IN VIVO ANTIBACTERIAL ACTIVITY OF FK037, A NOVEL PARENTERAL BROAD-SPECTRUM CEPHALOSPORIN

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FK037 has potent therapeutic activity against lethal systemic infections and experimental local infections due to a wide variety of Gram-positive and Gram-negative bacteria such as staphylococci, *Streptococcus pneumoniae*, Enterobacteriaceae and *Pseudomonas aeruginosa* in mice. In murine systemic infections, FK037 was the most effective of the cephalosporins and imipenem tested against highly methicillin-resistant *Staphylococcus aureus* (H-MRSA). It was more effective than ceftazidime against selected strains of *S. aureus* and Enterobacteriaceae, except *Serratia marcescens* and *P. aeruginosa* against which FK037 was as effective as ceftazidime and was as effective as cefpirome against all organisms tested, except MRSA and *P. aeruginosa* against which FK037 was more effective than cefpirome. These results correlated well with its *in vitro* activity. In murine local infections, with few exceptions, FK037 was more effective than ceftazidime and cefpirome against *Klebsiella* pneumonia in ED_{50} values and against methicillin-sensitive *S. aurens* (MSSA) subcutaneous abscess, pyelonephritis with *Staphylococcus epidermidis*, *E. coli* and *P. aeruginosa*, intrauterine infections with *S. aureus* and *E. coli* in reducing the number of viable bacteria in the abscess, kidneys and uterus. It is noteworthy that the therapeutic effects of FK037 were more potent than had been anticipated from its *in vitro* activity against local infections with staphylococci and *P. aeruginosa* when compared with ceftazidime or cefpirome. In addition, the therapeutic effects of FK037 were equipotent or superior to those of cefpirome and ceftazidime against pneumonia due to MSSA, *K. pneumoniae* and *P. aeruginosa* in reducing the number of viable bacteria in the lungs in mice using an *in vivo* pharmacokinetic model simulating human plasma concentrations after drip infusion of usual clinical doses (0.25 to 1.0 g for MSSA, 0.063 to 0.125 g for *K. pneumoniae* and 1.0 to 2.0 g for *P. aeruginosa*). FK037 induced an *in vivo* post-antibiotic effect (PAE) of 3.4 hours against a thigh infection with MSSA in neutropenic mice. These results strongly suggest that it has potential for clinical use against various infections due to bacteria which include staphylococci and *P. aeruginosa*.

FK037, a new parenteral cephalosporin regarded as a so-called fourth generation cephalosporin, offers some advantages over the commercially available parenteral cephalosporins[1]. It demonstrated extremely potent, broad-spectrum activity against clinical isolates of Gram-positive bacteria which include highly methicillin-resistant staphylococci, and Gram-negative bacteria which include *Pseudomonas*

aeruginosa. It is noteworthy that FK037 was the most active of all the cephalosporins tested against highly methicillin-resistant *Staphylococcus aureus* (H-MRSA) and provided significant activity against *Citrobacter* and *Enterobacter* resistant to the so-called third-generation cephalosporins. To confirm its *in vivo* activity, the therapeutic effects of FK037 were compared with those of the reference drugs on systemic infections and various local infections such as experimental pneumonia, subcutaneous abscess, ascending pyelonephritis and intrauterine infection in mice. Moreover, in order to predict its clinical efficacy in an experimental infection model, the therapeutic effects of FK037 were compared with those of the reference drugs against pneumonia in an *in vivo* pharmacokinetic model in which human plasma concentrations after drip infusion of FK037 were reproduced in murine plasma.

Materials and Methods

Drugs

FK037 (lot 30198P, 520197P, 330189S) and cefpirome (lot 331199S, 331108S, 214176S) were synthesized in the New Drug Research Laboratories of Fujisawa Pharmaceutical Co., Ltd., Osaka. The other drugs were commercially procured: Ceftazidime (lot 96271, 80141, 70291, 07031) from Tanabe Pharmaceutical Co., Ltd., Osaka; cefoperazone (lot RB987, SC108F) from Toyama Chemical Co., Ltd., Toyama prefecture; ceftizoxime (lot 200195G) from Fujisawa Pharmaceutical Co., Ltd., Osaka; cefuzonam (lot 165-2) from Takeda Chemical Ind., Ltd., Osaka; flomoxef (lot BC05, FM8235) from Shionogi & Co., Ltd., Osaka; and imipenem/cilastatin (imipenem, lot F177K) and methicillin (lot D023K, C022K) from Banyu Pharmaceutical Co., Ltd., Tokyo.

Animals

ICR-strain male or female mice, aged 4 or 5 weeks, weighing 19 to 26 g (Japan SLC Co.) were housed in cages and were given food and water *at libitum*.

Bacteria

The strains used for the experiments were as follows: Methicillin-sensitive *S. aureus* 2550 and *S. aureus* 47 (MSSA), low level methicillin-resistant *S. aureus* 9098 (L-MRSA), high level methicillin-resistant *S. aureus* 9002 (H-MRSA), *Staphylococcus epidermidis* 7022, *Streptococcus pneumoniae* FP1284, *Escherichia coli* 22 and *E. coli* 29, *Klebsiella pneumoniae* 1 and *K. pneumoniae* FP221, *Proteus mirabilis* 4, *Citrobacter freundii* 3033, *Enterobacter cloacae* 3020, *Serratia marcescens* 4003 and *P. aeruginosa* 7001. All these strains were previously screened for pathogenicity. These strains except MRSA and *S. pneumoniae* were passaged twice on a Trypticase soy agar (BBL) slant. MRSA was grown on Mueller-Hinton agar containing methicillin 3.13 μg/ml at the first passage to avoid loss of methicillin resistance, followed by growth on the same agar medium without the drug. *S. pneumoniae* was passaged twice in Mueller-Hinton broth (Difco) containing 5% horse serum for 15 hours.

Antibiotic Susceptibility Testing

MIC values were determined by the agar dilution method. Mueller-Hinton agar (Difco) was used for non-fastidious aerobic bacteria. This medium supplemented with 5% defibrinated horse blood was used for *S. pneumoniae*. The non-fastidious Gram-negative bacteria were precultured in Mueller-Hinton broth; staphylococci in Trypticase soy broth; *S. pneumoniae* in Mueller-Hinton broth plus 10% horse serum. A hundred-fold dilutions of overnight cultures in Mueller-Hinton broth containing 10^4 colony forming units (cfu) were inoculated with a multipoint replicating apparatus onto agar plates containing serial two-fold dilutions of each antibiotic prior to incubation at 37°C for 18 hours. The MIC was read as the lowest drug concentration required to inhibit visible growth of the organism. Growth of less than 5 colonies was considered as negative.

Lethal Systemic Infection

All strains (except *S. aureus* 9002, *S. pneumoniae* FP1284 and *P. aeruginosa* 7001) were intraperitoneally inoculated in groups of 8 male mice aged 4 weeks each with 0.5 ml of bacterial suspension in 5% gastric mucin to be given about 1 to 5 minimum lethal dose (MLD). *S. aureus* 9002 and *P. aeruginosa* 7001 were inoculated in groups of 8 male mice immunosuppressed with intraperitoneal administration of cyclophosphamide at 200 mg/kg 4 days before infection. *S. pneumoniae* FP1284 suspended in saline was intravenously inoculated in groups of 8 mice each. The infected mice were treated subcutaneously with serially diluted drugs one hour after infection. However, mice infected with *S. aureus* 9002 and *P. aeruginosa* 7001 were subcutaneously given drugs twice at post-infection 1 and 3 hours. The survival of the infected mice was observed for 3 to 5 days, and the 50% effective dose (ED_{50}) was determined from the final survival rates by the Probit method[2].

Pneumonia

Groups of 8 mice each were exposed to bacterial aerosol containing *K. pneumoniae* FP221 at 1.8×10^9 cfu/ml in a chamber for 20 minutes. The infected mice were treated subcutaneously with 7 post-infection administrations (4, 24, 32, 48, 56, 72 and 80 hours) of serially diluted drugs. The ED_{50} was determined from the survival rates on post-infection day 7.

Subcutaneous Abscess

Groups of 7 male mice each were immunosuppressed with intraperitoneal cyclophosphamide at 200 mg/kg. On the fourth day, the animals were inoculated subcutaneously with 0.1 ml of *S. aureus* 47 (4.6×10^2 cfu) on their dorsal regions. The infected mice were subcutaneously treated with 5 administrations of the drugs at post-infection 5, 24, 32, 48 and 56 hours at sites remote from the infection sites. Pieces of shaved skin at the infection sites were aseptically removed on post-infection day 3, and the viable bacteria were counted after homogenization by a conventional plating method.

Ascending Pyelonephritis

Ascending pyelonephritis was induced by the method of OBANA[4]. Groups of each with 6 or 7 female mice aged for 4 or 5 weeks were deprived of drinking water overnight. Volumes of 0.1 ml bacterial suspension of *S. epidermidis* 7022 (1.5×10^5 cfu), *E. coli* 29 (2.4×10^5 cfu) or *P aeruginosa* 7001 (3.0×10^5 cfu) were inoculated into bladder of anesthetized mice through the urethra and the ureterostoma was closed with a clip for 5 hours to prevent flux of bacteria. The infected mice were subcutaneously treated with the drugs for 5 times at post-infection 5, 24, 32, 48 and 56 hours. The kidneys were aseptically removed from the infected mice at post-infection day 3 and the viable bacteria in the kidneys were counted after homogenization by a conventional plating method. The detection limit of the viable bacteria was 10 cfu per kidney in this model.

Intrauterine Infection

Intrauterine infection was induced by the method of OBANA[5]. Groups of 6 female mice each, aged 4 or 5 weeks, were anesthetized prior to surgical exposure of their uteri. To prevent bacterial flux, the cervix was ligated with an operation suture, taking care not to ligate the arteries. A volume of 0.025 ml of the bacterial suspension of *S. aureus* 47 (1.2×10^7 cfu) or *E. coli* 22 (1.3×10^5 cfu) was inoculated into the left uterine horns. The infected mice were treated subcutaneously with 5 administrations of the drugs at post-infection 5, 24, 32, 48 and 56 hours. The uterines were aseptically removed from the infected mice 3 days after infection, and the viable bacteria were counted after homogenization by a conventional plating method.

In Vivo Pharmacokinetic Model

1) Pneumonia: Groups of 5 male mice aged 4 weeks were immunosuppressed with 200 mg/kg cyclophosphamide (ip) 4 days before infection. For *K. pneumoniae* FP221, the mice were exposed to bacterial aerosol at 2.4×10^9 cfu/ml in a chamber for 20 minutes. Volumes of 0.05 ml each of the bacterial suspension of *S. aureus* 47 (2.2×10^7 cfu) or *P. aeruginosa* 7001 (2.2×10^2 cfu) in saline were intranasally inoculated in the anesthetized mice.

2) Doses and dosage regimens: The doses and dosing schedules of the drugs were determined to approximate the time-related concentration in human plasma produced by usual clinical treatment of drip infusion with 1.0 g over a 1-hour period. The doses for drip infusion with 0.063, 0.125, 0.5 and 2.0 g were calculated by multiplying each dose ratio with 1.0 g. Since drip infusion was impossible in mice and their plasma clearance was much faster than that of human, frequent subcutaneous injections at a 1-hour intervals were necessary. Human plasma concentrations of FK037 after 1.0 g drip-infusion were calculated from those after intravenous injection of 0.125 g[6]. Those of cefpirome, ceftazidime and flomoxef were referred to data reported previously[7~9]. Sixteen hours after the inoculation of bacteria, the mice were treated with the drugs according to the above dosage regimens.

3) Measurement of bacterial count: Immediately before, 3, 6 and 24 hours after the initial dosing, groups of 5 mice were sacrificed, and the lungs were aseptically removed. Each lung was homogenized and the number of viable bacteria was quantitated by a conventional plating method. The detection limit was 10 cfu per lung.

In Vivo Post-antibiotic Effect (*In Vivo* PAE)

In vivo PAE was studied by the method of GUDMUNDSSON[10]. Groups of 5 male mice each were immunosuppressed with intraperitoneal cyclophosphamide at 200 mg/kg and inoculated on the fourth day with 0.1 ml of *S. aureus* 47 (2.2×10^6 cfu) in the thigh of each mouse. Two hours later (time 0), a dose of 5 mg/kg of FK037 was subcutaneously injected in the treated groups. The infected mice were killed at selected intervals from -2 to 12 hours. Thighs were aseptically removed, and the viable bacteria were counted after homogenization by a conventional plating method. The drug concentrations in the thighs infected with *S. aureus* 5027 after subcutaneous dosing of FK037 at 80 mg/kg were determined by the disc-plate diffusion method using *Morgarella morganii* IFO 3848. The PAE was calculated by the following equation: PAE = T − C − M, where M is the time thigh levels exceeded the MIC; T is the time required for a mean cfu/thigh of treated mice to increase to 1 $\log_{10}$ above the count at time M; and C is the time needed for the cfu/thigh of control animals to increase of 1 $\log_{10}$.

Statistical Analysis

The parallel line assay was used to determine the significant differences between FK037 and reference drugs in ED_{50}s for lethal systemic infections. Tukey-type multiple comparison was used to determine the significant differences between the FK037-treated and reference drug-treated groups, and between the drug-treated and non-treated groups in $\log_{10}$ values of viable counts in local infections.

Results

Therapeutic Effects of FK037 on Lethal Systemic Infection

The therapeutic effects of FK037 against the 11 strains of 9 organisms were compared with those of the reference drugs (Table 1). Against *S. aureus* 2550 (MSSA), FK037 was as effective as cefpirome and flomoxef, and was significantly more effective than ceftazidime and cefuzonam. Against 2 MRSA strains, FK037 was the most effective of the drugs tested, including imipenem. Against *S. pneumoniae* FP1284, FK037 was as effective as cefpirome and was significantly more effective than flomoxef and ceftizoxime. Against the Gram-negative bacteria, except *S. marcescens* and *P. aeruginosa*, the therapeutic activity of FK037 was similar to that of cefpirome and was superior to each of ceftazidime, cefoperazone and flomoxef. These results correlated well with their MIC values. Against *S. marcescens* 4003, FK037 was as effective as cefpirome and ceftazidime, and was more effective than cefoperazone and flomoxef. Against *P. aeruginosa* 7001, FK037 was as effective as ceftazidime and elicited more potent effects than cefpirome and cefoperazone.

Therapeutic Effects of FK037 on Pneumonia

The therapeutic effects of FK037 on lethal pneumonia due to *K. pneumoniae* FP221 was compared

Table 1. Therapeutic effect of FK037 and reference drugs on lethal systemic infections in mice.

Organism	Challenge (cfu/mouse)	Antibiotic[b]	MIC[d] (μg/ml)	ED_{50} (95% confidence limit) (mg/kg)	
Staphylococcus aureus 2550 (MSSA)	1.3×10^8 (ip)	FK037	0.39	0.370	(0.183~0.701)
		Cefpirome	0.39	0.370	(0.183~0.701)
		Ceftazidime	3.13	2.50[e]	(0.673~2.32)
		Cefuzonam	0.2	1.25[e]	(1.66~3.77)
		Flomoxef	0.2	0.273	(0.183~0.376)
S. aureus 9098 (L-MRSA)	1.6×10^8 (ip)	FK037	6.25	4.92	(2.05~10.2)
		Cefpirome	25	19.7[e]	(8.22~40.8)
		Flomoxef	12.5	23.7[e]	(9.84~67.8)
		Imipenem	6.25	8.14	(3.15~19.2)
S. aureus 9002 (H-MRSA)[a]	1.6×10^7 (ip)	FK037[c]	25	9.98	(8.14~12.2)
		Cefpirome[c]	50	40.2[e]	(32.7~49.3)
		Flomoxef[c]	100	80.1[e]	(42.9~152)
		Imipenem[c]	50	26.1[e]	(13.8~38.6)
Streptococcus pneumoniae FP1284	6.4×10^3 (iv)	FK037	$\leqq$0.025	0.339	(0.173~0.662)
		Cefpirome	$\leqq$0.025	0.472	(0.242~0.952)
		Flomoxef	0.1	8.25[e]	(3.95~50.7)
		Ceftizoxime	0.05	3.83[e]	(1.86~9.84)
Escherichia coli 29	7.5×10^6 (ip)	FK037	$\leqq$0.025	0.012	(0.006~0.022)
		Cefpirome	$\leqq$0.025	0.016	(0.008~0.032)
		Ceftazidime	0.05	0.065[e]	(0.033~0.131)
		Cefoperazone	$\leqq$0.025	0.035[e]	(0.024~0.048)
		Ceftizoxime	$\leqq$0.025	0.011	(0.008~0.017)
		Flomoxef	0.05	0.229[e]	(0.153~0.336)
Klebsiella pneumoniae 1	2.0×10^7 (ip)	FK037	$\leqq$0.025	0.014	(0.010~0.021)
		Cefpirome	$\leqq$0.025	0.017	(0.011~0.026)
		Ceftazidime	0.1	0.055[e]	(0.029~0.106)
		Cefoperazone	0.2	0.157[e]	(0.075~0.326)
		Ceftizoxime	$\leqq$0.025	0.020	(0.013~0.030)
		Flomoxef	0.1	0.112[e]	(0.059~0.218)
Proteus mirabilis 4	2.3×10^7 (ip)	FK037	0.05	0.296	(0.192~0.455)
		Cefpirome	0.1	0.212	(0.138~0.325)
		Flomoxef	0.2	1.76[e]	(1.17~2.42)
Citrobacter freundii 3033	3.4×10^7 (ip)	FK037	0.05	0.047	(0.023~0.088)
		Cefpirome	$\leqq$0.025	0.047	(0.023~0.088)
		Ceftazidime	0.39	0.383[e]	(0.192~0.761)
		Cefoperazone	0.39	1.81[e]	(0.865~3.69)
		Flomoxef	0.2	16.9[e]	(11.0~25.9)
Enterobacter cloacae 3020	9.0×10^5 (ip)	FK037	$\leqq$0.025	0.010	(0.008~0.012)
		Cefpirome	$\leqq$0.025	0.009	(0.006~0.012)
		Ceftazidime	0.2	0.057[e]	(0.038~0.084)
		Cefoperazone	0.39	0.215[e]	(0.076~0.470)
		Flomoxef	6.25	1.33[e]	(0.193~3.80)
Serratia marcescens 4003	1.0×10^6 (ip)	FK037	0.2	1.02	(0.514~2.04)
		Cefpirome	0.1	0.738	(0.378~1.45)
		Ceftazidime	0.2	1.20	(0.608~2.40)
		Cefoperazone	3.13	>160[e]	—
		Flomoxef	3.13	20.0[e]	(10.2~39.2)
Pseudomonas aeruginosa 7001[a]	1.9×10^3 (ip)	FK037[c]	1.56	30.7	(20.8~58.1)
		Cefpirome[c]	3.13	103[e]	(49.4~634)
		Ceftazidime[c]	1.56	40.0	(26.5~60.4)
		Cefoperazone[c]	3.13	200[e]	(115~∞)

[a] Mice were immunosuppressed by an intraperitoneal dose of 200 mg/kg cyclophosphamide 4 days before challenge; [b] antibiotic was given subcutaneously 1 hour after challenge ([c] 1 and 3 hours after challenge); [d] agar dilution method (stamp method, 10^4 cfu/spot, Mueller-Hinton agar); [e] statistical significances refer to parallel line assay for paired differences, significant difference from FK037 ($P<0.05$).

Table 2. Therapeutic effect of FK037 and reference drugs on pneumonia with *Klebsiella pneumoniae* FP221[a] in mice.

Antibiotic[b]	MIC[c] (μg/ml)	ED_{50} (95% confidence limit) (mg/kg)
FK037	0.025	0.428 (0.150～ 1.02)
Cefpirome	0.025	0.509 (0.197～ 1.20)
Ceftazidime	0.1	1.94[d] (0.316～ 4.05)
Flomoxef	0.1	6.28[d] (2.73 ～17.3)

[a] Mice were exposed to bacterial aerosal containing *K. pneumoniae* FP221 at 1.8×10^9 cfu/ml in a chamber for 20 minutes; [b] antibiotic was subcutaneously given 4, 24, 32, 48, 56, 72 and 80 hours after inhalation of bacteria; [c] agar dilution method (stamp method, 10^4 cfu/spot, Mueller-Hinton agar); [d] statistical significances refer to parallel line assay for paired differences, significant difference from FK037 ($P<0.05$).

Table 3. Therapeutic effect of FK037 and reference drugs on subcutaneous abscess with *Staphylococcus aureus* 47 (MSSA)[a] in mice.

Antibiotic	Dose[b] (mg/kg)	MIC[c] (μg/ml)	Log. cfu/abscess (mean±S.E.)
FK037	100	1.56	2.03±0.14[d]
	10		5.91±0.32[d]
Cefpirome	100	0.78	3.36±0.46[d]
	10		6.73±0.05
Ceftazidime	100	6.25	6.29±0.15
	10		7.17±0.18
Flomoxef	100	0.39	3.20±0.42[d]
	10		6.71±0.08
Control			8.35±0.07[e]

[a] Immunosuppressed mice (cyclophosphamide, 200 mg/kg, ip 4 days before inoculation) were subcutaneously inoculated with 0.1 ml of *S. aureus* 47 (4.6×10^2 cfu) on their dorsal regions; [b] antibiotic was subcutaneously administered at post-infection 5, 24, 32, 48 and 56 hours in sites remote from the infection sites; [c] agar dilution method (stamp method, 10^4 cfu/spot, Mueller-Hinton agar); [d] statistical significances refer to Tukey-type multiple comparison for paired differences, significant difference ($P<0.05$) from control, [e] significant difference ($P<0.05$) from FK037.

Table 4. Therapeutic effect of FK037 and reference drugs on ascending pyelonephritis[a] with *Staphylococcus epidermidis* 7022[b] in mice.

Antibiotic	Dose[c] (mg/kg)	MIC[d] (μg/ml)	Log. cfu/kidney (mean±S.E.)
FK037	50	0.78	2.77±0.39[e]
	10		3.75±0.58
Cefpirome	50	0.39	3.44±0.67[e]
	10		4.14±0.52
Flomoxef	50	0.39	3.73±0.44
	10		4.49±0.40
Control			6.01±0.28[f]

[a] Ascending pyelonephritis was induced by the method of OBANA[4]; [b] 0.1 ml bacterial suspension of *S. epidermidis* 7022 (1.5×10^5 cfu) was inoculated into bladder of anesthetized mice through the urethra, and the ureterostoma was closed with a clip for 5 hours; [c] antibiotic was subcutaneously given at post-infection 5, 24, 32, 48 and 56 hours; [d] agar dilution method (stamp method, 10^4 cfu/spot, Mueller-Hinton agar); [e] statistical significances refer to Tukey-type multiple comparison for paired differences, significant difference ($P<0.05$) from control, [f] significant difference ($P<0.05$) from FK037.

with those of cefpirome, ceftazidime and flomoxef (Table 2). All non-treated control mice died on post-infection day 3 in this model. FK037 was as effective as cefpirome and more effective than ceftazidime and flomoxef and this finding which correlated well with their MICs.

Therapeutic Effects of FK037 on Subcutaneous Abscess

Against a subcutaneous abscess due to *S. aureus* 47 (MSSA), FK037 significantly decreased the viable cell counts in the skin to 5.91 and 2.03 $\log_{10}$ at the respective doses of 10 and 100 mg/kg when compared with 8.35 $\log_{10}$ in the control mice (Table 3). The therapeutic effect of FK037 was superior to that of ceftazidime which did not significantly decrease the viable cell counts, and this finding can be explained by the difference in their MICs. Although the MIC of FK037 was higher value than that of cefpirome and flomoxef, FK037 at 100 mg/kg elicited an effect slightly superior to the two drugs since FK037 decreased the viable cell counts about 10-fold more than the drugs.

Therapeutic Effects of FK037 on Ascending Pyelonephritis

Against pyelonephritis with *S. epidermidis* 7022 (Table 4), FK037 decreased the viable cell counts to 3.75 $\log_{10}$ and significantly to 2.77 $\log_{10}$ in the kidneys at the respective doses of 10 and 50 mg/kg respectively when compared with 6.01 $\log_{10}$ in the control mice. Although FK037 registered a higher

MIC value than those of cefpirome and flomoxef, FK037 indicated an equipotent therapeutic effect to cefpirome and slightly superior to flomoxef which did not significantly decrease the viable cell counts at 50 mg/kg. Against *E. coli* 29 (Table 5), FK037 decreased the viable cell counts to 2.41 $\log_{10}$ at 0.5 mg/kg when compared with 6.88 $\log_{10}$ in the control mice, and the infected kidneys in 2 of 6 mice were sterilized at 2.5 mg/kg. The therapeutic effects of FK037 were equipotent to those of cefpirome and ceftazidime. Against *P. aeruginosa* 7001 (Table 6),

Table 5. Therapeutic effect of FK037 and reference drugs on ascending pyelonephritis[a] with *Escherichia coli* 29[b] in mice.

Antibiotic	Dose[c] (mg/kg)	MIC[d] (μg/ml)	Log. cfu/kidney (mean $\pm$ S.E.)
FK037	2.5	$\leqq$0.025	$\leqq$1.43[e]
	0.5		2.41 $\pm$ 0.30[e]
Cefpirome	2.5	$\leqq$0.025	$\leqq$1.87[e]
	0.5		3.03 $\pm$ 0.44[e]
Ceftazidime	2.5	0.05	$\leqq$1.59[e]
	0.5		3.14 $\pm$ 0.39[e]
Control			6.88 $\pm$ 0.27[e]

[a] Ascending pyelonephritis was induced by the method of OBANA[4]; [b] 0.1 ml bacterial suspension of *E. coli* 29 (2.4×10^5 cfu) was inoculated into bladder of anesthetized mice through the urethra, and the ureterostoma was closed with a clip for 5 hours; [c] antibiotic was subcutaneously given at post-infection 5, 24, 32, 48 and 56 hours; [d] agar dilution method (stamp method, 10^4 cfu/spot, Mueller-Hinton agar); [e] no statistical significances refer to Tukey-type multiple comparison for paired differences from control.

Table 6. Therapeutic effect of FK037 and reference drugs on ascending pyelonephritis[a] with *Pseudomonas aeruginosa* 7001[b] in mice.

Antibiotic	Dose[c] (mg/kg)	MIC[d] (μg/ml)	Log. cfu/kidney (mean $\pm$ S.E.)
FK037	50	1.56	2.72 $\pm$ 0.79[e]
	10		5.68 $\pm$ 0.56
Cefpirome	50	3.13	5.18 $\pm$ 0.62[e]
	10		6.59 $\pm$ 0.20
Ceftazidime	50	1.56	4.51 $\pm$ 0.58[e]
	10		6.11 $\pm$ 0.32
Cefoperazone	50	3.13	6.79 $\pm$ 0.39[f]
	10		7.28 $\pm$ 0.08
Control			8.78 $\pm$ 0.14[f]

[a] Ascending pyelonephritis was induced by the method of OBANA[4]; [b] 0.1 ml bacterial suspension of *P. aeruginosa* 7001 (3.0×10^5 cfu) was inoculated into bladder of anesthetized mice through the urethra, and the ureterostoma was closed with a clip for 5 hours; [c] antibiotic was subcutaneously given at post-infection 5, 24, 32, 48 and 56 hours; [d] agar dilution method (stamp method, 10^4 cfu/spot, Mueller-Hinton agar); [e] statistical significances refer to Tukey-type multiple comparison for paired differences, significant difference ($P<0.05$) from control, [f] significant difference ($P<0.05$) from FK037.

Table 7. Therapeutic effect of FK037 and reference drugs on intrauterine infection[a] with *Staphylococcus aureus* 47[b] in mice.

Antibiotic	Dose[c] (mg/kg)	MIC[d] (μg/ml)	Log. cfu/uterus (mean $\pm$ S.E.)
FK037	20	1.56	3.67 $\pm$ 0.21[e]
	5		6.03 $\pm$ 0.22
Cefpirome	20	0.78	3.94 $\pm$ 0.57[e]
	5		5.73 $\pm$ 0.14
Flomoxef	20	0.39	4.89 $\pm$ 0.43[e]
	5		5.73 $\pm$ 0.26
Control			7.41 $\pm$ 0.20[f]

[a] Intrauterine infection was induced by the method of OBANA[5]; [b] 0.025 ml bacterial suspension of *S. aureus* 47 (1.2×10^7 cfu) was inoculated into the left uterine horn; [c] antibiotic was subcutaneously given at post-infection 5, 24, 32, 48 and 56 hours; [d] agar dilution method (stamp method, 10^4 cfu/spot, Mueller-Hinton agar); [e] statistical significances refer to Tukey-type multiple comparison for paired differences, significant difference ($P<0.05$) from control, [f] significant difference ($P<0.05$) from FK037.

Table 8. Therapeutic effect of FK037 and reference drugs on intrauterine infection[a] with *Escherichia coli* 22[b] in mice.

Antibiotic	Dose[c] (mg/kg)	MIC[d] (μg/ml)	Log. cfu/uterine (mean $\pm$ S.E.)
FK037	2	0.025	2.61 $\pm$ 0.45[e]
	0.5		4.13 $\pm$ 0.59[e]
Cefpirome	2	0.05	2.70 $\pm$ 0.38[e]
	0.5		6.40 $\pm$ 0.19
Ceftazidime	2	0.1	3.73 $\pm$ 0.59[e]
	0.5		6.82 $\pm$ 0.66
Control			8.18 $\pm$ 0.24[f]

[a] Intrauterine infection was induced by the method of OBANA[5]; [b] 0.025 ml bacterial suspension of *E. coli* 22 (1.3×10^5 cfu) was inoculated into the left uterine horn; [c] antibiotic was subcutaneously given at post-infection 5, 24, 32, 48 and 56 hours; [d] agar dilution method (stamp method, 10^4 cfu/spot, Mueller-Hinton agar); [e] statistical significances refer to Tukey-type multiple comparison for paired differences, significant difference ($P<0.05$) from control, [f] significant difference ($P<0.05$) from FK037.

FK037 decreased the viable cell counts in the kidneys to 5.68 $\log_{10}$ and significantly to 2.72 $\log_{10}$ at the respective doses of 10 and 50 mg/kg when compared with 8.78 $\log_{10}$ of the control mice. The therapeutic effects of FK037 were more potent than those of cefpirome and cefoperazone at both doses. Similarly, FK037 elicited a slightly more potent effect than ceftazidime and cefpirome at 50 mg/kg, although the MIC of FK037 was the same as that of ceftazidime.

Therapeutic Effects of FK037 on Intrauterine Infection

Against intrauterine infection with *S. aureus* 47 (Table 7), FK037 decreased the viable cell counts in the uterus to 6.03 $\log_{10}$ and significantly to 3.67 $\log_{10}$ at the respective doses of 5 and 20 mg/kg when compared with 7.41 $\log_{10}$ of the control mice. The therapeutic effects of FK037 were equipotent to those

Table 9. Therapeutic effect of FK037 and reference drugs on pneumonia with *Staphylococcus aureus* 47[a] in mice in an *in vivo* pharmacokinetic model simulating human plasma concentrations[b].

Dose equivalent to human (drip infusion)	Antibiotic[c]	MIC[d] (μg/ml)	Log. cfu/lung at time after initial dosing (mean ± S.E.)		
			3 hours	6 hours	24 hours
0.25 g	FK037	1.56	N.D.[e]	5.67 ± 0.15	5.15 ± 0.20
	Cefpirome	0.78	N.D.	5.58 ± 0.10	5.07 ± 0.10
	Flomoxef	0.39	N.D.	6.16 ± 0.21	5.62 ± 0.12
0.5 g	FK037		5.22 ± 0.24	4.81 ± 0.26	3.88 ± 0.24
	Cefpirome		5.21 ± 0.35	5.25 ± 0.21	3.20 ± 0.08[f]
	Flomoxef		4.93 ± 0.11[f]	5.04 ± 0.32	3.31 ± 0.10[f]
1.0 g	FK037		5.35 ± 0.23	4.10 ± 0.29[f]	3.13 ± 0.13[f]
	Cefpirome		5.23 ± 0.33	4.89 ± 0.14	3.16 ± 0.09[f]
	Flomoxef		5.11 ± 0.21	4.50 ± 0.07[f]	3.32 ± 0.39[f]
Control			6.44 ± 0.15	6.47 ± 0.33[g]	6.75 ± 0.17[g]

[a] 0.05 ml bacterial suspension of *S. aureus* 47 (2.2×10^7 cfu) in saline was intranasally inoculated in the anesthetized immunosuppressed mice (cyclophosphamide, 200 mg/kg, ip, 4 days before inoculation); [b] human plasma concentrations after drip infusion of antibiotic were reproduced in murine plasma; [c] mice were subcutaneously given 10 times with antibiotic at a 1-hour intervals 16 hours after inoculation; [d] agar dilution method (stamp method, 10^4 cfu/spot, Mueller-Hinton agar); [e] not determined; [f] statistical significances refer to Tukey-type multiple comparison for paired differences, significant difference ($P<0.05$) from control, [g] significant difference ($P<0.05$) from FK037.

Table 10. Therapeutic effect of FK037 and reference drugs on pneumonia with *Klebsiella pneumoniae* FP221[a] in mice in an *in vivo* pharmacokinetic model simulating human plasma concentrations[b].

Dose equivalent to human (drip infusion)	Antibiotic[c]	MIC[d] (μg/ml)	Log. cfu/lung at time after initial dosing (mean ± S.E.)		
			3 hours	6 hours	24 hours
0.063 g	FK037	0.025	2.30 ± 0.15	1.66 ± 0.09[e]	1.10 ± 0.10[e]
	Cefpirome	0.025	2.63 ± 0.18	2.31 ± 0.12	1.38 ± 0.12
	Ceftazidime	0.1	2.39 ± 0.10	2.57 ± 0.13[f]	3.52 ± 0.54
0.125 g	FK037		1.97 ± 0.09[e]	1.92 ± 0.15[e]	≦1.06
	Cefpirome		2.29 ± 0.10	1.81 ± 0.09[e]	≦1.22
	Ceftazidime		2.04 ± 0.10[e]	2.15 ± 0.13	1.18 ± 0.07[e]
Control			4.56 ± 0.07[f]	4.95 ± 0.24[f]	7.66 ± 0.38[f]

[a] Immunosuppressed mice (cyclophosphamide, 200 mg/kg, ip, 4 days before inhalation) were exposed to bacterial aerosole of *K. pneumoniae* FP221 (2.4×10^9 cfu/ml) in a chamber for 20 minutes; [b] human plasma concentrations after drip infusion of antibiotic were reproduced in murine plasma; [c] mice were subcutaneously given 10 times with antibiotic at a 1-hour intervals 16 hours after inhalation; [d] agar dilution method (stamp method, 10^4 cfu/spot, Mueller-Hinton agar); [e] statistical significances refer to Tukey-type multiple comparison for paired differences, significant difference ($P<0.05$) from control, [f] significant difference ($P<0.05$) from FK037.

Table 11. Therapeutic effect of FK037 and reference drugs on pneumonia with *Pseudomonas aeruginosa* 7001[a] in mice in an *in vivo* pharmacokinetic model simulating human plasma concentrations[b].

Dose equivalent to human (drip infusion)	Antibiotic[c]	MIC[d] (μg/ml)	Log. cfu/lung at time after initial dosing (mean±S.E.)		
			3 hours	6 hours	24 hours
1.0 g	FK037	1.56	5.31±0.08	5.38±0.45[e]	8.63±0.13
	Cefpirome	1.56	5.60±0.14	5.64±0.20	8.60±0.08
	Ceftazidime	0.78	6.01±0.26	5.76±0.14	8.69±0.09
2.0 g	FK037		4.79±0.17[e]	4.97±0.07[e]	8.01±0.39
	Cefpirome		5.28±0.07[e]	5.36±0.24[e]	8.70±0.13
	Ceftazidime		5.53±0.22	5.57±0.14	8.52±0.06
Control			7.34±0.21[f]	8.44±0.10[f]	Dead

[a] 0.05 ml bacterial suspension of *P. aeruginosa* 7001 (2.2×10^2 cfu) in saline was intranasally inoculated in the anesthetized immunosuppressed mice (cyclophosphamide, 200 mg/kg, ip, 4 days before inoculation); [b] human plasma concentrations after drip infusion of antibiotic were reproduced in murine plasma; [c] mice were subcutaneously given 10 times with antibiotic at a 1-hour intervals 16 hours after inoculation; [d] agar dilution method (stamp method, 10^4 cfu/spot, Mueller-Hinton agar); [e] statistical significances refer to Tukey-type multiple comparison for paired differences, significant difference ($P<0.05$) from control, [f] significant difference ($P<0.05$) from FK037.

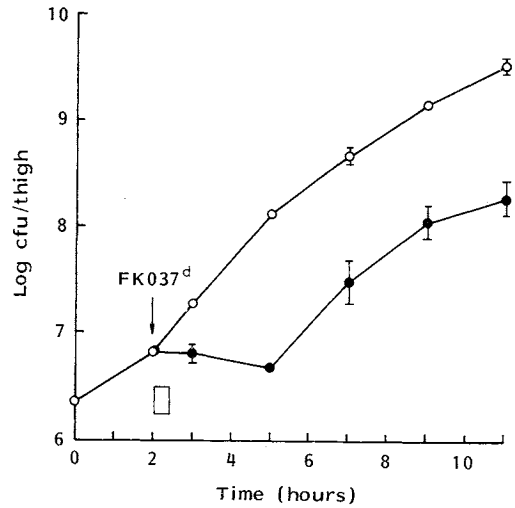
of cefpirome and were slightly superior to those of flomoxef, although its MIC value was higher than those of the two drugs. Against *E. coli* 22 (Table 8), FK037 also significantly decreased the viable cell counts to 4.13 and 2.61 $\log_{10}$ at the respective doses of 0.5 and 2 mg/kg when compared with 8.18 $\log_{10}$ of the control mice. The therapeutic effects of FK037 were superior to those of cefpirome and ceftazidime which did not significantly decrease the viable cell counts at 0.5 mg/kg, and this result can be explained by the higher MIC values of the latter.

Therapeutic Effects of FK037 in an *In Vivo* Pharmacokinetic Model

The therapeutic effects of FK037 on *S. aureus* 47 (MSSA) (Table 9) were compared with those of cefpirome and flomoxef at doses equivalent to 0.25, 0.5 and 1.0 g in humans. FK037 exhibited dose-dependent therapeutic effects although the viable counts in lungs from FK037-treated mice only at the dose equivalent to 1.0 g were significantly lower than those from control mice. The therapeutic effects of FK037 were almost the same with those of cefpirome and flomoxef at all the doses tested.

Fig. 1. *In vivo* post-antibiotic effect of FK037 in a thigh infection[a] with *Staphylococcus aureus* 47[b] in neutropenic mice[c].

○ Control, ● FK037, □ time thigh levels exceeded the MIC.



[a] *In vivo* PAE was studied by the method of GUDMUNDSSON[10]; [b] 0.1 ml bacterial suspension of *S. aureus* 47 (2.2×10^6 cfu) was inoculated in the thigh of mouse; [c] mice were immunosuppressed with intraperitoneal cyclophosphamide at 200 mg/kg 4 days before inoculation; [d] FK037 (5 mg/kg) was subcutaneously injected 2 hours after inoculation.

Against *K. pneumoniae* FP221 (Table 10), the therapeutic effects of FK037 were compared with those of cefpirome and ceftazidime at doses equivalent to 0.063 and 0.125 g in humans. The viable counts in the lungs from FK037-treated mice at both doses were significantly lower than those from the control mice at most sampling points and did not indicate any increases even 24 hours after the initiation of dosing.

The therapeutic effects of FK037 were similar to those of cefpirome and more potent than those of ceftazidime at 0.063 g dose. Against *P. aeruginosa* 7001 (Table 11), the therapeutic effects of FK037 were compared with those of cefpirome and ceftazidime at doses equivalent to 1.0 and 2.0 g in humans. The viable counts in the lungs from FK037-treated mice at doses equivalent to 1.0 and 2.0 g were significantly lower than those from the control mice 6 hours after the initation of dosing. All mice that had received FK037 which could not suppress the bacterial re-growth in the lungs, were still alive 24 hours after the initiation of dosing, although all the control mice died. The therapeutic effects of FK037 were similar to those of cefpirome and ceftazidime, although FK037 like cefpirome had a higher MIC than ceftazidime.

In Vivo Post-antibiotic Effect (*In Vivo* PAE) of FK037

As shown in Fig. 1, FK037 produced *in vivo* PAE of 3.4 hours against a thigh infection with MSSA in neutropenic mice.

Discussion

The potent therapeutic activity of FK037 against a wide range of bacteria, including *S. aureus* and *P. aeruginosa*, were confirmed in our present lethal systemic infection models in accord with its potent *in vitro* activity[1]. Against MRSA, it is noteworthy that FK037 indicated the highest activity of the tested drugs, including imipenem, in systemic infections. The detailed anti-MRSA activity of FK037 will be present in another paper[11]. With regards to other bacteria, the therapeutic activity of FK037 correlated well with its *in vitro* activity when compared with that of the reference drugs. FK037 indicated potent activity similar to cefpirome, a so-called fourth-generation cephalosporin, against most organisms, but elicited a higher effect against MRSA and *P. aeruginosa*. Moreover, FK037 manifested a higher activity than ceftazidime against most organisms, except that its therapeutic activities against *S. marcescens* and *P. aeruginosa* were similar to those of ceftazidime. In addition to its effects against lethal systemic infections, FK037 demonstrated potent therapeutic effects in several experimental local infections such as acute pneumonia due to *K. pneumoniae*, ascending pyelonephritis due to *S. epidermidis*, *E. coli* or *P. aeruginosa*, subcutaneous abscess due to *S. aureus* and intrauterine infections due to *S. aureus* or *E. coli*. These results suggest that FK037 might be useful for treating various infections in patients. On comparison of its MICs with those of the reference drugs, FK037 displayed excellent therapeutic effects as expected. However, in some infections due to staphylococci and *P. aeruginosa*, FK037 elicited effects more active than expected from its MICs. For instance, FK037 indicated therapeutic effects which were either superior or similar to those of cefpirome and ceftazidime even its MICs were similar to or higher than those of the latter drugs. These results might be due to higher bactericidal activity of FK037 at sub-MIC since the pharmacokinetic properties of these drugs are similar[1,12]. Against pneumonia with MSSA, *K. pneumoniae* or *P. aeruginosa* in an *in vivo* pharmacokinetic model in mice simulating human plasma concentrations, FK037 was therapeutically effective at doses equivalent to probable clinical dose of 1.0 g or less. Moreover, findings that the therapeutic effects of FK037 were equipotent or superior to those of cefpirome, ceftazidime and flomoxef support its expected clinical potential.

In conclusion, FK037 exhibited potent therapeutic effects on several experimental infections on the basis of its high antibacterial activity as a fourth-generation cephalosporin and displayed certain advantages over the reference drugs.

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