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

YASUHIRO MINE, YUJI WATANABE, HIROSHI SAKAMOTO, KAZUO HATANO, KYOICHIRO KUNO, YASUYUKI HIGASHI, TOSHIAKI KAMIMURA†, YOSHIMI MATSUMOTO† and SHUICHI TAWARA†

Product Development Laboratories and New Drug Research Laboratories†, Fujisawa Pharmaceutical Co., Ltd., Osaka, Japan

FUMIO MATSUMOTO

Kanagawa Prefectural Nursing and Hygienic School Hospital, Yokohama, Japan

SHOGO KUWAHARA

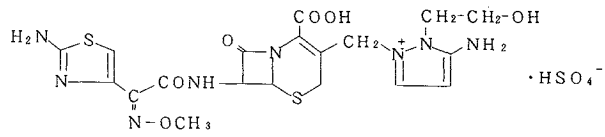
Toho University School of Medicine, Tokyo, Japan

(Received for publication May 25, 1992)

FK037 is a new parenteral cephalosporin, which offers some advantages over the commercially available parenteral cephalosporins. It demonstrated potent broad-spectrum activity against clinical isolates of Gram-positive bacteria including methicillin-resistant staphylococci, and Gram-negative bacteria including *Pseudomonas aeruginosa*. Against clinical isolates of aerobic Gram-positive bacteria, FK037, like cefpirome, demonstrated more potent activity than ceftazidime, cefoperazone and ceftizoxime. It is noteworthy that FK037, on the basis of the MIC_{90}s, was the most active of all the cephalosporins tested against methicillin-resistant *Staphylococcus aureus* (MRSA). It was similar in activity to cefpirome against methicillin-sensitive *S. aureus* (MSSA). Against clinical isolates of aerobic Gram-negative bacteria, FK037, like cefpirome, was superior to cefoperazone, similar to ceftazidime and inferior to ceftizoxime in activity. Against *P. aeruginosa*, FK037 was superior to cefoperazone, similar or slightly superior to cefpirome and inferior to ceftazidime in activity. However, FK037 exhibited significant activity against *Citrobacter* and *Enterobacter* which were highly resistant to ceftazidime, cefoperazone and ceftizoxime. FK037 had an advantage in that its bactericidal activity against *S. aureus*, *Escherichia coli* and *P. aeruginosa* at sub-MICs (1/2 or 1/4 the MIC) was much stronger than those of cefpirome and ceftazidime. Moreover, it exhibited potent bactericidal activity against MSSA, MRSA and *P. aeruginosa* in a pharmacokinetic *in vitro* model simulating human plasma concentrations after intravenous dosage of 0.125, 1.0 and 1.0 g, respectively. FK037 inhibited essential penicillin-binding proteins (PBPs), 1, 2 and 3 of *S. aureus* with a 50% inhibitory concentration (I_{50}) of 0.58 μg/ml or lower. Of essential PBPs 3, 1a and 1b of *E. coli* and *P. aeruginosa*, FK037 inhibited PBP 3 at the lowest I_{50} (0.03 and 0.04 μg/ml, respectively) and PBPs 1a and 1b with I_{50} values of 2.7 μg/ml or lower. FK037, like cefpirome, was highly stable to hydrolysis by various β-lactamases except Ic cephalosporinase from *Bacteroides fragilis*, and had extremely low affinity for β-lactamases. Therefore, FK037 was more potent than ceftazidime in activity against β-lactamase-producing bacteria except *P. aeruginosa* and *Serratia marcescens*. The ability of FK037 to penetrate the outer membrane of *E. coli* was slightly higher than that of ceftazidime, but slightly lower than that of cefpirome.

Great improvement in antibacterial activity, spectrum and β-lactamase stability has been achieved in the so-called third generation parenteral cephalosporins over the last decade. However, most third generation cephalosporins displayed a weak activity against staphylococci and *Pseudomonas aeruginosa*. Cefpirome

Fig. 1. Chemical structure of FK037.



(HR 810), as described by JONES *et al.*[1], is a "fourth generation cephalosporin", due to its enhanced activity against staphylococci, Enterobacteriaceae and *P. aeruginosa*, when compared to third generation cephalosporins. We also recently succeeded in addressing this deficiency of third generation cephalosporins with FK037 (Fig. 1) as evidenced by its *in vitro* activity against methicillin-resistant *Staphylococcus aureus*[2~5].

The *in vitro* antibacterial activity of FK037 in comparison to that of the reference drugs is the subject of this report.

Materials and Methods

Drugs

FK037 (lot 301186S, 330189S, 510194P, 520197P, 530198P), cefpirome (lot 262166S, 314176S, 331108S, 331199S) and a chromogenic cephalosporin FR18419 (lot K5001) were synthesized in the New Drug Research Laboratories of Fujisawa Pharmaceutical Co., Ltd., Osaka. The other drugs were commercially procured: ceftazidime (lot 07031, 80141, 96271, 73241, 70291, 000115C, B3605LF, B8035LC, B8635LC) from Tanabe Pharmaceutical Co., Ltd., Osaka; cefoperazone (lot RB987, TF276G) from Toyama Chemical Co., Ltd., Toyama prefecture; ceftizoxime (lot 20017XG) and ampicillin (lot 200195G) from Fujisawa Pharmaceutical Co., Ltd., Osaka; flomoxef (lot NR04, FM8235, CDH01) and cephaloridine (lot RL02) from Shionogi & Co., Ltd., Osaka; cefuzonam (lot 117-1) from Takeda Chemical Ind., Osaka; and methicillin (lot C022K) from Banyu Pharmaceutical Co., Ltd., Tokyo.

Bacteria

Stock strains from the culture collection in our laboratories were used in this study. Clinical isolates of various species of bacteria were obtained from several hospitals in Japan. *S. aureus* 2562M2 was a methicillin-susceptible subclone isolated from *S. aureus* 2562 (L-MRSA) during subcultivation in drug-free media. The criteria for drug resistance were MICs (minimum inhibitory concentrations) of 12.5 to 100 μg/ml of methicillin for L-MRSA and L-methicillin-resistant coagulase-negative staphylococci (L-MRCNS), $\geqq$200 μg/ml of methicillin for H-MRSA and H-MRCNS; $\geqq$1.56 μg/ml of ampicillin for ampicillin-resistant *Streptococcus pneumoniae*; $\geqq$6.25 μg/ml of ampicillin for ampicillin-resistant *Neisseria gonorrhoeae* and *Haemophilus influenzae*; and $\geqq$25 μg/ml of cefoperazone or ceftazidime for cefoperazone- or ceftazidime-resistant Gram-negative strains.

Determination of MICs

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*, *Streptococcus pyogenes*, *Streptococcus agalactiae*, "Viridans" group streptococci and *Corynebacterium diphtheriae*, and with heated 5% defibrinated horse blood (chocolate agar) for *Neisseria* species and *H. influenzae*, respectively. GAM agar (Nissui) was used for the anaerobic bacteria. The non-fastidious Gram-negative bacteria were precultured in Mueller-Hinton broth (Difco); staphylococci, *Enterococcus faecalis* and *Branhamella catarrhalis* in Trypticase soy broth (BBL); *S. pneumoniae* on Mueller-Hinton agar with 5% defibrinated horse blood; *S. pyogenes, S. agalactiae*, the "Viridans" group streptococci and *C. diphtheriae* in Trypticase soy broth plus 5% horse serum; *Neisseria* species on Mueller-Hinton agar with 5% defibrinated horse blood (chocolate agar); *H. influenzae* in Trypticase soy

broth plus 5% Fildes enrichment; and anaerobic bacteria in GAM broth (Nissui). 10^4 cfu/spot were inoculated with a multipoint replicating apparatus onto agar plates containing serial 2-fold dilutions of each antibiotic prior to incubation at 37°C for 18 hours. Incubation was carried out in an atmosphere of 5% CO_2 for streptococci, *H. influenzae*, *Neisseria* species and *C. diphtheriae*, whereas anaerobic bacteria were incubated in an anaerobic system Model 1024 (Forma) at 37°C for 24 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.

Determination of Minimum Bactericidal Concentration (MBC)

MBC values were determined by the microdilution broth method in Mueller-Hinton broth supplemented with $CaCl_2$ at 50 mg/liter and $MgCl_2$ at 25 mg/liter. The drugs dissolved in the medium at 100 μg/ml were serially diluted 2-fold with an automatic diluter AD-2 (Dainippon Seiki), and 100 μl of each broth was dispersed into 96-well multiwell trays with a dispenser (MIC 2000 system; Dynatech). The strains precultured on Mueller-Hinton agar at 37°C for 18 hours were suspended in saline for turbidity adjustment to McFarland 0.5 standard. About 1 μl samples of the suspension were inoculated into broths containing drugs with an automatic inoculator (MIC 2000 system; Dynatech) to achieve a final concentration at about 10^6 cfu/ml. After incubation at 37°C for 18 hours, the MIC was read as the lowest drug concentration that inhibited visible bacterial growth. About 1 μl of the culture broth in each well was inoculated onto Mueller-Hinton agar with an automatic inoculator. Media for *Proteus* species contained 3% agar to prevent swarming on the agar surface. After incubation at 37°C for 18 hours, the MBC which killed 99.9% of inoculated bacteria was read as the lowest drug concentration that inhibited colony formation.

Bactericidal Activity at Constant Concentrations

Overnight cultures of *S. aureus* 209P JC1 in Trypticase soy broth (BBL), and *Escherichia coli* NIHJ JC2 and *P. aeruginosa* 7001 in Mueller-Hinton broth were diluted in fresh Mueller-Hinton broth to achieve a final concentration of about 10^6 cfu/ml prior to incubation with shaking at 37°C for 1 hour. The bacteria were then exposed to drugs at 1/4 to the MIC at 37°C, and the viable bacterial counts were determined after incubation for 2, 4 or 6 hours. MICs were determined by the macrodilution broth method in the same volume of culture with the time-kill study after incubation for 18 hours.

Bactericidal Activity in an *In Vitro* Pharmacokinetic Model

Overnight cultures in Mueller-Hinton broth were diluted in the same fresh media to a final concentration of 10^6 cfu/ml and incubated with shaking at 37°C for 1 hour. The bacteria were then exposed to drugs at the estimated human plasma concentrations achieved after intravenous dosing of 0.125 g to 1.0 g, concentrations which were achieved by a stepwise dilution with fresh medium as described by NISHIDA[6]. The human plasma concentrations of FK037 were calculated on the basis of the data on healthy volunteers receiving intravenous FK037 (0.125 g)[7], whereas those of cefpirome, ceftazidime and flomoxef were referred to previously published data[8~10]. Viable cells were counted at various time intervals and adjusted by dilution factors.

Affinity to Penicillin-binding Proteins (PBPs)

PBP assays were performed by the modified method of SPRATT[11,12]. Membrane fractions containing PBPs were prepared from bacteria after sonication with a sonicator Model 200M (Kubota) and ultracentrifugation at 100,000 × *g*. For preparation of membrane fractions from *S. aureus*, lysostaphin (Sigma) treatment at 50 μg/ml at 30°C for 30 minutes was performed before sonication. The membrane fractions from various strains were exposed to drugs at 30°C for 10 minutes. ^{14}C-Labeled benzylpenicillin (^{14}C-PCG, specific activity at 58.5 mCi/mmol, Amersham) was used for detection of unsaturated PBPs by pretreatment with drugs. The labeled PBPs were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) with 10% acrylamide and 0.12% bisacrylamide. The gels were processed for fluorography and the concentrations of drugs which inhibited the ^{14}C-PCG binding to 50% of non-treated PBPs (I_{50}) were determined as the affinity of drugs to PBPs after scanning the X-ray film with a densitometer Model CS-930 (Shimadzu).

β-Lactamase Assay

After disruption by sonication, β-lactamases from several strains were purified by gel filtration *via* a Sephadex G-100 column. Enzymatic hydrolysis rates of drugs were determined with a spectrophotometer Model UV-265FW (Shimadzu) at 37°C in a 67 mM phosphate buffer (pH 7.0). The extinction coefficients per 1 cm at 50 μg/ml used were as follows; 1.69 for FK037 (260 nm); 0.643 for cefpirome (260 nm); 0.708 for ceftazidime (260 nm); 0.438 for cefoperazone (260 nm); 0.66 for flomoxef (260 nm); 1.318 for cephaloridine (260 nm); 0.096 for ampicillin (240 nm); and 1.97 for FR18419 (498 nm). *Km* and Vmax values were obtained by a least-square fit of the initial steady-state velocities at different substrate concentrations. *Ki* values were alternatively determined for the enzyme-stable drugs by measuring the inhibition of enzymatic degradation of a chromogenic cephalosporin, FR18419. Relative Vmax and relative Vmax/*Km* values of FK037 and the reference drugs were expressed as percentage of those of cephaloridine for cephalosporinase (CSase) and those of ampicillin for penicillinase (PCase).

Outer Membrane Permeability of *E. coli* to Drugs

The permeability coefficients of FK037, cefpirome and ceftazidime in *E. coli* FP1719 (TEM-9-β-lactamase producing strain) were determined by the method of ZIMMERMANN and ROSSELET[13].

Results

Antibacterial Spectrum of FK037

The activities of FK037 against 43 stock strains of aerobic organisms are shown in Table 1. FK037 had potent antibacterial activity against an extensive range of organisms, including staphylococci and *P. aeruginosa*. This antibacterial activity was similar to that of cefpirome, except that it was less active against *E. faecalis*. FK037 was more potent than ceftazidime against most Gram-positive bacteria and Gram-negative bacteria, however, it was less active against glucose-nonfermenting organisms including *P. aeruginosa*. Further, FK037 was superior and equipotent to ceftizoxime against most Gram-positive bacteria and Gram-negative bacteria, respectively. FK037 was more active than ceftizoxime against *P. aeruginosa* and *Pseudomonas putida*. In addition, FK037 displayed a potent activity against Gram-positive and Gram-negative anaerobic organisms (Table 2). Its activity approximated to that of cefpirome, was greater than that of ceftazidime, but was weaker than that of ceftizoxime.

Antibacterial Activity of FK037 against Clinical Isolates

The activities of FK037 against various clinical isolates were compared with those of cefpirome, ceftazidime, cefoperazone, ceftizoxime, flomoxef and cefuzonam. Table 3 shows the drug concentration necessary to inhibit 50% or 90% of the test strains and the MIC range. FK037 displayed advantages over ceftazidime, cefoperazone and ceftizoxime in its activities against *S. aureus* and coagulase-negative staphylococci. In addition, the MIC_{90} of FK037 (25 μg/ml) was the lowest of all the cephalosporins tested (except flomoxef) against MRSA and MRCNS. However, the activity of FK037 against MSSA and MSCNS was similar or slightly inferior to those of cefpirome, flomoxef and cefuzonam. FK037, like the other drugs except cefpirome and cefoperazone, was not active against *E. faecalis*. Against most clinical isolates of Gram-negative bacteria, FK037 had excellent *in vitro* activity similar to that of cefpirome, superior to those of ceftazidime, flomoxef and cefoperazone, and inferior to that of ceftizoxime. It is noteworthy that the MIC_{90}s of FK037, like cefpirome, against *Citrobacter freundii* and *Enterobacter* species were less than 3.13 μg/ml, whereas those of ceftazidime, cefoperazone, flomoxef and ceftizoxime were 25 μg/ml or higher. FK037 was also active against *P. aeruginosa*, and its activity was superior to that of cefoperazone, slightly superior to cefpirome, and slightly inferior to ceftazidime.

Table 1. Antibacterial spectrum of FK037 and reference drugs against aerobic organisms.

Organism	MIC (μg/ml)			
	FK037	Cefpirome	Ceftazidime	Ceftizoxime
Staphylococcus aureus 209P JC1	0.39	0.2	6.25	6.25
S. aureus Smith	0.78	0.78	6.25	12.5
S. aureus 8020 (MRSA)	6.25	12.5	50	>100
S. epidermidis 89	0.39	0.2	6.25	0.39
Streptococcus pyogenes S-23[a]	≦0.025	≦0.025	0.2	≦0.025
S. agalactiae FP1275[a]	0.05	0.1	0.39	0.2
S. mitis 3002[a]	0.2	0.2	3.13	0.39
S. pneumoniae 8001[a]	≦0.025	≦0.025	0.1	≦0.025
Enterococcus faecalis 0115	100	12.5	>100	>100
Neisseria gonorrhoeae PCL-783[b]	≦0.025	≦0.025	0.05	≦0.025
N. gonorrhoeae 3008 (ABPC^R)[b]	≦0.025	≦0.025	0.1	≦0.025
N. meningitidis Holland 68[b]	≦0.025	≦0.025	≦0.025	≦0.025
Moraxella (*Branhamella*) *catarrhalis* 4011	0.78	0.78	0.1	0.39
Escherichia coli NIHJ JC2	≦0.025	≦0.025	0.1	≦0.025
E. coli 3107	≦0.025	≦0.025	0.39	≦0.025
E. coli 4050 (CZX^R)	0.05	0.05	3.13	1.56
Citrobacter freundii 3015	0.1	0.1	1.56	0.39
Salmonella typhi T-287	≦0.025	≦0.025	0.1	≦0.025
S. paratyphi A-1015	≦0.025	≦0.025	0.1	≦0.025
S. typhimurium 1406	≦0.025	≦0.025	0.05	≦0.025
Shigella dysenteriae A-1 Shiga	≦0.025	≦0.025	0.1	0.05
S. flexneri 1a EW-8	≦0.025	0.05	0.2	≦0.025
S. sonnei I EW-33	≦0.025	≦0.025	0.05	≦0.025
Klebsiella pneumoniae NCTC 418	≦0.025	≦0.025	0.05	≦0.025
Enterobacter cloacae 3015	0.1	0.2	0.39	0.2
E. aerogenes 3013	0.2	0.1	0.39	0.2
Serratia marcescens 3059	6.25	25	6.25	3.13
Proteus mirabilis 1	0.05	0.1	0.1	≦0.025
P. vulgaris IAM 1025	0.05	0.05	0.05	≦0.025
Morganella morganii 4023	0.1	0.1	0.78	1.56
Providencia rettgeri 14	0.78	0.1	0.39	≦0.025
P. stuartii 4001	0.2	0.2	0.39	≦0.025
Pseudomonas aeruginosa IAM 1095	25	25	6.25	>100
P. aeruginosa NCTC 10490	6.25	12.5	3.13	6.25
P. cepacia ATCC 25416	25	25	3.13	6.25
P. putida FP720	0.39	0.39	1.56	3.13
Xanthomonas maltophilia ATCC 13637	50	25	3.13	100
Flavobacterium meningosepticum FP1045	6.25	6.25	50	12.5
Haemophilus influenzae 57[b]	0.05	≦0.025	0.05	≦0.025
H. influenzae 3002 (ABPC^R)[b]	0.05	≦0.025	0.05	≦0.025
Alcaligenes faecalis NCTC 655	50	12.5	3.13	25
A. denitrificans subsp. *xylosoxydans* FP1040	25	25	1.56	3.13

Agar dilution method (stamp method): Mueller-Hinton agar, 10^4 cfu/spot, 37°C, 18 hours; [a] supplemented with 5% defibrinated horse blood ([b] chocolate agar), 5% CO_2.

ABPC: ampicillin, CZX: ceftizoxime.

Antibacterial Activity of FK037 against β-Lactam-resistant and β-Lactamase-producing Organisms

Antibacterial activities of FK037 and reference drugs against methicillin-, ampicillin-, cefoperazone- and ceftazidime-resistant organisms are shown in Table 4. The activity of FK037 against L-MRSA, H-MRSA, L-MRCNS and H-MRCNS was superior to that of cefpirome (except L-MRCNS), ceftazidime

Table 2. Antibacterial spectrum of FK037 and reference drugs against anaerobic organisms.

Organism	MIC (μg/ml)			
	FK037	Cefpirome	Ceftazidime	Ceftizoxime
Peptostreptococcus asaccharolyticus Z1003	0.78	0.78	0.39	≦0.025
P. prevotii ATCC 9321	0.39	0.39	12.5	0.78
P. magnus ATCC 14956	3.13	3.13	6.25	1.56
P. productus ATCC 27340	0.78	0.78	3.13	0.78
Propionibacterium acnes ATCC 11828	0.2	0.39	1.56	0.05
Eubacterium limosum ATCC 8486	≦0.025	≦0.025	1.56	3.13
Clostridium tetani FP390	0.1	0.1	0.78	0.2
C. perfringens SAKAI	0.2	0.05	0.1	0.1
C. difficile FP885	25	25	25	>100
Veillonella parvula ATCC 10790	0.2	0.78	3.13	0.2
Bacteroides fragilis Ju-13	25	25	50	1.56
B. fragilis FP431	1.56	1.56	3.13	0.39
B. distasonis KVO-450	25	25	25	0.78
B. vulgatus W-6	25	25	25	0.39
B. thetaiotaomicron No. 11	>100	>100	>100	25
Porphyromonas asaccharolyticus Rm-1	≦0.025	0.1	1.56	≦0.025
Tissierella praeacuta ATCC 25539	0.2	0.2	0.39	0.05
Fusobacterium necrophorum W-12	0.39	0.39	0.39	0.05
F. nucleatum B-1	0.78	0.78	1.56	0.1
F. varium ATCC 8501	25	50	100	1.56
F. varium FP355	1.56	1.56	12.5	0.39

Agar dilution method (stamp method): GAM agar (Nissui), Anaerobic system model 1024 (Forma), 10^4 cfu/spot, 37°C, 24 hours.

and cefoperazone. Against ampicillin-resistant *N. gonorrhoeae* and *H. influenzae*, FK037, like the reference drugs, had an excellent activity. However, the activity of FK037 and the reference drugs was lower against ampicillin-resistant *S. pneumoniae* than that against ampicillin-sensitive *S. pneumoniae*. FK037 exhibited potent activities against cefoperazone-resistant Gram-negative bacteria except *Enterobacter aerogenes*, *Xanthomonas maltophilia* and *P. aeruginosa*. The activity of FK037 against these organisms was equipotent to that of cefpirome. Against ceftazidime-resistant Gram-negative bacteria, FK037 demonstrated potent activities against *C. freundii* and *Enterobacter* species, and moderate activities against *Flavobacterium meningosepticum* and *Acinetobacter calcoaceticus*. However, it was inactive against *Serratia marcescens*, *X. maltophilia*, *P. aeruginosa* and *Alcaligenes denitrificans* subsp. *xylosoxydans*.

The mean MICs of FK037 against several Gram-negative strains producing one or two β-lactamases were compared with those of cefpirome, ceftazidime and cefoperazone (Table 5). FK037 had potent activity against the strains producing TEM-1 PCase, IV PCase, Ia CSase, Ib CSase, TEM-1 PCase+Ia CSase (except *S. marcescens*) and TEM-1 PCase+Ib CSase, and a moderate activity against the *P. aeruginosa* strains producing PSE-1 and OXA-2 PCase and the *S. marcescens* strains producing Ia CSase and TEM-1 PCase+Ia CSase. However, it had poor activity against *P. aeruginosa* strains producing Id CSase. The activity of FK037 against these strains was similar to that of cefpirome; FK037 was slightly better vs. OXA-2 PCase and slightly less vs. Ia CSase. FK037 had more potent activity than ceftazidime against all strains except the CSase producers of *P. aeruginosa* and *S. marcescens*. Similarly, FK037 was more potent than cefoperazone against all strains except the OXA-2 PCase producing *P. aeruginosa*.

Comparison of Broth Dilution MBCs and MICs of FK037

The microdilution broth MBCs and MICs of FK037 and the reference drugs against 5 strains each

Table 3. Antibacterial activity of FK037 and reference drugs against clinical isolates.

Organism (No. of strains)	Antibiotic	MIC (μg/ml)		
		Range	50%	90%
Staphylococcus aureus (48) (MSSA)	FK037	0.78～3.13	1.56	3.13
Methicillin: ≦6.25 μg/ml	Cefpirome	0.39～6.25	0.78	1.56
	Flomoxef	0.2～6.25	0.78	1.56
	Cefuzonam	0.39～6.25	0.78	3.13
	Ceftazidime	6.25～50	12.5	25
	Cefoperazone	1.56～12.5	3.13	12.5
	Ceftizoxime	1.56～＞100	6.25	＞100
S. aureus (76) (MRSA)	FK037	1.56～25	12.5	25
Methicillin: ≧12.5 μg/ml	Cefpirome	0.78～100	50	100
	Flomoxef	0.78～100	6.25	50
	Cefuzonam	0.78～＞100	50	＞100
	Ceftazidime	12.5～＞100	＞100	＞100
	Cefoperazone	3.13～＞100	＞100	＞100
	Ceftizoxime	12.5～＞100	＞100	＞100
Coagulase-negative staphylococci	FK037	0.1～6.25	0.39	3.13
(145) (MSCNS)	Cefpirome	0.1～25	0.39	1.56
Methicillin: ≦6.25 μg/ml	Flomoxef	0.2～100	0.78	3.13
	Cefuzonam	0.1～12.5	0.39	1.56
	Ceftazidime	1.56～50	6.25	25
	Cefoperazone	0.39～25	1.56	3.13
	Ceftizoxime	0.2～＞100	1.56	50
Coagulase-negative staphylococci (37)	FK037	1.56～100	3.13	25
(MRCNS)	Cefpirome	0.78～100	6.25	100
Methicillin: ≧12.5 μg/ml	Flomoxef	0.78～100	6.25	25
	Cefuzonam	0.78～＞100	6.25	＞100
	Ceftazidime	6.25～＞100	50	＞100
	Cefoperazone	1.56～＞100	12.5	＞100
	Ceftizoxime	1.56～＞100	＞100	＞100
Streptococcus pyogenes[a] (42)	FK037	≦0.025	≦0.025	≦0.025
	Cefpirome	≦0.025	≦0.025	≦0.025
	Flomoxef	0.2～0.39	0.2	0.39
	Ceftazidime	0.1～0.2	0.2	0.2
	Cefoperazone	0.05～0.2	0.1	0.2
	Ceftizoxime	≦0.025	≦0.025	≦0.025
S. agalactiae[a] (11)	FK037	0.05	0.05	0.05
	Cefpirome	0.05～0.1	0.1	0.1
	Flomoxef	0.39～0.78	0.78	0.78
	Ceftazidime	0.39～0.78	0.39	0.78
	Cefoperazone	0.1～0.2	0.2	0.2
	Ceftizoxime	0.2	0.2	0.2
S. pneumoniae[a] (42)	FK037	≦0.025～0.39	≦0.025	0.1
	Cefpirome	≦0.025～0.39	≦0.025	0.1
	Flomoxef	0.1～0.39	0.2	0.2
	Ceftazidime	0.1～6.25	0.2	0.78
	Cefoperazone	≦0.025～0.1	0.05	0.1
	Ceftizoxime	≦0.025～1.56	0.05	0.2
Viridans group streptococci[a] (37)	FK037	≦0.025～1.56	0.1	0.78
	Cefpirome	≦0.025～1.56	0.1	0.39
	Flomoxef	0.05～6.25	0.78	3.13
	Ceftazidime	0.1～25	1.56	6.25
	Cefoperazone	0.1～6.25	0.78	3.13
	Ceftizoxime	0.05～12.5	0.39	6.25

Table 3. (Continued)

Organism (No. of strains)	Antibiotic	MIC (μg/ml)		
		Range	50%	90%
Enterococcus faecalis (42)	FK037	25～>100	100	>100
	Cefpirome	6.25～100	12.5	50
	Flomoxef	50～>100	100	>100
	Ceftazidime	>100	>100	>100
	Cefoperazone	25～100	25	50
	Ceftizoxime	>100	>100	>100
Clostridium difficile[b] (21)	FK037	25～>100	100	100
	Cefpirome	25～100	50	100
	Flomoxef	3.13～25	12.5	25
	Ceftazidime	25～>100	100	100
	Cefoperazone	12.5～>100	>100	>100
	Ceftizoxime	>100	>100	>100
Neisseria gonorrhoeae[c] (40)	FK037	≦0.025～0.1	≦0.025	0.05
	Cefpirome	≦0.025～0.2	≦0.025	0.05
	Flomoxef	0.1～0.78	0.39	0.39
	Ceftazidime	≦0.025～0.1	0.05	0.1
	Cefoperazone	≦0.025～0.2	≦0.025	0.1
	Ceftizoxime	≦0.025	≦0.025	≦0.025
Escherichia coli (42)	FK037	≦0.025～0.2	0.05	0.1
	Cefpirome	≦0.025～0.2	0.05	0.1
	Flomoxef	0.05～1.56	0.05	0.2
	Ceftazidime	0.05～12.5	0.2	0.39
	Cefoperazone	0.05～3.13	0.2	0.78
	Ceftizoxime	≦0.025～3.13	0.05	0.2
Klebsiella pneumoniae (42)	FK037	≦0.025～0.2	0.05	0.1
	Cefpirome	≦0.025～0.2	0.05	0.1
	Flomoxef	0.05～0.2	0.05	0.1
	Ceftazidime	0.1～1.56	0.2	0.39
	Cefoperazone	0.1～12.5	0.2	1.56
	Ceftizoxime	≦0.025～0.2	≦0.025	0.05
Klebsiella oxytoca (21)	FK037	≦0.025～6.25	≦0.025	0.78
	Cefpirome	≦0.025～6.25	0.05	0.78
	Flomoxef	≦0.025～0.2	0.05	0.2
	Ceftazidime	0.05～100	0.1	0.39
	Cefoperazone	0.1～50	0.78	25
	Ceftizoxime	≦0.025～3.13	≦0.025	0.05
Proteus mirabilis (41)	FK037	≦0.025～0.2	0.05	0.1
	Cefpirome	0.05～0.2	0.1	0.2
	Flomoxef	0.1～0.78	0.2	0.39
	Ceftazidime	0.05～0.2	0.1	0.1
	Cefoperazone	0.39～3.13	0.78	1.56
	Ceftizoxime	≦0.025～0.05	≦0.025	≦0.025
P. vulgaris (42)	FK037	≦0.025～1.56	0.2	1.56
	Cefpirome	0.05～6.25	0.39	3.13
	Flomoxef	0.2～0.78	0.39	0.78
	Ceftazidime	≦0.025～1.56	0.1	0.2
	Cefoperazone	0.1～>100	1.56	6.25
	Ceftizoxime	≦0.025～0.2	≦0.025	0.05
Other indole(+) *Proteus* (63) (*Morganella morganii* (21), *P. stuartii* (21), *P. rettgeri* (21))	FK037	≦0.025～6.25	0.05	1.56
	Cefpirome	≦0.025～1.56	0.1	0.39
	Flomoxef	0.05～6.25	0.39	3.13
	Ceftazidime	≦0.025～6.25	0.1	1.56
	Cefoperazone	0.2～25	0.78	6.25
	Ceftizoxime	≦0.025～6.25	≦0.025	0.39

Table 3. (Continued)

Organism (No. of strains)	Antibiotic	MIC (μg/ml)		
		Range	50%	90%
Citrobacter freundii (26)	FK037	≦0.025～12.5	0.1	3.13
	Cefpirome	≦0.025～3.13	0.1	1.56
	Flomoxef	0.2～>100	12.5	100
	Ceftazidime	0.2～>100	0.78	100
	Cefoperazone	0.1～>100	0.78	100
	Ceftizoxime	0.1～>100	0.78	50
Enterobacter cloacae (29)	FK037	≦0.025～12.5	0.1	3.13
	Cefpirome	≦0.025～6.25	0.1	1.56
	Flomoxef	0.2～>100	50	>100
	Ceftazidime	0.1～>100	0.39	100
	Cefoperazone	≦0.025～>100	0.39	50
	Ceftizoxime	≦0.025～>100	0.2	100
E. aerogenes (42)	FK037	≦0.025～25	0.1	1.56
	Cefpirome	≦0.025～50	0.1	0.78
	Flomoxef	0.1～>100	25	100
	Ceftazidime	0.05～100	0.78	50
	Cefoperazone	≦0.025～>100	1.56	25
	Ceftizoxime	≦0.025～50	1.56	50
Serratia marcescens (34)	FK037	≦0.025～6.25	0.78	6.25
	Cefpirome	≦0.025～6.25	0.78	3.13
	Flomoxef	0.39～>100	25	>100
	Ceftazidime	0.1～3.13	0.78	3.13
	Cefoperazone	0.39～>100	25	>100
	Ceftizoxime	0.05～25	0.78	12.5
Pseudomonas aeruginosa (110)	FK037	0.39～>100	6.25	25
	Cefpirome	0.39～>100	6.25	50
	Ceftazidime	0.39～>100	3.13	12.5
	Cefoperazone	0.39～>100	12.5	50
Haemophilus influenzae[c] (42)	FK037	0.05～0.39	0.1	0.2
	Cefpirome	0.05～0.2	0.1	0.2
	Flomoxef	0.39～6.25	0.78	3.13
	Ceftazidime	0.1～0.78	0.2	0.39
	Cefoperazone	≦0.025～0.2	≦0.025	0.2
	Ceftizoxime	≦0.025～0.39	≦0.025	0.1
Moraxella (*Branhamella*) *catarrhalis* (41)	FK037	0.05～3.13	0.78	1.56
	Cefpirome	0.05～1.56	0.78	1.56
	Flomoxef	≦0.025～0.39	0.1	0.39
	Ceftazidime	≦0.025～0.2	0.1	0.2
	Cefoperazone	0.05～3.13	0.78	3.13
	Ceftizoxime	≦0.025～0.39	0.39	0.39
Bacteroides fragilis[b] (42)	FK037	0.39～>100	25	>100
	Cefpirome	0.78～>100	25	>100
	Flomoxef	0.1～12.5	0.78	6.25
	Ceftazidime	0.78～>100	25	>100
	Cefoperazone	0.1～>100	50	>100
	Ceftizoxime	0.05～>100	3.13	100

Agar dilution method (stamp method): Mueller-Hinton agar, 10^4 cfu/spot, 37°C, 18 hours; [a] supplement with 5% defibrinated horse blood ([c] chocolate agar), 5% CO_2; [b] GAM agar (Nissui), Anaerobic system model 1024 (Forma), 37°C, 24 hours.

are shown in Table 6. The mean MBCs of FK037 were similar to the mean MICs against all organisms tested except *S. marcescens*. The mean MBCs of FK037 against MSSA, *S. epidermidis* and Gram-negative bacteria except *S. marcescens* and *P. aeruginosa* ranged from 0.1 to 1.56 μg/ml and were equipotent to

Table 4. Antibacterial activity of FK037 and reference drugs against β-lactam-resistant organisms.

Organism (No. of strains)	Mean MIC (μg/ml)			
	FK037	Cefpirome	Ceftazidime	Cefoperazone
Methicillin-resistant				
L-MRSA[a] (49) (12.5~100 μg/ml)	6.71	11.0	79.7	56.0
H-MRSA[a] (27) (≧200 μg/ml)	20.9	71.6	>100	>100
L-MRCNS[b] (21) (12.5~100 μg/ml)	2.93	3.34	32.6	8.41
H-MRCNS[b] (16) (≧200 μg/ml)	17.7	32.4	>100	62.1
Ampicillin-resistant				
Streptococcus pneumoniae (5) (≧1.56 μg/ml)	2.72	1.56	21.8	N.D.[c]
Neisseria gonorrhoeae (7) (≧6.25 μg/ml)	0.03	0.03	0.04	0.08
Haemophilus influenzae (8) (≧6.25 μg/ml)	0.13	0.10	0.16	0.11
Cefoperazone-resistant (≧25 μg/ml)[d]				
Proteus vulgaris (2)	0.78	3.13	0.39	70.7
Serratia marcescens (19)	2.17	1.74	1.62	89.6
Citrobacter freundii (1)	0.39	1.56	1.56	100
Enterobacter cloacae (1)	0.78	0.39	12.5	25
E. aerogenes (1)	25	50	3.13	>100
Xanthomonas maltophilia (5)	100	50	8.25	50
Pseudomonas aeruginosa (118)	36.4	39.8	12.2	68.7
P. cepacia (11)	9.72	11.7	2.59	38.9
P. putida (10)	2.21	3.59	2.72	33.0
Alcaligenes faecalis (2)	4.42	6.25	0.55	35.4
Acinetobacter calcoaceticus (10)	1.92	2.54	3.59	46.7
Ceftazidime-resistant (≧25 μg/ml)				
Serratia marcescens (8)	>100	84.1	70.7	91.7
Citrobacter freundii (26)	2.66	1.37	78.7	51.4
Enterobacter cloacae (23)	2.94	1.39	71.8	43.0
E. aerogenes (16)	1.06	0.55	45.9	14.2
Xanthomonas maltophilia (14)	>100	>100	95.2	55.2
Pseudomonas aeruginosa (35)	>100	>100	51.0	>100
Flavobacterium meningosepticum (19)	19.4	24.1	>100	33.5
Alcaligenes denitrificans subsp. *xylosoxydans* (2)	>100	>100	50	>100
Acinetobacter calcoaceticus (2)	25	35.4	50	>100

Agar dilution method (stamp method): Mueller-Hinton agar, 10^4 cfu/spot, 37°C, 18 hours.

[a] MRSA: Methicillin-resistant *Staphylococcus aureus*. [b] MRCNS: Methicillin-resistant coagulase-negative staphylococci. [c] Not determined. [d] Cefoperazone-resistant and ceftazidime-sensitive (≦12.5 μg/ml) strain (except *P. aeruginosa*).

those of cefpirome, but superior to those of flomoxef, ceftazidime and cefoperazone. Against L-MRSA and H-MRSA, the mean MBCs of FK037 were 14.4 and 25 μg/ml, respectively, but superior to those of the other tested drugs. FK037, like cefpirome, was more active than the other drugs against *S. marcescens*. The mean MBC of FK037 against *P. aeruginosa* was equipotent to that of cefoperazone, superior to that of cefpirome and flomoxef, but inferior to that of ceftazidime.

Bactericidal Activity of FK037

Bactericidal Activity at Constant Concentrations

Against *S. aureus* 209P JC1 (Fig. 2), FK037 had bactericidal activity even at concentrations of 1/4 the MIC and 1/2 the MIC. The activity of FK037 was more potent than that of cefpirome and ceftazidime.

Table 5. Antibacterial activity of FK037 and reference drugs against β-lactamase-producing organisms.

β-Lactamase	Organism (No. of strains)	Mean MIC (μg/ml)			
		FK037	Cefpirome	Ceftazidime	Cefoperazone
III PCase (TEM-1)[a]	*Escherichia coli* (9)	0.07	0.06	0.17	2.3
	Klebsiella pneumoniae (7)	0.07	0.1	0.22	5.13
	Serratia marcescens (4)	0.28	0.33	0.39	21
V PCase (PSE-1)	*Pseudomonas aeruginosa* (6)	11.1	11.1	3.13	>100
V PCase (OXA-2)	*Pseudomonas aeruginosa* (2)	35.4	70.7	2.21	12.5
IV PCase	*Klebsiella pneumoniae* (7)	0.07	0.09	0.35	3.81
Ia CSase[b]	*Citrobacter freundii* (12)	2.78	0.98	59.5	37.5
	Enterobacter cloacae (12)	0.98	0.37	10.5	7.02
	Serratia marcescens (4)	6.25	3.13	2.21	>100
Ib CSase	*Escherichia coli* (6)	0.1	0.06	2.21	0.62
Id CSase	*Pseudomonas aeruginosa* (5)	87.1	66	18.9	100
TEM-1PCase+Ia CSase	*Citrobacter freundii* (3)	0.39	0.31	7.87	9.92
	Enterobacter cloacae (1)	1.56	0.39	12.5	25
	Serratia marcescens (4)	29.7	17.7	14.9	>100
TEM-1 PCase+Ib CSase	*Escherichia coli* (2)	0.2	0.2	3.13	3.13
PSE-1 PCase+Id CSase	*Pseudomonas aeruginosa* (1)	>100	100	100	>100
OXA-2 PCase+Id CSase	*Pseudomonas aeruginosa* (1)	>100	>100	25	>100

Agar dilution method (stamp method): Mueller-Hinton agar, 10^4 cfu/spot, 37°C, 18 hours; [a] PCase, penicillinase; [b] CSase, cephalosporinase.

Table 6. Comparison of broth dilution MBCs and MICs of FK037 and reference drugs.

Organism (No. of strains)		Mean MBC/Mean MIC (μg/ml)				
		FK037	Cefpirome	Flomoxef	Ceftazidime	Cefoperazone
Staphylococcus aureus (MSSA)[a]	(5)	1.56/1.56	1.56/1.18	0.68/0.68	9.47/8.25	2.72/2.72
S. aureus (L-MRSA)[b]	(5)	14.4/14.4	50.0/33.0	28.7/16.5	>100/>100	>100/87.1
S. aureus (H-MRSA)[c]	(5)	25.0/21.8	87.1/75.8	66.0/50.0	>100/>100	>100/>100
S. epidermidis	(5)	1.18/0.78	1.03/0.59	3.13/1.56	12.5/7.18	2.06/0.90
Escherichia coli	(5)	0.10/0.09	0.10/0.09	0.20/0.13	0.34/0.34	0.39/0.34
Citrobacter freundii	(5)	1.36/1.03	0.90/0.78	25.0/21.8	19.0/16.5	19.0/10.9
Klebsiella pneumoniae	(5)	0.20/0.15	0.13/0.11	0.26/0.26	0.45/0.45	0.45/0.45
Enterobacter cloacae	(5)	0.52/0.45	0.39/0.34	21.8/14.4	2.72/1.18	2.72/2.06
Serratia marcescens	(5)	18.9/4.74	19.0/4.74	>100/43.5	37.9/6.25	>100/43.5
Proteus mirabilis	(5)	0.13/0.11	0.17/0.15	0.52/0.45	0.15/0.10	19.0/10.9
P. vulgaris	(5)	0.34/0.26	0.78/0.59	0.68/0.68	0.26/0.22	3.59/2.37
Pseudomonas aeruginosa	(5)	6.25/6.25	12.5/7.18	>100/>100	2.72/1.79	7.18/6.25

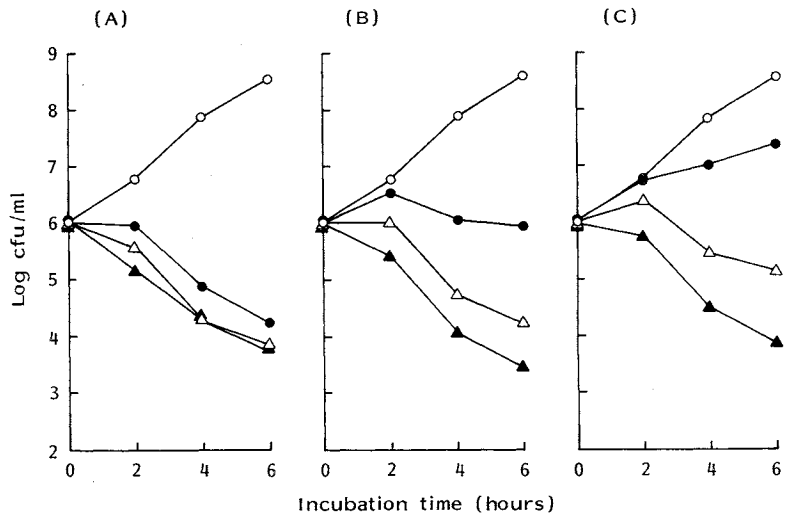
Microdilution broth method: Mueller-Hinton broth supplemented with $CaCl_2$ at 50 mg/liter and $MgCl_2$ at 25 mg/liter, 10^6 cfu/ml, 37°C, 18 hours. MBC: Killing 99.9% of the inoculum. Methicillin-MIC: [a] MSSA (≦6.25 μg/ml); [b] L-MRSA (12.5～100 μg/ml); [c] H-MRSA (≧200 μg/ml).

Against *E. coli* NIHJ JC2 (Fig. 3), FK037, like cefpirome and ceftazidime, elicited excellent bactericidal activity at 1/2 the MIC or higher. Against *P. aeruginosa* 7001 (Fig. 4), the bactericidal activity of FK037 was relatively superior to those of cefpirome and ceftazidime.

Bactericidal Activity in an *In Vitro* Pharmacokinetic Model

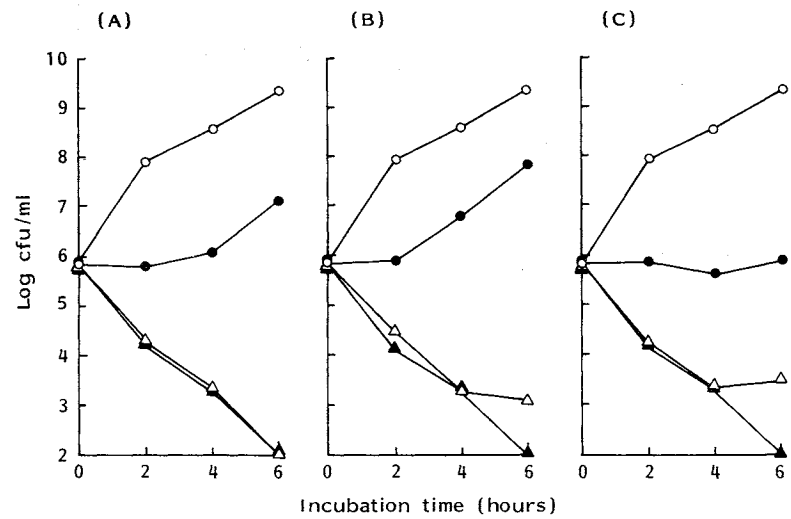
To estimate the drug efficacy in human subjects, bactericidal activity was also studied in an *in vitro* pharmacokinetic model simulating human plasma concentrations after an intravenous dosage of 0.125 g against MSSA, and 1.0 g against MRSA and *P. aeruginosa* (Fig. 5). Against *S. aureus* 2562M2 (MSSA),

Fig. 2. Bactericidal activity of FK037 and reference drugs against *Staphylococcus aureus* 209P JC1.
○ Control, ● $\frac{1}{4}$ MIC, △ $\frac{1}{2}$ MIC, ▲ 1 MIC.



(A) FK037 (MIC: 0.78 μg/ml), (B) cefpirome (MIC: 0.39 μg/ml), (C) ceftazidime (MIC: 6.25 μg/ml).

Fig. 3. Bactericidal activity of FK037 and reference drugs against *Escherichia coli* NIHJ JC2.
○ Control, ● $\frac{1}{4}$ MIC, △ $\frac{1}{2}$ MIC, ▲ 1 MIC.

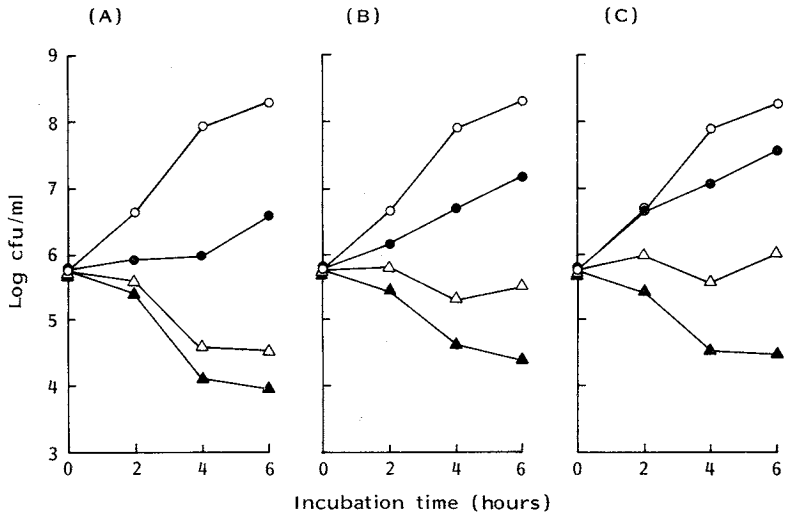


(A) FK037 (MIC: 0.05 μg/ml), (B) cefpirome (MIC: 0.05 μg/ml), (C) ceftazidime (MIC: 0.2 μg/ml).

FK037, even at 0.125 g, was highly bactericidal during the 8 hour-incubation, although its activity was slightly lower than that of flomoxef. Against *S. aureus* 5027 (MRSA), FK037 showed an excellent bactericidal activity even at 1.0 g dose. However, cefpirome and flomoxef at 1.0 g doses showed only bacteriostatic activity for 3 hours followed by regrowth. Against *P. aeruginosa* 7129, FK037, even at 1.0 g, was highly bactericidal for the 8 hours incubation. Its bactericidal activity was similar to that of ceftazidime. However, regrowth of *P. aeruginosa* occurred 4 hours after incubation with cefpirome.

Fig. 4. Bactericidal activity of FK037 and reference drugs against *Pseudomonas aeruginosa* 7001.

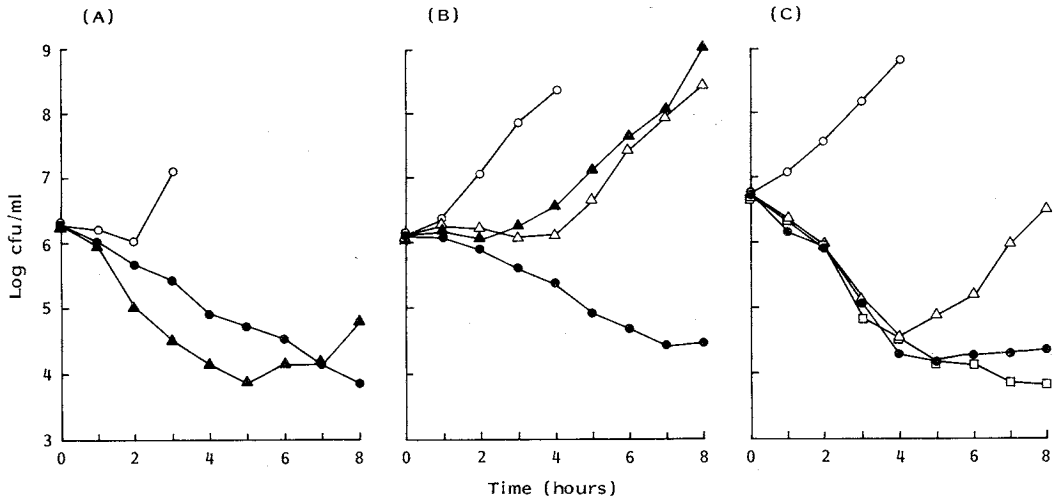
○ Control, ● $\frac{1}{4}$ MIC, △ $\frac{1}{2}$ MIC, ▲ 1 MIC.



(A) FK037 (MIC: 1.56 μg/ml), (B) cefpirome (MIC: 1.56 μg/ml), (C) ceftazidime (MIC: 1.56 μg/ml).

Fig. 5. Bactericidal activity of FK037 and reference drugs in a pharmacokinetic *in vitro* model simulating human plasma concentrations after an intravenous dosage.

○ Control, ● FK037, ▲ flomoxef, △ cefpirome, □ ceftazidime.



(A) *Staphylococcus aureus* 2562M2 (MSSA), 0.125 g (1 hour drip infusion), MIC: FK037, 1.56 μg/ml, flomoxef, 0.78 μg/ml; (B) *Staphylococcus aureus* 5027 (MRSA), 1.0 g (1 hour drip infusion), MIC: FK037, 25 μg/ml, flomoxef, 200 μg/ml, cefpirome, 100 μg/ml; (C) *Pseudomonas aeruginosa* 7129, 1.0 g (1 hour drip infusion), MIC: FK037, 25 μg/ml, cefpirome, 25 μg/ml, ceftazidime, 12.5 μg/ml.

Affinity of FK037 for the Penicillin-binding Proteins (PBPs)

The affinities of FK037 and of the reference drugs to PBPs of *S. aureus* 2562M2 (MSSA), *E. coli* NIHJ JC2 and *P. aeruginosa* IAM 1095 are expressed as concentrations of drugs required to reduce

Table 7. Affinity of FK037 and reference drugs for the penicillin-binding proteins (PBPs).

Organism	PBP	I_{50} (μg/ml)[a]			
		FK037	Cefpirome	Flomoxef	Ceftazidime
Staphylococcus aureus 2562M2	1	0.07	0.13	0.54	N.D.
	2	0.06	0.14	0.12	N.D.
	3	0.58	2.80	0.11	N.D.
	4	14	>25	0.01	N.D.
	MIC (μg/ml)	0.78	0.39	0.39	
Escherichia coli NIHJ JC2	1a	2.7	>5	0.14	0.80
	1bs	1.5	0.36	0.61	1.3
	2	7.3	2.5	>25	>25
	3	0.03	0.01	0.07	0.05
	4	3.9	5	2.1	>25
	5	>25	>5	0.13	>25
	6	21	>5	0.16	>25
	MIC (μg/ml)	$\leqq$0.025	$\leqq$0.025	0.1	0.1
Pseudomonas aeruginosa IAM 1095	1a	0.08	<0.04	N.D.	0.11
	1b	2.7	5.2	N.D.	7.3
	2	>25	>25	N.D.	>25
	3	0.04	<0.04	N.D.	0.10
	4	0.11	0.07	N.D.	2.2
	5/6	>25	>25	N.D.	>25
	MIC (μg/ml)	25	25		6.25

PBP assay: the modified method of SPRATT. [a] Concentrations of drug required to reduce ^{14}C-benzylpenicillin binding by 50%. N.D.: not determined.

^{14}C-PCG binding by 50%. As shown in Table 7, FK037 inhibited PBPs 1, 2 and 3 of *S. aureus* at I_{50} values of 0.58 μg/ml or lower, which is similar to that of cefpirome and flomoxef, although FK037 and cefpirome were inferior to flomoxef in PBP 4 affinity. Against the main PBPs of *E. coli*, FK037 inhibited PBP 3 to the greatest extent (I_{50} 0.03 μg/ml), which is similar to that obtained with cefpirome, flomoxef and ceftazidime. PBPs 1bs and 1a were inhibited at 1.5 and 2.7 μg/ml of FK037, respectively. The inhibition of PBP 1bs by FK037 is similar to ceftazidime and inferior to cefpirome and flomoxef, while against PBP 1a, FK037 had greater inhibition than cefpirome and inferior inhibition to that of flomoxef and ceftazidime. FK037 inhibited the PBP 3 of *P. aeruginosa* at the I_{50} value of 0.04 μg/ml, PBP 1a at 0.08 μg/ml and PBP 1b at 2.7 μg/ml. The affinities of FK037 to the main PBPs of *P. aeruginosa* were nearly equipotent to those of cefpirome and ceftazidime.

Stability and Affinity of FK037 to Various β-Lactamases

Relative Vmax, *Km* and Vmax/*Km* values of FK037 for various β-lactamases were compared with those of cefpirome, ceftazidime and cefoperazone (Table 8). Based on these values, FK037 was stable to CSase and PCase, but was unstable to Ic CSase from *Bacterioides fragilis*. The stability of FK037 to these enzymes was markedly superior to that of cefoperazone and similar to that of cefpirome, except for IV and OXA-2 PCase, where FK037 was more stable, and Ia CSase from *Enterobacter cloacae* where FK037 was less stable than cefpirome. Except for Ib CSase, FK037 was less stable than ceftazidime to all the CSases. Based on the *Km* values, FK037 had similar affinities as cefpirome to the β-lactamases. When compared with ceftazidime, the affinities of FK037 were lower for Ia CSase (*E. cloacae*) and Ib CSase, but higher

Table 8. Stability and affinity of FK037 and reference drugs to β-lactamases.

β-Lactamase (organism)	Antibiotic	Vmax[a]	*Km* (μg/ml)	Vmax/*Km*[a]
Ia cephalosporinase	FK037	1.9	650	0.78
(*Serratia marcescens*)	Cefpirome	0.85	330	0.68
	Ceftazidime	0.16	>1,000[b]	<0.04
	Cefoperazone	4.9	14	92
Ia cephalosporinase	FK037	0.11	25	1.2
(*Enterobacter cloacae*)	Cefpirome	0.36	330	0.29
	Ceftazidime	0.006	2.7[b]	0.64
	Cefoperazone	0.73	5.9	34
Ib cephalosporinase	FK037	0.45	200[b]	0.65
(*Escherichia coli*)	Cefpirome	0.06	32	0.50
	Ceftazidime	0.03	2.8[b]	3.2
	Cefoperazone	1.1	130	2.5
Ic cephalosporinase	FK037	49	430	2.7
(*Proteus vulgaris*)	Cefpirome	55	330	3.9
	Ceftazidime	2.9	>1,000	<0.07
	Cefoperazone	26	8.3	72
Ic cephalosporinase	FK037	77	160	19
(*Bacteroides fragilis*)	Cefpirome	160	250	24
	Ceftazidime	79	500	6.0
	Cefoperazone	140	50	110
Id cephalosporinase	FK037	1.0	20[b]	1.5
(*Pseudomonas aeruginosa*)	Cefpirome	0.75	31[b]	0.69
	Ceftazidime	0.08	30[b]	0.08
	Cefoperazone	12	13	29
II penicillinase	FK037	0.39	>1,000[b]	<0.01
(*Proteus mirabilis*)	Cefpirome	0.50	>1,000[b]	<0.01
	Ceftazidime	0.98	>1,000[b]	<0.01
	Cefoperazone	3.8	20	0.20
III penicillinase TEM-1	FK037	2.0	>1,000[b]	<0.02
(*Escherichia coli*)	Cefpirome	6.25	>1,000	<0.05
	Ceftazidime	0.72	>1,000[b]	<0.01
	Cefoperazone	33	125	2.1
IV penicillinase	FK037	1.9	>1,000[b]	<0.03
(*Klebsiella pneumoniae*)	Cefpirome	4.4	500	0.16
	Ceftazidime	<0.05	>1,000[b]	<0.01
	Cefoperazone	20	15	24
V penicillinase OXA-2	FK037	6.3	68	0.54
(*Pseudomonas aeruginosa*)	Cefpirome	120	350[b]	2.0
	Ceftazidime	1.6	>530[b]	<0.02
	Cefoperazone	2.2	13	0.95
V penicillinase PSE-1	FK037	1.3	>1,000[b]	<0.01
(*Pseudomonas aeruginosa*)	Cefpirome	2.3	>1,000[b]	<0.01
	Ceftazidime	3.2	>530[b]	<0.03
	Cefoperazone	0.86	1.1	3.7
Penicillinase	FK037	0.20	>1,000[b]	<0.01
(*Staphylococcus aureus*)	Cefpirome	0.31	>1,000[b]	<0.01
	Ceftazidime	3.9	>1,000[b]	<0.01
	Cefoperazone	0.29	0.24	1.9

[a] Relative value (%) to that of cephaloridine (for CSase) or ampicillin (for PCase); [b] *Ki* values substituted for *Km* values.

for OXA-2 PCase. The affinities of FK037 were extremely lower than those of cefoperazone for all the β-lactamases.

Ability of FK037 to Penetrate the Outer Membrane of *E. coli*

The ability of FK037 to penetrate the outer membrane of *E. coli* was slightly higher than that of ceftazidime, but slightly lower than that of cefpirome (Table 9).

Table 9. Outer membrane permeability of *Escherichia coli* to FK037 and the reference drugs.

Antibiotic	Permeability coefficient[a] (1/minute/1.54×10^7 cells)
FK037	3.39×10^{-3}
Cefpirome	4.03×10^{-3}
Ceftazidime	2.76×10^{-3}

[a] Method of ZIMMERMANN and ROSSELET.

Discussion

FK037 displayed a broader spectrum of activity than the so-called third generation cephalosporins such as ceftazidime, cefoperazone and ceftizoxime, and its spectrum of activity was similar to that of cefpirome. A major improvement of FK037 over the third generation cephalosporins was the increased anti-staphylococcal activity. In particular, the improved activity of FK037 against MRSA is one of its major characteristics. The anti-staphylococcal activity of FK037 can be attributed to its high affinity for PBPs 1, 2 and 3 of *S. aureus*, and PBP 2a of MRSA[4]. The detailed anti-MRSA activity of FK037 will be presented in another paper[14]. The anti-pseudomonal activity is another major attribute of FK037. The activity of FK037 was slightly better than cefoperazone and cefpirome but inferior to ceftazidime. Although the affinities of FK037 to PBPs 3, 1a and 1b of *P. aeruginosa* were nearly equipotent to those of ceftazidime, FK037 MICs were higher than those of ceftazidime. This discrepancy may be based on the poorer stability of FK037 to pseudomonal β-lactamases or its poorer outer membrane permeability compared to ceftazidime. In addition, the potent activity of FK037 against *C. freundii* and *Enterobacter* species resistant to most third generation cephalosporins may also be related to higher stability to β-lactamases, its higher affinity to essential PBPs and its more efficient outer membrane permeability. The high β-lactamase stability of FK037 was due to low Vmax and high *Km* values, indicating that FK037 can not be easily recognized and degraded by most β-lactamases. On the basis of its antibacterial profiles, FK037, like cefpirome, should be considered a "fourth generation cephalosporin" as described by JONES *et al.*[1]. The MBCs of FK037 were similar to the MICs against the various bacterial species tested except *S. marcescens*. In particular, it is noteworthy that the MBCs of FK037 against both L- and H-MRSA (14.4 and 25 μg/ml, respectively) were the lowest among the cephalosporins tested. Potent bactericidal activity of FK037 by time-kill studies was noted even at a concentration of 1/2 or 1/4 the MIC against *S. aureus*, *E. coli* and *P. aeruginosa*, which was superior to that of cefpirome and ceftazidime except against *E. coli*. These results suggest that the bactericidal activity of FK037 is more potent than could be anticipated from the MICs, especially against MRSA and *P. aeruginosa*. In an *in vitro* pharmacokinetic model simulating human plasma concentrations after an intravenous dose, FK037 showed excellent bactericidal activity for 8 hours against MRSA (MIC 25 μg/ml) after administration of a 1.0 g dose in this model. Cefpirome and flomoxef showed only bacteriostatic activity up to 3 hours after which bacterial regrowth occurred. In addition, FK037 even at a 1.0 g dose was bactericidal against *P. aeruginosa* (MIC 25 μg/ml) up to 8 hours after administration. It was as potent as ceftazidime and superior to cefpirome which exhibited the bacterial re-growth 3 hours after administration. The excellent bactericidal activities of FK037 in this system suggest that FK037 might be useful for treating MRSA and pseudomonal infections in humans.

From these results, clinical application of FK037 may be expected in patients with infections caused by a wide range of pathogenic bacteria.

References

1) JONES, R. N.; C. THORNSBERRY, A. L. BARRY, L. AYERS, S. BROWN, J. DANIEL, P. C. FUCHS, T. L. GAVAN, E. H. GERLACH, J. M. MATSEN, L. B. RELLER & H. M. SOMMERS: Disk diffusion testing, quality control guidelines, and antimicrobial spectrum of HR810, a fourth-generation cephalosporin, in clinical microbiology laboratories. J. Clin. Microbiol. 20: 409~412, 1984

2) MINE, Y.; Y. WATANABE, H. SAKAMOTO, K. HATANO, T. KAMIMURA, F. MATSUMOTO & S. KUWAHARA: FK037, a novel parenteral broad-spectrum cephalosporin: I. *In vitro* antibacterial activity. Program and Abstracts of the 31st Intersci. Conf. on Antimicrob. Agents Chemother., No. 849, p. 240, Chicago, Sept. 29～Oct. 2, 1991
3) MINE, Y.; Y. WATANABE, H. SAKAMOTO, T. KAMIMURA, S. TAWARA, F. MATSUMOTO & S. KUWAHARA: FK037, a novel parenteral broad-spectrum cephalosporin: II. *In vivo* antibacterial activity. Program and Abstracts of the 31st Intersci. Conf. on Antimicrob. Agents Chemother., No. 850, p. 240, Chicago, Sept. 29～Oct. 2, 1991
4) MINE, Y.; Y. WATANABE, H. SAKAMOTO, T. KAMIMURA, F. MATSUMOTO & S. KUWAHARA: FK037, a novel parenteral broad-spectrum cephalosporin: III. Excellent activity against methicillin-resistant staphylococci. Program and Abstracts of the 31st Intersci. Conf. on Antimicrob. Agents Chemother., No. 851, p. 241, Chicago, Sept. 29～Oct. 2, 1991
5) MINE, Y.; H. SAKAMOTO, K. HATANO, Y. HIGASHI, T. KAMIMURA, F. MATSUMOTO & S. KUWAHARA: FK037, a novel parenteral broad-spectrum cephalosporin: IV. Animal pharmacokinetic. Program and Abstracts of the 31st Intersci. Conf. on Antimicrob. Agents Chemother., No. 852, p. 241, Chicago, Sept. 29～Oct. 2, 1991
6) NISHIDA, M.; T. MURAKAWA, T. KAMIMURA & N. OKADA: Bactericidal activity of cephalosporins in an *in vitro* model simulating serum levels. Antimicrob. Agents Chemother. 14: 6～12, 1978
7) NAKASHIMA, M. & T. UEMATSU: Single dose study of FK037 in healthy volunteers. Research Report. CLR910014 of Clinical Pharmacology of Fujisawa Pharmaceutical Co., Ltd., 1991
8) Symposium on New Drug HR810 (Cefpirome: CPR). The 38th General Meeting of Japan Society of Chemotherapy, p. 59, May 19, 1990 (Nagasaki)
9) Symposium on New Drug SN401 (Ceftazidime). The 30th General Meeting of Japan Society of Chemotherapy, p. 75, June 30, 1982 (Tokyo)
10) Symposium on New Drug 6315-S (Flomoxef: FMOX). The 34th General Meeting of Japan Society of Chemotherapy, p. 78, June 7, 1986 (Kurashiki)
11) SPRATT, B. G. & A. B. PARDEE: Penicillin-binding proteins and cell shape in *E. coli*. Nature 254: 516～517, 1975
12) SHIGI, Y.; H. KOJO, M. WAKASUGI & M. NISHIDA: Differences between ceftizoxime and its stereoisomer in antibacterial activity and affinity for penicillin-binding proteins. Antimicrob. Agents Chemother. 19: 393～396, 1981
13) ZIMMERMANN, W. & A. ROSSELET: Function of the outer membrane of *Escherichia coli* as a permeability barrier to beta-lactam antibiotics. Antimicrob. Agents Chemother. 12: 368～372, 1977
14) MINE, Y.; T. WATANABE, H. SAKAMOTO, K. HATANO, K. KUNO, T. KAMIMURA, S. TAWARA, Y. MATSUMOTO, F. MATSUMOTO & S. KUWAHARA: Excellent activity of FK037, a novel parenteral broad-spectrum cephalosporin, against methicillin-resistant staphylocci. J. Antibiotics 46: 99～119, 1993