COMPARISON OF ANTIBACTERIAL ACTIVITY OF A NEW CEPHALOSPORIN, CEFTIZOXIME (FK 749) WITH OTHER CEPHALOSPORIN ANTIBIOTICS

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(Received for publication August 27, 1979)

FK 749 is a distinctive new parenteral cephalosporin antibiotic with a broad antibacterial spectrum which is more potently active against a wide variety of Gram-negative bacilli, including the opportunistic pathogens such as *Citrobacter* and *Enterobacter* species and *Serratia marcescens*, than SCE 963, T 1551 and cefmetazole. The activity of FK 749 against *Escherichia coli*, *Klebsiella pneumoniae*, indole-positive and -negative *Proteus* species, *Haemophilus influenzae* and *Streptococcus pyogenes* was by far superior to that of the three other antibiotics. These test organisms were not resistant to FK 749. The antibacterial activity of FK 749 against *Pseudomonas aeruginosa* was almost the same as that of ticarcillin but was inferior to that of gentamicin and T 1551. The bactericidal activity of FK 749 against *E. coli*, *K. pneumoniae* and *Proteus mirabilis* was more potent than that of the three other antibiotics. FK 749, like cefmetazole, was extremely stable to β-lactamases. In studies in mice, the therapeutic effect of subcutaneous injection of FK 749 against various infections due to Gram-negative bacilli was by far superior to that of SCE 963, T 1551 and cefmetazole, was almost the same as that of SCE 963 and cefmetazole against *Staphylococcus aureus* infection and that of ticarcillin against *P. aeruginosa* infection.

Since the advent of cefazolin[1], various other cephalosporins[2~4] and cephamycins[5] have been developed in Japan. Of these, cefotiam (SCE 963)[3], cefoperazone (T 1551)[4] and cefmetazole (CS 1170)[5] are reported to be antibacterial against some strains of Gram-negative bacteria resistant to currently available cephalosporins.

In a continuous effort to develop better cephalosporins, a great number of derivatives have been synthesized and evaluated in the Fujisawa Research Laboratories. Recently, we have succeeded in developing ceftizoxime (FK 749), a distinctive new cephalosporin antibiotic (Fig. 1), which is potently active against both Gram-positive and Gram-negative bacteria including the opportunistic pathogens. In this study we compared the *in vitro* and *in vivo* antibacterial activities of FK 749 with those of three other new Japanese antibiotics and report here the results.

Materials and Methods

1. Antibiotics

The antibiotics used in this study included ceftizoxime (FK 749, Fujisawa, Japan), cefotiam (SCE 963, Takeda, Japan), cefoperazone (T 1551, Toyama, Japan), cefmetazole (CS 1170, Sankyo, Japan),

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ticarcillin (Beecham, England) and gentamicin (Schering, U.S.A.).

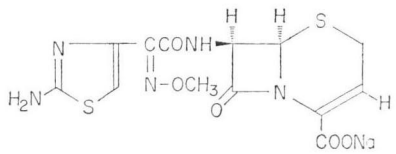
2. Bacterial strains

Standard strains maintained in our laboratory were used in this study. Clinical isolates of various species of bacteria were supplied by several hospitals in Japan.

3. Antibiotic susceptibility

Minimum inhibitory concentration (MIC) was determined by the agar dilution method using Heart infusion agar (HI agar, Difco), unless otherwise specified. For the testing of *Haemophilus influenzae* and *Streptococci*, the medium was supplemented with 5% defibrinized horse blood. The inoculum was grown in Trypticase soy broth (BBL) overnight at 37°C for all cultures except the above bacteria; each broth was supplemented with 10% Fildes-enrichment (Difco) for *H. influenzae* and *Streptococci*. An overnight broth culture and decimal dilutions thereof were streaked or spot-inoculated onto the agar media containing graded concentrations of the test antibiotics. MIC was estimated after incubation at 37°C for 20 hours. For the testing of *Bacteroides fragilis*, the incubation was performed by the GAS-PAK method at 37°C; GAM-broth (Nissui) and GAM-agar (Nissui) were used for the preculture and test culture, respectively.

Fig. 1. Chemical structure of ceftizoxime (FK 749).



Sodium(6R,7R)-7-[(Z)-2-methoxyimino-2-(2-aminothiazole-4-yl)acetamido]-8-oxo-5-thia-1-azabicyclo[4,2,0]oct-2-ene-2-carboxylate.

4. Minimum bactericidal concentration (MBC)

Each HI-broth containing graded concentrations of the test antibiotics was inoculated with test organisms to obtain a concentration of about 10^6 colony forming units (C.F.U.)/ml. After incubating at 37°C for 18 hours, the MBC was estimated as the lowest antibiotic concentration to kill 99.9% of the bacterial cells inoculated.

5. Stability to β-lactamases

(1) Preparation of β-lactamase: The cells were grown at 37°C in HI-broth to which penicillin G was added as an inducer. After overnight incubation, the cells were harvested by centrifugation, washed once and suspended in 0.067 M phosphate buffer (pH 7.0). The cell suspensions were sonicated at 20 Kc for 20 minutes. After cellular debris was removed by centrifugation, the supernatant was subjected to gel filtration on a Sephadex G100 column. The column was equilibrated with 0.067 M phosphate buffer (pH 7.0) and eluted with the same buffer. The enzyme-containing fractions were pooled and stored at −20°C.

(2) Assay of β-lactamase activity: β-Lactamase activity was determined with a Hitachi 200-20 spectrophotometer equipped with a thermostatted cell holder. The enzyme was mixed in a 1-cm quartz cuvette with 150 μg of substrate and 200 μmoles of 0.067 M phosphate buffer (pH 7.0) to a final volume of 3.0 ml and incubated at 37°C. The rate of hydrolysis of the β-lactam ring was followed by the change in UV-absorption at 240 nm for penicillin G and at 260 nm for cephalosporins. The relative initial rates of hydrolysis was expressed as a percentage of the rate hydrolysis of cephaloridine for cephalosporinase and that of penicillin G for penicillinase.

6. Therapeutic effect on experimental infections in mice

Each organism cultured overnight on Trypticase soy agar (BBL) at 37°C, was suspended in a 5% mucin solution to obtain specified cell counts. Male JCL-ICR strain mice aged 4 weeks (18.5~21.5 g) were used and each group consisted of 10 mice. Mice were inoculated intraperitoneally with 0.5 ml of the suspension and were given a single subcutaneous dose of the test antibiotics 1 hour after challenge. The mean ED_{50} value was found by the probit method from the number of mice surviving 4 days of observation.

Results

1. Antibacterial Activity

The antibacterial activity of FK 749 against clinical isolates was compared with that of SCE 963,

T 1551 and cefmetazole (Table 1).

The MICs of FK 749 for 84 strains of *S. aureus* ranged from 1.56 to 25 μg/ml (mean: 4.07 μg/ml) and were somewhat inferior to SCE 963 and cefmetazole (mean: 1.37 and 1.78 μg/ml, respectively). No strains of *S. aureus* were resistant to any of the test antibiotics including FK 749. In contrast, the MICs of FK 749 for 40 strains of *Staphylococcus epidermidis* ranged from 0.2 to ≧200 μg/ml (mean: 1.92 μg/ml). The activity of FK 749 against this group of organism was superior to that of cefmetazole and T-1551 but was inferior to that of SCE 963. As a whole, strains of *S. pyogenes* were susceptible to all the antibiotics, especially to FK 749. The MICs of FK 749 for 40 strains of *S. pyogenes* were 0.025 μg/ml or lower, and the drug was the most effective of all the antibiotics tested against this organism.

In general, marked differences between FK 749 and the reference antibiotics were found in antibacterial activity against Gram-negative bacteria:

The mean MICs against 84 strains of *E. coli* were 0.11 μg/ml for FK 749, 0.42 μg/ml for SCE 963, 0.90 μg/ml for T 1551 and 2.28 μg/ml for cefmetazole. The activity of FK 749 against these strains was superior to that of the other antibiotics tested. No strains of *E. coli* were resistant to FK 749. The activity of FK 749 against 84 strains of *K. pneumoniae* was the most potent, with a mean MIC of 0.048 μg/ml, followed in descending order by SCE 963 (0.73 μg/ml), cefmetazole (1.91 μg/ml) and T 1551 (1.94 μg/ml). None of the 84 strains of *K. pneumoniae* were resistant to FK 749, but 6 strains were resistant to T 1551 (MIC: ≧100 μg/ml).

The MICs of FK 749 for 84 strains of *P. mirabilis* ranged from ≦0.025 to 1.56 μg/ml. The activity of FK 749 against these strains was the most potent; the mean MICs were 0.029 μg/ml for FK 749, 1.10 μg/ml for SCE 963, 2.16 μg/ml for T 1551, and 4.68 μg/ml for cefmetazole. FK 749 was also the most effective of all the antibiotics against indole-positive *Proteus* species; the mean MICs of FK 749 were 0.048 μg/ml for *P. vulgaris*, 0.46 μg/ml for *P. morganii*, 0.033 μg/ml for *P. rettgeri* and 0.045 μg/ml for *P. inconstans* B. No strains were resistant to FK 749.

The mean MIC of FK 749 for 42 strains of *C. freundii* was 1.42 μg/ml. Of these, only 3 were highly resistant (MIC: 100 μg/ml or higher). In contrast, the MICs for these strains were 5.95 μg/ml for SCE 963, 2.14 μg/ml for T 1551 and 93.6 μg/ml for cefmetazole. Strains resistant to SCE 963, cefmetazole and T 1551 were found more frequently than those to FK 749. The MICs of FK 749 ranged from 0.05 to 100 μg/ml (mean: 1.02 μg/ml) for 42 strains of *E. aerogenes* and from 0.1 to ≧200 μg/ml (2.08 μg/ml) for *E. cloacae*. The activity of FK 749 against *Enterobacter* species was the most potent of all the antibiotics tested. Cefmetazole was scarcely active against this bacterial species.

Most of the 42 strains of *S. marcescens* were resistant to SCE 963, cefmetazole and T 1551, however, these strains were susceptible to FK 749 and the MICs ranged from 0.1 to 25 μg/ml (mean: 1.97 μg/ml). The 40 strains of *H. influenzae* were highly susceptible to FK 749 and the activity of FK 749 against these strains was the most potent (mean MIC: ≦0.025 μg/ml) of all the drugs tested. The MICs of FK 749 for *Bacteroides fragilis* (16 strains) ranged from 0.39 to 50 μg/ml (mean: 7.43 μg/ml). The activity of FK 749 against these strains was the most potent, followed in descending order by cefmetazole, T 1551 and SCE 963.

The antibacterial activity of FK 749 against *P. aeruginosa* was compared with that of ticarcillin (TIPC), T 1551 and gentamicin (GM). The activity of FK 749 against 42 strains of *P. aeruginosa* was similar to that of ticarcillin but was inferior to that of T 1551 and gentamicin.

Table 1. Susceptibility distribution of clinical isolates to FK 749 and related antibiotics.

Organism	Antibiotic	No. of organisms with a MIC (μg/ml) of:													Mean MIC
		≦0.025	0.05	0.1	0.2	0.39	0.78	1.56	3.13	6.25	12.5	25	50	100	≧200	
S. aureus (84 strains)	FK 749							8	47	21	5	3				4.07
	SCE 963						18	64	2							1.37
	Cefmetazole							68	16							1.78
	T 1551							4	53	26	1					3.81
S. epidermidis (40 strains)	FK 749				7	7	9	4	2	4	1	1		1	4	1.92
	SCE 963				4	11	11	8	3	2					1	0.93
	Cefmetazole							14	19	3	1	1	1	1		3.29
	T 1551						2	14	12	7	2	1	1		1	3.59
*S. pyogenes** (40 strains)	FK 749	40														≦0.025
	SCE 963		16	24												0.074
	Cefmetazole				1	17	21	1								0.57
	T 1551			3	14	23										0.28
E. coli (84 strains)	FK 749	1	22	45	9	1	2		2	2						0.11
	SCE 963			1	25	36	13	5	2	2						0.42
	Cefmetazole						8	41	25	6	1	1	2			2.28
	T 1551		1	2	9	27	18	8	7	3	4	5				0.90
K. pneumoniae (84 strains)	FK 749	29	35	13	6	1										0.048
	SCE 963			3	8	25	23	14	9		1		1			0.73
	Cefmetazole					2	11	43	19	7	2					1.91
	T 1551				8	14	25	10	3	3	6	6	3	5	1	1.94
P. mirabilis (84 strains)	FK 749	71	11	1				1								0.029
	SCE 963					14	32	25	10	2	1					1.10
	Cefmetazole							1	46	28	5	4				4.68
	T 1551						7	40	30	6		1				2.16
P. vulgaris (42 strains)	FK 749	24	9	2	3	2	1	1								0.048
	SCE 963									1	2	2	4	3	30	>200
	Cefmetazole						1	1	20	16	4					4.42
	T 1551							12	7	8	9	1	1	2	2	6.57
P. morganii (42 strains)	FK 749		7	9	6	5	4	1	1	1	3	4	1			0.46
	SCE 963					10	6	6	5	3		1	1	1	9	5.04
	Cefmetazole									18	21	1	2			10.1
	T 1551						6	15	10	3	2	2	4			3.23
P. rettgeri (42 strains)	FK 749	31	9	1			3		3		10	10	2	2	2	0.033
	SCE 963	2	2	4	1	1	5	5	4			5	14	5	2	4.49
	Cefmetazole					2				17	11					13.1
	T 1551					3	4	1	3			1	1		1	5.48

P. inconstans B (42 strains)	FK 749	22	7	10	2	1										0.045
	SCE 963	1		2	4	5	6	6	7	6	2		2	1		1.54
	Cefmetazole						3	9	16	4	3	4	2	1		4.35
	T 1551						4	8	5	4	7	10	4			6.90
C. freundii (42 strains)	FK 749			1	7	16	6	2					7	1	2	1.42
	SCE 963					3	6	10	8	5				1	9	5.95
	Cefmetazole											2	17	11	12	93.6
	T 1551				2	13	14	2		1			4	3	3	2.14
E. aerogenes (42 strains)	FK 749		4	8	7	2	3	1	1	6	5	1	2	2		1.02
	SCE 963						6	7	3	5	1	4	1	1	14	17.4
	Cefmetazole											1		2	39	>200
	T 1551				2	11	9	1	2	6	3	3	1		4	2.83
E. cloacae (39 strains)	FK 749			2	9	5	5	4		4	2	1	1	1	5	2.08
	SCE 963						1	2	6	3	1	4	5	3	14	49.1
	Cefmetazole													3	36	>200
	T 1551			1	2	6	13	3		3		2	1	2	6	3.60
S. marcescens (42 strains)	FK 749			2	2	6	5	8	7	3	4	5				1.97
	SCE 963												2		40	>200
	Cefmetazole										1	2	3	3	33	>200
	T 1551								2		1	1	4	4	30	>200
*H. influenzae*** (40 strains)	FK 749	35	3	2												≦0.025
	SCE 963	2		1		1	11	22	2	1						1.01
	Cefmetazole						1		8	25	6					5.73
	T 1551	24	13	1			2									0.032
*B. fragilis**** (16 strains)	FK 749					1			6	3	1	2	3			7.43
	SCE 963											1	9	6		62.1
	Cefmetazole										5	2	9			29.7
	T 1551									1	4	6		4	1	31.0
P. aeruginosa (42 strains)	FK 749											15	18	8	1	46.8
	TIPC										3	26	6	4	3	37.2
	T 1551									8	25	6	2		1	13.8
	GM							8	22	9				1	2	4.35

*: Supplemented with 10% horse blood

**: HI agar+5% horse blood (chocolate agar)

***: GAM agar (Nissui), 37°C, 24 hours, Gas Pak method

Inoculum size; 10^6 C.F.U./ml, Stamp method. HI agar (Difco), 37°C, 20 hours.

2. Antibacterial Activity of FK 749 against Ampicillin-Resistant Strains of *E. coli* and *K. pneumoniae*

Ampicillin-resistant clinical isolates of *E. coli* and *K. pneumoniae* were classified into those whose resistance was chromosomal and those whose resistance was mediated by an R-plasmid. The antibacterial activity of FK 749 against these strains was compared with that of SCE 963, T 1551 and cefmetazole in two sizes of inocula (10^6 and 10^8 C.F.U./ml) (Table 2).

FK 749 was the most active of all the antibiotics against both types of resistant strains of *E. coli*. The mean MICs of FK 749 were 0.081 μg/ml for R-plasmid-harboring strains and 0.1 μg/ml for chromosomally resistant strains, when a bacterial suspension of 10^6 C.F.U./ml was used as the inoculum. At 10^8 C.F.U./ml, the activity of FK 749 against these resistant strains decreased slightly but the MICs were still in achievable serum levels, *i.e.* 0.65 μg/ml for R-plasmid-harboring strains and 1.36 μg/ml for chromosomally resistant strains. In contrast, with the same size of inoculum, the activities of SCE 963 and T 1551 were markedly lower; mean MICs of SCE 963 being 11.7 μg/ml and 17.7 μg/ml, and those of T 1551 being 213 μg/ml and >400 μg/ml, respectively.

Table 2. Antibacterial activity of FK 749 and related antibiotics against ampicillin-resistant strains of *E. coli* and *K. pneumoniae*.

Mean MIC; μg/ml

Antibiotic	R-plasmid		Chromosomal	
	E. coli 11 strains	*K. pneumoniae* 10 strains	*E. coli* 10 strains	*K. pneumoniae* 10 strains
FK 749	0.081[a] (0.65)	0.030 (0.14)	0.10 (1.36)	0.037 (0.68)
SCE 963	0.39 (11.7)	0.59 (25)	0.90 (17.7)	0.68 (9.47)
Cefmetazole	1.38 (5.17)	1.11 (2.92)	2.92 (5.08)	1.92 (8.84)
T 1551	3.13 (213)	25 (>400)	10.2 (>400)	1.36 (187)

[a] Inoculum size: 10^6 C.F.U./ml (10^8 cells/ml). HI-agar (Difco), 37°C, 20 hours. Stamp method.

The antibacterial activity of FK 749 against both types of resistant strains of *K. pneumoniae* was also superior to that of the other antibiotics at both sizes of inocula. The mean MICs of FK 749 for these strains of *K. pneumoniae* were about the same as those of *E. coli* given above.

3. Minimum Bactericidal Concentration (MBC)

The MBCs of FK 749 against *E. coli, K. pneumoniae* and *P. mirabilis* were compared with those of SCE 963, cefmetazole and T 1551 (Table 3). The bactericidal activity of FK 749 against 8 strains of *E. coli* was the most potent of all the drugs tested, with a mean MBC of 2.5 μg/ml, and was 2.7~5 times lower than that of the three other antibiotics. Similar results were obtained with 7 strains of *K. pneumoniae*. The activity of FK 749 was the most potent of all the test drugs (mean MBC: 0.12 μg/ml) and was respectively about 2.8, 39 and 158 times lower than that of SCE 963, cefmetazole and T 1551. Generally, *P. mirabilis* was somewhat resistant to the bactericidal activity of these antibiotics. The MBC of FK 749 for the 5 strains, however, was 22 μg/ml and showed a definite bactericidal effect. The MBCs of the other antibiotics were 100 μg/ml or higher; they had no marked bactericidal activity.

4. Stability of β-Lactamases

The hydrolysis of FK 749 by cephalosporinase (β-lactamase Ia) and penicillinase (β-lactamase III) was compared with that of the reference antibiotics. The relative initial rates of hydrolysis were expressed in percentage of hydrolysis of cephaloridine for cephalosporinase and in that of penicillin G for penicillinase. Table 4 shows that FK 749 was extremely stable to both types of β-lactamases

Table 3. Minimum bactericidal concentration of FK 749 and related antibiotics.

Organism		FK 749	SCE 963	Cefmetazole	T 1551
E. coli 8 strains	MBC	2.5 (0.39~25)	12.5 (3.13~100)	13 (3.13~100)	6.7 (0.78~>100)
	MIC	0.59 (0.1~12.5)	3.3 (0.39~100)	8.8 (1.56~100)	4.1 (0.39~>100)
K. pneumoniae 7 strains	MBC	0.12 (0.05~0.2)	3.3 (1.56~12.5)	4.7 (3.13~6.25)	19 (3.13~100)
	MIC	0.05 (≦0.025~0.1)	0.59 (0.39~3.13)	3.13 (1.56~6.25)	2.5 (0.39~12.5)
P. mirabilis 5 strains	MBC	22 (12.5~50)	>100 (50~>100)	>100 (>100)	>100 (100~>100)
	MIC	0.04 (≦0.025~0.39)	0.68 (0.39~1.56)	7.2 (1.56~25)	1.4 (0.78~1.56)

HI-broth, 37°C, 20 hours. Mean MBC or MIC; μg/ml. ( ); Distribution range.
MBC was determined as the lowest concentration that kills 99.9% of the inoculum (10^5 C.F.U./ml).

as well as cefmetazole. In contrast, SCE 963 was hydrolysed at significant rates by cephalosporinase and was also more susceptible to penicillinase than FK 749 and cefmetazole. T 1551 was hydrolysed at substantial rates by cephalosporinase and was markedly hydrolysed by penicillinase.

Table 4. Stability of FK 749 and related antibiotics to β-lactamase.

Antibiotic	Cephalosporinase	Penicillinase
FK 749	1.3	0.07
SCE 963	18.5	4.2
Cefmetazole	<0.1	0.2
T 1551	65.2	12.9
Cephaloridine	100	18.9
Penicillin G	<0.1	100

Substrate concentration: 50 μg/ml
37°C, 0.067 M potassium phosphate buffer (pH 7.0).
UV assay, relative initial velocity:
Cephaloridine =100 for cephalosporinase,
Penicillin G =100 for penicillinase
Cephalosporinase: *S. marcescens* No. 78
Penicillinase: *E. coli* No. 18 (R^+)

5. Protective Effect on Infections in Mice

The protective effect of FK 749 and the reference antibiotics in experimental infections was studied in mice according to the procedure described under Materials and Methods.

As shown in Table 5, the ED_{50} values of FK 749 for infections due to *S. aureus* Nos. 47 and 44 were 4.03 and 3.53 mg/kg respectively. The protective effect of FK 749 on infections due to *E. coli* No. 29 was almost the same as that of T 1551, but was superior to that of SCE 963 and cefmetazole. The ED_{50} values of FK 749 for infections due to *P. mirabilis* No. 73 and *P. vulgaris* No. 63 were 0.255 and 0.038 mg/kg respectively, and were superior to those of T 1551, SCE 963 and cefmetazole. Of all the antibiotics tested only FK 749 was effective (ED_{50}, 4.28 mg/kg) against infection due to *S. marcescens* No. 31. The ED_{50} value of FK 749 for *P. aeruginosa* No. 93 was 50.1 mg/kg and the protective effect was nearly the same as that of T 1551 and ticarcillin.

Discussion

Although many new cephalosporin and cephamycin derivatives have been developed and applied in the treatment of infections, new and more useful antibiotics which are potently effective against various species of Gram-negative pathogens are still urgently needed in chemotherapy. In our screening for better cephalosporin antibiotics, we have recently developed a distinctive new parenteral cephalo-

Table 5. Protective effect of FK 749 and related antibiotics on mice infections.

(ED_{50}; mg/kg)

Organism	Mucin %	FK 749	SCE 963	Cefmetazole	T 1551
S. aureus 47 (2.0×10^7/mouse)	5	4.03 (1.20~9.30)	2.50 (1.04~5.53)	3.91 (1.84~8.29)	*18.7 (9.65~43.3)
S. aureus 44 (5.3×10^7/mouse)	5	3.53 (1.69~5.94)	1.76 (0.415~3.61)	3.27 (1.41~6.34)	*9.01 (3.08~30.9)
E. coli 29 (1.2×10^4/mouse)	2.5	0.020 (0.006~0.031)	*0.595 (0.465~0.819)	*2.50 (1.17~3.97)	0.026 (0.012~0.041)
P. mirabilis 73 (2.1×10^7/mouse)	5	0.255 (0.141~0.401)	*2.06 (1.10~3.89)	*13.9 (7.48~26.7)	*5.64 (2.32~29.3)
P. vulgaris 63 (9.4×10^5/mouse)	5	0.038 (0.025~0.061)	*6.05 (3.68~9.51)	*4.42 (2.43~8.15)	*0.695 (0.455~1.18)
S. marcescens 31 (4.9×10^4/mouse)	5	4.28 (2.75~7.57)	*>62.5	*>62.5	*>62.5
			Ticarcillin	Gentamicin	
P. aeruginosa 93 (3.7×10^4/mouse)	5	50.1 (23.4~127)	69.1 (44.3~128)	**3.91 (2.69~5.68)	36.9 (17.7~81.0)

Mouse; ICR strain, male, 4 W (20.0±1.5 g), 10 mice/group
Infection; 2.5 or 5% Mucin suspension, i.p.
Therapy; 1 hour after challenge, s.c.
( ); 95% confidence limit
Significant difference; *: FK 749 > control antibiotics
**: FK 749 < control antibiotics

sporin, FK 749, which has high antibacterial potency and which meets the actual requirements for treatment of infections due to Gram-negative pathogens including the opportunistic pathogens[6].

FK 749 is distinguished by its exceptional antibacterial activity against frequently encountered pathogens such as *E. coli, K. pneumoniae, Proteus* species, *H. influenzae* and *S. pyogenes* and also against *Enterobacter* and *Citrobacter* species and *S. marcescens*. The excellent antibacterial activity of FK 749 against Gram-negative bacterial species is well evidenced by the following main characteristics; high permeability of the outer membrane of the bacterial cell (H. KOJO, Y. SHIGI, M. NISHIDA, unpublished data) and stability to hydrolysis by β-lactamase located in the periplasm.

In contrast, the activity of FK 749 against *P. aeruginosa* is similar to that of ticarcillin and is the weakest against this species of Gram-negative bacilli. It is generally considered that the susceptibility of *P. aeruginosa* to β-lactam antibiotics is decreased due to limited permeability of antibiotics to the bacterial cells. However, the practical worth of FK 749 against pseudomonal infection remains to be clinically evaluated.

In view of the fact our studies of FK 749 showed the drug to be more potent than other newly developed Japanese antibiotics in various *in vitro* and *in vivo* tests against Gram-negative bacilli except *P. aeruginosa*, we strongly believe that FK 749 has considerable potential for clinical application.

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