.39	≤0.1	0.2
B. fragilis (29)	T-1982	0.78–50	1.56	6.25
	Cefmetazole	0.78–25	6.25	12.5
	Cefoxitin	1.56–25	6.25	12.5
	Cefazolin	1.56–>100	12.5	50
	Cefoperazone	1.56–100	6.25	25

TABLE 2. Effect of inoculum size on antibacterial activities of T-1982 and other β-lactam antibiotics

Strain	Inoculum size (CFU/ml)	MIC (μg/ml)				
		T-1982	Cefmetazole	Cefoxitin	Cefazolin	Cefoperazone
S. aureus FDA209P	6.0×10^5	12.5	1.56	3.13	0.78	1.56
	6.0×10^3	12.5	1.56	3.13	0.2	1.56
	6.0×10^1	12.5	1.56	1.56	0.2	1.56
E. coli NIHJ	3.1×10^6	≤0.1	0.78	1.56	1.56	≤0.1
	3.1×10^4	≤0.1	0.78	1.56	0.78	≤0.1
	3.1×10^2	≤0.1	0.78	1.56	0.78	≤0.1
E. coli TK-16	3.1×10^6	0.2	0.78	3.13	3.13	0.2
	3.1×10^4	≤0.1	0.39	1.56	1.56	≤0.1
	3.1×10^2	≤0.1	0.39	1.56	0.78	≤0.1
E. coli TK-3[a]	1.9×10^6	0.2	3.13	12.5	100	>200
	1.9×10^4	≤0.1	1.56	3.13	25	25
	1.9×10^2	≤0.1	0.78	1.56	12.5	6.25
K. pneumoniae Y-41	1.8×10^6	0.39	1.56	12.5	1.56	3.13
	1.8×10^4	0.2	0.78	3.13	1.56	0.2
	1.8×10^2	≤0.1	0.78	3.13	1.56	0.2
K. pneumoniae Y-4[a]	1.1×10^6	0.39	3.13	6.25	25	>200
	1.1×10^4	0.2	0.78	6.25	12.5	25
	1.1×10^2	0.2	0.78	6.25	6.25	12.5
S. marcescens IID620	1.7×10^6	0.2	3.13	6.25	>200	3.13
	1.7×10^4	≤0.1	1.56	3.13	>200	0.39
	1.7×10^2	≤0.1	0.78	3.13	>200	0.2
S. marcescens W-22[a]	2.0×10^6	3.13	200	>200	>200	>200
	2.0×10^4	1.56	100	200	>200	50
	2.0×10^2	1.56	50	50	>200	12.5
S. marcescens W-26[a]	1.5×10^6	0.39	25	25	>200	>200
	1.5×10^4	0.2	12.5	25	>200	25
	1.5×10^2	0.2	6.25	12.5	>200	12.5
P. vulgaris GN76[a]	1.0×10^6	1.56	6.25	6.25	>200	200
	1.0×10^4	1.56	3.13	6.25	>200	6.25
	1.0×10^2	0.39	3.13	3.13	>200	1.56

[a] β-Lactamase producing strain.

TABLE 3. Correlation between MICs and MBCs of T-1982 and other β-lactam antibiotics

Organism	Inoculum size (CFU/ml)	MBC/MIC (μg/ml)				
		T-1982	Cefmetazole	Cefoxitin	Cefazolin	Cefoperazone
S. aureus FDA209P	1.0×10^4	25/12.5	1.56/1.56	3.13/3.13	0.39/0.2	1.56/0.78
	1.0×10^6	>100/12.5	>100/6.25	>100/3.13	100/0.78	>100/0.78
E. coli NIHJ JC-2	1.8×10^4	0.39/0.39	1.56/1.56	12.5/12.5	1.56/1.56	0.2/0.2
	1.8×10^6	0.39/0.39	6.25/3.13	25/25	3.13/3.13	0.2/0.2
E. coli TK-16	5.4×10^4	0.2/0.1	0.39/0.39	3.13/3.13	1.56/1.56	0.1/0.1
	5.4×10^6	0.2/0.1	0.78/0.78	6.25/6.25	6.25/3.13	0.1/0.1
K. pneumoniae Y-50	1.0×10^4	≤0.1/≤0.1	0.78/0.78	1.56/1.56	1.56/1.56	0.2/0.2
	1.0×10^6	≤0.1/≤0.1	0.78/0.78	3.13/3.13	3.13/3.13	3.13/3.13
K. pneumoniae Y-4	2.0×10^4	0.39/0.2	1.56/0.78	6.25/6.25	12.5/12.5	25/25
	2.0×10^6	6.25/0.78	12.5/6.25	12.5/12.5	100/50	>100/>100
S. marcescens IID620	1.2×10^4	≤0.1/≤0.1	1.56/1.56	12.5/6.25	>100/>100	0.78/0.78
	1.2×10^6	0.39/≤0.1	12.5/6.25	50/25	>100/>100	100/1.56
S. marcescens W-108	3.0×10^4	25/12.5	>100/>100	>100/>100	>100/>100	>100/>100
	3.0×10^6	100/25	>100/>100	>100/>100	>100/>100	>100/>100
P. vulgaris GN3027	1.1×10^4	≤0.1/≤0.1	0.78/0.78	1.56/1.56	100/50	0.2/0.2
	1.1×10^6	0.2/0.2	1.56/1.56	3.13/3.13	>100/>100	50/50

an inoculum size of 10^4 CFU/ml, the MBCs of T-1982 for most of the test strains were either the same or only twice as high as the MICs. When the inoculum was increased to 10^6 CFU/ml, the MBC of T-1982 for *Staphylococcus aureus* FDA209P was eight times the MIC. However, the MBCs of cefmetazole, cefoxitin, cefazolin, and cefoperazone against this strain were 16- to 128 times higher than the MICs.

Stability to β-lactamase. The stabilities of T-1982 and six β-lactam antibiotics to β-lactamase are shown in Table 4, and the activities of these

TABLE 4. Stability of T-1982 in the presence of various β-lactamases

Enzyme	β-Lactamase class[a]	Substrate specificity (sp act)[b]						
		Cephaloridine	T-1982	Cefmetazole	Cefoxitin	Cefazolin	Cefoperazone	Penicillin G
Chromosomally mediated cephalosporinase								
E. coli GN5482	Ib	100 (90)	<0.1	<0.1	<0.1	130	0.9	22
C. freundii GN346	Ia	100 (2,900)	<0.01	<0.01	<0.01	150	0.3	15
S. marcescens W-8	Ia	100 (2,200)	<0.01	<0.01	<0.01	94	3	21
P. vulgaris GN76	Ic	100 (300)	<0.02	<0.02	<0.02	440	3	9
R-plasmid-mediated penicillinase								
E. coli TK-3	III (TEM)	115	<0.01	<0.01	<0.01	21	16	100 (300)
Pseudomonas aeruginosa GN3379	Vc	18	<0.05	<0.05	<0.05	2	0.1	100 (100)
Chromosomally mediated penicillinase from *K. pneumoniae* Y4	IV	41	<0.02	<0.02	<0.02	4	13	100 (250)

[a] Class of β-lactamase is based on the classification of Richmond (9).

[b] The rate of hydrolysis is expressed as a relative rate of hydrolysis, taking the absolute rate of cephaloridine or penicillin G as 100. Specific activity is expressed as units per milligram of protein.

TABLE 5. Antibacterial activities against β-lactamase-producing strains

Strain	MIC (μg/ml)				
	T-1982	Cefmetazole	Cefoxitin	Cefazolin	Cefoperazone
E. coli TK-3	≤0.1	1.56	3.13	25	25
E. coli GM5482	6.25	50	50	50	1.56
K. pneumoniae Y-4	0.2	0.78	6.25	12.5	25
C. freundii GN346	50	>200	>200	>200	6.25
S. marcescens W-8	50	>200	100	>200	100
P. vulgaris GN76	1.56	3.13	6.25	>200	6.25
P. aeruginosa GN3379	>200	>200	>200	>200	50

agents against various β-lactamase-producing organisms are shown in Table 5. T-1982 was as stable as cefmetazole (6) and cefoxitin (7) to typical cephalosporinases produced by *E. coli* GN5482, *C. freundii* GM346, and *Serratia marcescens* W-8 and to cefuroximase (1) produced by *P. vulgaris* GN76, the class Ic of β-lactamase of Richmond (5). T-1982 was also stable to R plasmid-mediated penicillinase types I and IV and the *K. pneumoniae* Y-4 chromo-

TABLE 6. In vivo antibacterial activity of T-1982

Strain	Challenge dose (CFU per mouse)	Challenge LD_{50}[a]	Antibiotic	MIC (μg/ml)	ED_{50} (mg/kg)[b]
S. aureus F-31	1.1×10^{8}[c]	26	T-1982	12.5	39.5 (22.6–69.1)
			Cefmetazole	1.56	4.37 (2.82–6.77)
			Cefoxitin	0.2	2.37 (1.74–3.22)
			Cefazolin	≤0.1	0.33 (0.24–0.47)
			Cefoperazone	0.39	1.87 (1.34–2.52)
E. coli TK-16	2.0×10^{7}[d]	11	T-1982	≤0.1	2.74 (1.77–4.25)
			Cefmetazole	0.78	21.8 (17.7–26.8)
			Cefoxitin	1.56	30.0 (21.1–42.6)
			Cefazolin	1.56	14.2 (10.5–19.2)
			Cefoperazone	0.1	3.89 (2.32–6.54)
E. coli TK-89	1.1×10^{7}[c]	6	T-1982	1.56	4.53 (2.06–9.97)
			Cefmetazole	6.25	83.1 (67.0–103)
			Cefoxitin	25	89.4 (54.2–148)
			Cefazolin	400	>1,000
			Cefoperazone	>800	>1,000
K. pneumoniae Y-41	3.1×10^{2}[c]	310	T-1982	≤0.1	17.9 (10.5–28.9)
			Cefmetazole	0.78	220 (116–418)
			Cefoxitin	3.13	279 (180–432)
			Cefazolin	1.56	153 (80.5–291)
			Cefoperazone	0.2	105 (68.6–161)
P. vulgaris GN3027	8.0×10^{6}[c]	24	T-1982	≤0.1	5.79 (3.31–10.1)
			Cefmetazole	0.78	59.4 (37.6–93.9)
			Cefoxitin	1.56	73.6 (47.2–115)
			Cefazolin	50	>800
			Cefoperazone	0.2	17.8 (9.62–32.9)
S. marcescens IID620	1.0×10^{7}[c]	136	T-1982	≤0.1	4.89 (2.57–9.29)
			Cefmetazole	1.56	54.2 (28.7–102)
			Cefoxitin	3.13	85.7 (56.0–131)
			Cefazolin	>200	>800
			Cefoperazone	1.56	28.9 (20.5–40.7)

[a] LD_{50}, 50% Lethal dose.
[b] ED_{50}, 50% Effective dose. 95% Confidence limits are given in parentheses.
[c] Bacterial cells were suspended in saline containing 5% mucin.
[d] Bacterial cells were suspended in saline.

some-mediated penicillinase, which are classes III, Vc, and IV (5), respectively. The stability of T-1982 to β-lactamase contributed to its antibacterial activity, and T-1982 was more potent against β-lactamase-producing strains than cefmetazole, cefoxitin, or cefazolin. Cefoperazone was more active than T-1982 and other antibiotics against *E. coli* GN5482 and *C. freundii* GN346.

In vivo antibacterial activity. The therapeutic activities of T-1982 were compared with those of cefmetazole, cefoxitin, cefazolin, and cefoperazone in mice experimentally infected with *Staphylococcus aureus* F-31, *E. coli* TK-16, *E. coli* TK-89, *K. pneumoniae* Y-41, *P. vulgaris* GN3027, and *Serratia marcescens* IID620 (Table 6).

Against infections with *Staphylococcus aureus* F-31, T-1982 was less active than the other antibiotics. Against infections with gram-negative bacteria, T-1982 was much more effective than any of the other antibiotics. This included infections with *E. coli* TK-89, which proved resistant to both cefazolin and cefoperazone.

DISCUSSION

Antibiotics of the cephamycin group, such as cefoxitin (7) and cefmetazole, have been reported to have better antibacterial activities against gram-negative bacteria than the cephalosporins currently available and also to show a higher resistance to β-lactamase (6). T-1982, a new semisynthetic cephamycin developed by the Research Laboratory of Toyama Chemical Co., Ltd., has greater antibacterial activity against a wide range of *Enterobacteriaceae* isolates than does cefmetazole, cefoxitin, cefazolin, or cefoperazone and has almost the same antibacterial activity against *P. morganii, C. freundii*, and *Enterobacter* spp. as that of cefoperazone. T-1982 possesses a high degree of resistance to enzymatic hydrolysis by chromosomal and R-plasmid-mediated β-lactamases and has greater antibacterial activity against β-lactamase-producing strains than does cefmetazole or cefoxitin. In some instances, cefoperazone was more active than T-1982. It is likely that this is owing to the low inoculum size. T-1982 showed excellent in vivo activity against gram-negative bacteria, which included cefazolin-resistant strains.

The pharmacokinetic characteristics and toxicity of T-1982 to experimental animals will be described elsewhere.

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