# *IN VITRO* ANTIBACTERIAL ACTIVITY OF FK482, A NEW ORALLY ACTIVE CEPHALOSPORIN

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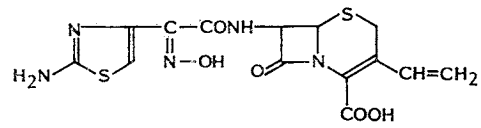
FK482 is a new orally active cephem antibiotic which offers some advantages over the commercially available oral β-lactam antibiotics. It displayed a broad spectrum of activity *in vitro* against stock strains of Gram-positive and Gram-negative aerobes and anaerobes. FK482 was more active *in vitro* than cefixime (CFIX), cefaclor (CCL) or cephalexin (CEX) against clinical isolates of Gram-positive organisms such as methicillin-sensitive *Staphylococcus aureus*, coagulase-negative Staphylococci including *Staphylococcus epidermidis* and strains of the *Streptococcus* group. Moderate activity was found against methicillin-resistant *S. aureus* and *Enterococcus faecalis*. Against clinical isolates of many Gram-negative species, including opportunistic pathogens, FK482 had good *in vitro* activity similar or slightly inferior to that of CFIX but superior to that of CCL or CEX. However, it was clearly inferior to CFIX in activity against *Serratia marcescens*, and was inactive against *Pseudomonas aeruginosa*. Strains of *S. aureus* resistant to methicillin were moderately susceptible to FK482. All tested strains of *Klebsiella pneumoniae* resistant to CCL and CEX were susceptible to FK482, as were all the strains of *Escherichia coli*, *Proteus mirabilis*, *Haemophilus influenzae* and *Branhamella catarrhalis* resistant to amoxicillin (AMPC). FK482, like CFIX, was relatively stable to all type of β-lactamases except *Bacteroides fragilis* and its stability was superior to that of CCL or CEX. The antibacterial activity of FK482 against CSH2 strains containing ampicillin-resistance plasmids was not affected by the presence of the ampicillin resistance determinants. FK482 showed higher affinity for the penicillin-binding proteins (PBPs) (3, 2 and 1) of *S. aureus* than did CFIX, CCL and CEX. FK482 also showed very high affinity for the PBPs (2 and 3) of *E. faecalis* and PBPs (3, 1a, 4, 2 and 1bs) of *E. coli*. The bactericidal activity of FK482 against *S. aureus* was almost as strong as that of AMPC and superior to that of CCL or CEX. Against Gram-negative bacteria such as *E. coli*, *K. pneumoniae* and *P. mirabilis*, FK482 was similar to CFIX and superior to CCL and CEX in bactericidal activity.

Great improvements in antibacterial activity, spectrum and β-lactamase stability have been made in the parenteral cephem class of antibiotics over the last decade. There has not, however, been paralleled progress in orally active cephem antibiotics. At the 22nd Interscience Conference on Antimicrobial Agents and Chemotherapy in 1982, we reported a distinctive new broad spectrum, oral cephalosporin, cefixime (CFIX)[1~3], with potent antibacterial activity against Gram-negative bacteria and good stability to β-lactamases from Gram-negative bacteria similar to injectable newer cephem antibiotics. In addition, T-2588[4~8], a new oral prodrug with similar antibacterial properties was reported at the

24th Interscience Conference on Antimicrobial Agents and Chemotherapy in 1984. Both drugs were tolerated well and showed good clinical efficacy in clinical trials. Although both drugs had advantages in their spectrum of activity against clinical isolates of Gram-negative bacteria including opportunistic pathogens, both were very weakly active against Staphylococci. We recently succeeded in addressing this deficiency with FK482 (Fig. 1), a new cephem antibiotic for oral use, with advantages over CFIX, T-2588, cefaclor (CCL) and cephalexin (CEX) in *in vitro* activity against Gram-positive organisms. In this report we compare the *in vitro* activities of FK482 with the activities of CFIX, CCL, CEX and amoxicillin (AMPC).

Fig. 1. Chemical structure of FK482.



(6*R*,7*R*)-7-[(*Z*)-2-(2-amino-4-thiazolyl)-2-(hydroxyimino)acetamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo[4,2,0]oct-2-ene-2-carboxylic acid.

## Materials and Methods

### Antibiotics

The antibiotics used in this study were FK482 and CFIX (Fujisawa Research Laboratories, Osaka, Japan), CCL and CEX (Eli Lilly and Company, Indianapolis, U.S.A.), and AMPC (Beecham Laboratories, Betchworth, UK).

### Bacterial Strains

Stock strains from the culture collection in our laboratories were used in this study. *Escherichia coli* CSH2 ($Nal^r$) and 13 strains of *E. coli* CSH2 harboring R-plasmids specifying ampicillin-resistance were provided by Dr. T. YOKOTA of Juntendo University. Clinical isolates of various species of bacteria were obtained from several hospitals in Japan.

### Antibiotic Susceptibility Testing

The MICs of the test antibiotics were determined by the agar dilution method. Mueller-Hinton agar (Difco Laboratories, Detroit, U.S.A.) was used for nonfastidious aerobic bacteria. This medium, supplemented with 5% defibrinated horse blood (chocolate agar) or 5% defibrinated horse blood, was used for *Neisseria* species and *Haemophilus influenzae*, or *Streptococcus pneumoniae*, *Streptococcus pyogenes*, strains of the "Viridans" group Streptococci and *Corynebacterium diphtheriae*. GAM agar (Nissui, Tokyo, Japan) was used for testing the anaerobic bacteria. The nonfastidious aerobic organisms were precultured in Mueller-Hinton broth (Difco); Staphylococci, *Enterococcus faecalis* and *Branhamella catarrhalis* were precultured in Trypticase soy broth (BBL Microbiology System, Cockeysville, U.S.A.); *S. pneumoniae* was precultured in Mueller-Hinton broth plus 5% horse serum; *S. pyogenes*, the "Viridans" group Streptococci, *C. diphtheriae* and *Neisseria* species were precultured in Trypticase soy broth plus 5% horse serum; *H. influenzae* was precultured in Trypticase soy broth plus 5% Fildes enrichment; and anaerobic bacteria were precultured in GAM broth (Nissui). $10^3$ cfu were inoculated with a multipoint replicating apparatus onto agar plates containing serial 2-fold dilutions of each antibiotic and incubated 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* and at 37°C for 24 hours by GasPak method (BBL) for anaerobic bacteria. The MIC was the lowest antibiotic concentration that inhibited macroscopic colonial growth during this incubation.

### Determination of Broth Dilution MICs and MBCs

Broth dilution MICs were determined in Mueller-Hinton broth. Overnight cultures were diluted in this broth to give an inoculum of $10^5$ cfu/ml. Test tubes containing 9 ml of the culture dilutions plus 1 ml of serial 2-fold dilutions of the antibiotics were incubated at 37°C for 18 hours. After incubation, the lowest antibiotic concentration inhibiting visual bacteria growth was regarded as the MIC. MBCs were assayed by transferring 0.1 ml from each tube without visible growth in the broth

dilution MIC series to agar plates. After incubation at 37°C for 18 hours, the lowest antibiotic concentration at which the number of colonies on the plate was fewer than 10 (killing 99.9% of the inoculum) was regarded as the MBC.

Bactericidal Activity

Mueller-Hinton broths containing one-fourth the MIC, the MIC, or four times the MIC of each test drug were inoculated with *Staphylococcus aureus* 2558 or *E. coli* 3147 in a quantity sufficient to yield a final concentration of approximately $10^6$ cfu/ml. The cultures were incubated at 37°C. The number of viable cells was measured at regular intervals throughout the incubation period.

Preparation of β-Lactamases

The cells were grown at 37°C in Trypticase soy broth (GAM broth for *Bacteroides fragilis*) to which ampicillin was added as an inducer as needed. Cells from the exponential growth phase were harvested by centrifugation for 10 minutes at 5,000×*g*, washed once, and suspended in 0.067 M potassium phosphate buffer (pH 7.0). The cell suspensions were sonicated at 20 kilocycles for 10 minutes. After the cellular debris was removed by centrifugation, the supernatant was subjected to gel-filtration on a Sephadex G-100 column. The column was equilibrated with 0.06 M phosphate buffer (pH 7.0) and eluted with the same buffer. The enzyme fractions were pooled and stored at −20°C.

Assay of β-Lactamase Activity

β-Lactamase activity was determined with a Hitachi 200A spectrophotometer equipped with a thermostated cell holder. The enzyme was mixed in a 1-cm quartz cuvette with 150 μg of substrate and sufficient 0.2 mM phosphate buffer (pH 7.0) to make a final volume of 3 ml. The rate of hydrolysis of the β-lactam ring was followed at 37°C by the change in absorption at 240 nm for ampicillin and at 260 nm for cephalosporins. The relative initial rate of hydrolysis was expressed as percent of hydrolysis of cephaloridine for cephalosporinase and of ampicillin for penicillinase.

Assay of Affinity for Penicillin-binding Proteins (PBPs)

The affinity of the test antibiotic for the PBPs of *S. aureus* 209P JC-1, *E. faecalis* FP183 and *E. coli* NIHJ JC-2 was assayed by a modification of SPRATT's method as described previously[9].

Determination of Permeability Coefficients

The permeability coefficients of FK482, CCL, CEX and cephaloridine were determined by the method of ZIMMERMANN and ROSSELET[10].

## Results

### Antibacterial Spectrum of FK482

The activities of FK482 against stock strains of 27 species of aerobes are shown in Table 1. FK482 showed a broader spectrum and more potent activity *in vitro* than the reference drugs against Gram-positive and Gram-negative aerobes. FK482 was more active than CFIX, CCL and CEX against all 8 species of Gram-positive bacteria. It was superior to AMPC in activity against methicillin-sensitive *S. aureus*, methicillin-resistant *S. aureus* (MRSA) and *Staphylococcus epidermidis*, was as active as AMPC against *S. pneumoniae*, *S. pyogenes*, "Viridans" group Streptococci and *C. diphtheriae*, but was less active than AMPC against *E. faecalis*. Against Gram-negative bacteria including opportunistic pathogens, FK482 was superior in activity to CCL, CEX and AMPC and was similar or slightly inferior to CFIX. However, the drug was as inactive as other reference antibiotic against *Pseudomonas aeruginosa*. The antibiotic was also effective against Gram-positive and Gram-negative anaerobes, and its activity was nearly the same as that of AMPC and was greater than that of CFIX, CCL or CEX (Table 2).

Table 1. Antibacterial spectrum of FK482 and reference antibiotics against aerobes.

| Organism | MIC (μg/ml) | | | | |
|---|---|---|---|---|---|
| | FK482 | CFIX | CCL | CEX | AMPC |
| *Staphylococcus aureus* 209P JC-1 | 0.05 | 25 | 0.78 | 1.56 | 0.10 |
| *S. aureus* 2535 (MRSA) | 6.25 | 100 | 100 | 100 | 25 |
| *S. epidermidis* 89 | 0.10 | 6.25 | 0.78 | 1.56 | 0.39 |
| *Streptococcus pyogenes* S-23[a] | ≦0.025 | 0.10 | 0.20 | 0.78 | ≦0.025 |
| *S. pneumoniae* 4004[a] | ≦0.025 | 0.05 | 0.39 | 1.56 | ≦0.025 |
| *S. mitis* 3002[a] | 0.39 | 1.56 | 12.5 | 50 | 0.39 |
| *Corynebacterium diphtheriae* NIHJ A-7[a] | 0.20 | 12.5 | 0.39 | 0.78 | 0.20 |
| *Enterococcus faecalis* 115 | 6.25 | >100 | >100 | >100 | 0.78 |
| *Neisseria gonorrhoeae* PCL783[a] | ≦0.025 | ≦0.025 | 0.05 | 0.78 | 0.20 |
| *N. meningitidis* 68[a] | ≦0.025 | ≦0.025 | 0.05 | 0.78 | 0.05 |
| *Escherichia coli* NIHJ JC-2 | 0.10 | 0.10 | 3.13 | 6.25 | 3.13 |
| *Citrobacter freundii* 3029 | 3.13 | 3.13 | 25 | 50 | >100 |
| *Salmonella typhi* T-287 | 0.10 | ≦0.025 | 0.78 | 6.25 | 0.39 |
| *Shigella flexneri* Ia EW8 | 0.20 | 0.10 | 0.78 | 6.25 | 0.78 |
| *Klebsiella pneumoniae* NCTC 418 | 0.10 | ≦0.025 | 0.78 | 6.25 | 25 |
| *Enterobacter cloacae* 3036 | 12.5 | 25 | 50 | 100 | 50 |
| *E. aerogenes* 3026 | 1.56 | 1.56 | 25 | >100 | >100 |
| *Serratia marcescens* 3049 | 6.25 | 0.39 | >100 | >100 | 50 |
| *Proteus mirabilis* 1 | 0.10 | ≦0.025 | 1.56 | 12.5 | 0.78 |
| *P. vulgaris* IAM1025 | 0.20 | ≦0.025 | 6.25 | 12.5 | 6.25 |
| *Providencia rettgeri* 4004 | 0.20 | ≦0.025 | >100 | >100 | 100 |
| *Morganella morganii* 4017 | 12.5 | 0.78 | >100 | >100 | >100 |
| *Providencia stuartii* 52 | 0.10 | ≦0.025 | 25 | 25 | >100 |
| *Pseudomonas aeruginosa* IAM 1095 | >100 | 100 | >100 | >100 | >100 |
| *P. cepacia* ATCC 25416 | 25 | 3.13 | >100 | >100 | >100 |
| *Alcaligenes faecalis* NCTC 655 | 1.56 | 1.56 | 1.56 | 6.25 | 12.5 |
| *Haemophilus influenzae* 57[a] | 0.20 | ≦0.025 | 1.56 | 6.25 | 0.20 |

Agar dilution method (stamp method): Mueller-Hinton agar, $10^8$ cfu/spot, 37°C, 18~20 hours.

[a] Supplemented with 5% horse blood.

Abbreviations: CFIX; Cefixime, CCL; cefaclor, CEX; cephalexin, AMPC; amoxicillin.

## Susceptibility of Clinical Isolates to FK482

The activity of FK482 against clinical isolates was compared with that of CFIX, CCL, CEX and AMPC. Activity was expressed as the drug concentration necessary to inhibit 50% or 90% of the test strains (Table 3). FK482 had advantages over CFIX, CCL and CEX in activity against Gram-positive organisms such as methicillin-sensitive *S. aureus*, coagulase-negative Staphylococci, *S. pneumoniae*, *S. pyogenes* and "Viridans" group Streptococci, and was similar or slightly inferior to AMPC in activity against these Gram-positive bacteria. Furthermore, FK482 was the most active of the test drugs against moderately methicillin-resistant *S. aureus*, although this activity was not strong. FK482 also exhibited moderate activity against *E. faecalis* resistant to commercially available cephem antibiotics. This activity was affected by the test medium (Fig. 2); that is, in Mueller-Hinton agar (Difco) it was moderate, but in sensitivity test agar (Eiken Chemical Co., Ltd., Tokyo, Japan) it was almost the same as that of AMPC. Against clinical isolates of Gram-negative bacteria such as the *Neisseria* group, *B. catarrhalis*, *E. coli*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Proteus mirabilis*, *Proteus vulgaris*, *Providencia stuartii*, *Providencia rettgeri* and *H. influenzae*, FK482 had excellent *in vitro* activity which was similar or slightly weaker than that of CFIX and stronger than that

Table 2. Antibacterial spectrum of FK482 and reference antibiotics against anaerobes.

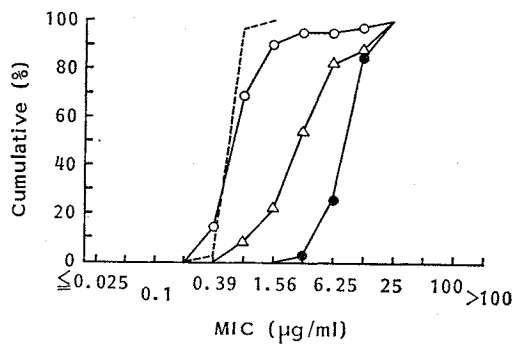
| Organism | MIC (μg/ml) | | | | |
|---|---|---|---|---|---|
| | FK482 | CFIX | CCL | CEX | AMPC |
| *Peptostreptococcus asaccharolyticus* Z1003 | 0.10 | 0.78 | 0.78 | 0.39 | ≦0.025 |
| *P. prevotii* ATCC 9321 | 0.78 | 6.25 | 1.56 | 1.56 | 0.20 |
| *P. magnus* ATCC 14956 | 0.39 | 25 | 3.13 | 6.25 | 0.20 |
| *P. productus* ATCC 27340 | 0.05 | 0.39 | 0.39 | 0.39 | 0.20 |
| *Streptococcus constellatus* ATCC 27513 | 1.56 | 6.25 | 25 | 12.5 | 0.39 |
| *Propionibacterium acnes* ATCC 11828 | 0.05 | 0.20 | 0.39 | 0.78 | 0.05 |
| *Eubacterium lentum* H-1 | ≦0.025 | 0.78 | 0.10 | 0.05 | ≦0.025 |
| *E. limosum* ATCC 8486 | 0.78 | 3.13 | 6.25 | 6.25 | 0.10 |
| *Clostridium perfringens* ATCC 3624 | 0.39 | 1.56 | 0.20 | 0.78 | ≦0.025 |
| *C. tetani* ATCC 10779 | 0.20 | 0.39 | 1.56 | 0.39 | 0.10 |
| *C. difficile* FP1007 | 25 | >100 | 100 | 50 | 0.39 |
| *Veillonella alcalescens* H-3 | 0.10 | 0.78 | 0.39 | 0.39 | 0.10 |
| *V. parvula* ATCC 10790 | 0.10 | 0.78 | 0.20 | 0.39 | 0.10 |
| *Bacteroides fragilis* Ju-13 | 1.56 | 3.13 | 25 | 50 | 1.56 |
| *B. fragilis* FP404 | 0.20 | 3.13 | 1.56 | 1.56 | 0.20 |
| *B. distasonis* KVO450 | 1.56 | 0.78 | 25 | 50 | 1.56 |
| *B. vulgatus* W-6 | 3.13 | 12.5 | 100 | 25 | 6.25 |
| *B. thetaiotaomicron* 11 | 25 | 100 | >100 | 50 | 25 |
| *B. asaccharolyticus* Rm1 | ≦0.025 | 0.10 | 0.39 | 0.39 | ≦0.025 |
| *B. praeacutus* ATCC 25539 | ≦0.025 | 0.10 | 0.39 | 0.20 | ≦0.025 |
| *Fusobacterium necrophorum* W-12 | 0.05 | 0.20 | 0.78 | 0.39 | ≦0.025 |
| *F. nucleatum* ATCC 25586 | 0.05 | 0.78 | 0.78 | 0.39 | 0.05 |
| *F. varium* ATCC 8501 | 1.56 | 0.78 | 50 | >100 | 0.78 |
| *F. mortiferum* FP355 | 1.56 | 0.78 | 100 | >100 | 1.56 |
| *F. russii* ATCC 25533 | 0.10 | 3.13 | 1.56 | 3.13 | 0.39 |

Agar dilution method (stamp method): GAM agar, GasPak, $10^8$ cfu/spot, 37°C, 24 hours.
Abbreviations: See footnote in Table 1.

of CCL, CEX or AMPC. FK482, like CFIX, displayed good $MIC_{50}$s, but poor $MIC_{90}$s against *Citrobacter freundii* and *Enterobacter aerogenes*. The drug, however, was clearly weaker than CFIX in activity against *Serratia marcescens* and *Enterobacter cloacae*, and was inactive against *P. aeruginosa*. Its activity against *Bacteroides fragilis* was nearly as strong as that of CFIX and AMPC.

Fig. 2. Effect of test medium on antibacterial activity of FK482 against *Enterococcus faecalis* ($n$=45).

● Mueller-Hinton agar (FK482), △ heart infusion agar (FK482), ○ sensitivity test agar (FK482), --- Mueller-Hinton agar (amoxicillin).




## Antibacterial Activity of FK482 against β-Lactam Resistant Strains

Clinical isolates of *S. aureus*, MIC of methicillin: 6.25~50 μg/ml; *E. coli*, MICs of CCL and CEX: ≧12.5 μg/ml, *K. pneumoniae*, MICs of CCL and CEX: ≧6.25 and ≧12.5 μg/ml respectively; and *E. coli*, *P. mirabilis*, *H. influenzae*, *B. catarrhalis* and *Neisseria gonorrhoeae*, MICs of AMPC: ≧12.5, ≧12.5, ≧3.13, ≧0.78 and ≧3.13 μg/ml, respectively, were chosen for testing cross-

Table 3. Antibacterial activity of FK482 and reference antibiotics against clinical isolates.

| Organism (No. of strains) | Antibiotic | MIC (μg/ml) | | |
|---|---|---|---|---|
| | | Range | 50% | 90% |
| *Staphylococcus aureus* (54) | FK482 | 0.1~1.56 | 0.39 | 0.78 |
| (MSSA) | CFIX | 6.25~25 | 12.5 | 25 |
| Methicillin: ≦3.13 μg/ml | CCL | 0.78~12.5 | 1.56 | 6.25 |
| | CEX | 1.56~25 | 3.13 | 12.5 |
| | AMPC | 0.1~1.56 | 0.2 | 0.39 |
| *S. aureus* (24) | FK482 | 1.56~25 | 6.25 | 25 |
| (MRSA) | CFIX | 100~>100 | >100 | >100 |
| Methicillin: 6.25~50 μg/ml | CCL | 3.13~>100 | >100 | >100 |
| | CEX | 25~>100 | >100 | >100 |
| | AMPC | 0.39~100 | 50 | 100 |
| *S. epidermidis* (49) | FK482 | ≦0.025~>100 | 0.1 | 100 |
| | CFIX | 1.56~>100 | 6.25 | >100 |
| | CCL | 0.39~100 | 1.56 | 25 |
| | CEX | 1.56~>100 | 3.13 | 50 |
| | AMPC | 0.05~50 | 0.39 | 12.5 |
| *S. haemolyticus* (9) | FK482 | 0.1~>100 | 0.39 | 100 |
| | CFIX | 6.25~>100 | 50 | >100 |
| | CCL | 0.39~50 | 1.56 | 50 |
| | CEX | 0.39~>100 | 3.13 | 100 |
| | AMPC | 0.1~50 | 0.78 | 25 |
| *S. saprophyticus* (10) | FK482 | 0.2~>100 | 0.2 | 3.13 |
| | CFIX | 12.5~>100 | 50 | >100 |
| | CCL | 0.78~50 | 3.13 | 6.25 |
| | CEX | 3.13~>100 | 6.25 | 12.5 |
| | AMPC | 0.2~12.5 | 0.39 | 6.25 |
| *S. hominis* (5) | FK482 | 0.2~0.39 | 0.39 | 0.39 |
| | CFIX | 12.5~50 | 12.5 | 50 |
| | CCL | 0.78~6.25 | 3.13 | 6.25 |
| | CEX | 1.56~25 | 3.13 | 25 |
| | AMPC | 0.39~1.56 | 0.39 | 1.56 |
| *Streptococcus pyogenes* (21)[a] | FK482 | ≦0.025 | ≦0.025 | ≦0.025 |
| | CFIX | ≦0.025~0.1 | 0.1 | 0.1 |
| | CCL | ≦0.025~0.2 | 0.2 | 0.2 |
| | CEX | 0.2~0.78 | 0.39 | 0.78 |
| | AMPC | ≦0.025 | ≦0.025 | ≦0.025 |
| *S. pneumoniae* (30)[a] | FK482 | ≦0.025~0.39 | 0.05 | 0.1 |
| | CFIX | 0.1~1.56 | 0.2 | 0.39 |
| | CCL | 0.2~1.56 | 0.39 | 0.78 |
| | CEX | 0.78~6.25 | 3.13 | 3.13 |
| | AMPC | ≦0.025~0.05 | ≦0.025 | ≦0.025 |
| Viridans group Streptococci (11)[a] | FK482 | 0.05~25 | 0.2 | 6.25 |
| | CFIX | 0.2~100 | 1.56 | 25 |
| | CCL | 1.56~>100 | 3.13 | 100 |
| | CEX | 3.13~100 | 12.5 | 100 |
| | AMPC | ≦0.025~1.56 | 0.1 | 1.56 |
| *Enterococcus faecalis* (45) | FK482 | 1.56~25 | 12.5 | 25 |
| | CFIX | 100~>100 | >100 | >100 |
| | CCL | 50~>100 | 100 | >100 |
| | CEX | 50~>100 | >100 | >100 |
| | AMPC | 0.39~1.56 | 0.78 | 0.78 |

Table 3. (Continued)

| Organism (No. of strains) | Antibiotic | MIC (μg/ml) | | |
|---|---|---|---|---|
| | | Range | 50% | 90% |
| *Neisseria gonorrhoeae* (27)[b] | FK482 | ≦0.025 | ≦0.025 | ≦0.025 |
| | CFIX | ≦0.025 | ≦0.025 | ≦0.025 |
| | CCL | 0.05~0.78 | 0.2 | 0.39 |
| | CEX | 0.39~6.25 | 3.13 | 3.13 |
| | AMPC | 0.1~0.78 | 0.2 | 0.39 |
| *N. meningitidis* (10)[b] | FK482 | ≦0.025 | ≦0.025 | ≦0.025 |
| | CFIX | ≦0.025 | ≦0.025 | ≦0.025 |
| | CCL | 0.05~0.1 | 0.1 | 0.1 |
| | CEX | 0.78~1.56 | 0.78 | 1.56 |
| | AMPC | ≦0.025~0.05 | 0.05 | 0.05 |
| *Branhamella catarrhalis* (36) | FK482 | 0.1~0.39 | 0.2 | 0.39 |
| | CFIX | ≦0.025~0.39 | 0.2 | 0.39 |
| | CCL | 0.2~6.25 | 1.56 | 3.13 |
| | CEX | 3.13~12.5 | 3.13 | 6.25 |
| | AMPC | ≦0.025~12.5 | 1.56 | 6.25 |
| *Haemophilus influenzae* (38)[b] | FK482 | 0.2~3.13 | 0.39 | 0.78 |
| | CFIX | ≦0.025~0.2 | 0.05 | 0.1 |
| | CCL | 1.56~25 | 6.25 | 12.5 |
| | CEX | 6.25~>100 | 50 | 100 |
| | AMPC | 0.2~1.56 | 0.39 | 0.78 |
| *Escherichia coli* (50) | FK482 | 0.05~0.78 | 0.2 | 0.39 |
| | CFIX | ≦0.025~1.56 | 0.2 | 0.39 |
| | CCL | 0.39~6.25 | 1.56 | 3.13 |
| | CEX | 1.56~12.5 | 6.25 | 12.5 |
| | AMPC | 0.78~>100 | 6.25 | >100 |
| *Klebsiella pneumoniae* (51) | FK482 | 0.05~1.56 | 0.2 | 0.39 |
| | CFIX | ≦0.025~0.39 | 0.05 | 0.2 |
| | CCL | 0.39~12.5 | 0.78 | 1.56 |
| | CEX | 3.13~25 | 6.25 | 6.25 |
| | AMPC | 3.13~>100 | 100 | >100 |
| *K. oxytoca* (20) | FK482 | 0.05~0.78 | 0.1 | 0.2 |
| | CFIX | ≦0.025~0.1 | 0.05 | 0.1 |
| | CCL | 0.39~12.5 | 0.78 | 0.78 |
| | CEX | 1.56~12.5 | 3.13 | 6.25 |
| | AMPC | 25~>100 | 100 | >100 |
| *Proteus mirabilis* (30) | KF482 | 0.05~0.2 | 0.1 | 0.2 |
| | CFIX | ≦0.025 | ≦0.025 | ≦0.025 |
| | CCL | 0.78~12.5 | 1.56 | 3.13 |
| | CEX | 12.5~25 | 12.5 | 12.5 |
| | AMPC | 0.39~>100 | 0.78 | 1.56 |
| *P. vulgaris* (20) | FK482 | 0.2~25 | 1.56 | 6.25 |
| | CFIX | ≦0.025~0.1 | ≦0.025 | 0.05 |
| | CCL | 6.25~>100 | >100 | >100 |
| | CEX | 25~>100 | >100 | >100 |
| | AMPC | 12.5~>100 | >100 | >100 |
| *Providencia stuartii* (19) | FK482 | ≦0.025~3.13 | 0.1 | 3.13 |
| | CFIX | ≦0.025~1.56 | 0.05 | 0.2 |
| | CCL | 0.39~>100 | 50 | >100 |
| | CEX | 1.56~>100 | 50 | >100 |
| | AMPC | 3.13~>100 | >100 | >100 |

Table 3. (Continued)

| Organism (No. of strains) | Antibiotic | MIC (μg/ml) | | |
|---|---|---|---|---|
| | | Range | 50% | 90% |
| *P. rettgeri* (20) | FK482 | ≦0.025~12.5 | 0.2 | 3.13 |
| | CFIX | ≦0.025~0.78 | 0.05 | 0.39 |
| | CCL | 12.5~>100 | >100 | >100 |
| | CEX | 6.25~>100 | >100 | >100 |
| | AMPC | 12.5~>100 | 100 | >100 |
| *Morganella morganii* (19) | FK482 | 3.13~100 | 12.5 | 50 |
| | CFIX | 0.1~>100 | 0.78 | 25 |
| | CCL | 100~>100 | >100 | >100 |
| | CEX | >100 | >100 | >100 |
| | AMPC | >100 | >100 | >100 |
| *Serratia marcescens* (20) | FK482 | 0.78~>100 | 100 | >100 |
| | CFIX | 0.2~50 | 6.25 | 50 |
| | CCL | >100 | >100 | >100 |
| | CEX | >100 | >100 | >100 |
| | AMPC | 50~>100 | >100 | >100 |
| *Citrobacter freundii* (20) | FK482 | 0.39~>100 | 3.13 | >100 |
| | CFIX | 0.78~>100 | 3.13 | >100 |
| | CCL | 12.5~>100 | 25 | >100 |
| | CEX | 25~>100 | 100 | >100 |
| | AMPC | 100~>100 | >100 | >100 |
| *Enterobacter cloacae* (20) | FK482 | 0.2~>100 | 50 | >100 |
| | CFIX | 0.1~>100 | 25 | >100 |
| | CCL | 12.5~>100 | >100 | >100 |
| | CEX | 50~>100 | >100 | >100 |
| | AMPC | 25~>100 | >100 | >100 |
| *E. aerogenes* (19) | FK482 | 0.2~>100 | 1.56 | >100 |
| | CFIX | ≦0.025~>100 | 1.56 | >100 |
| | CCL | 6.25~>100 | 50 | >100 |
| | CEX | 12.5~>100 | 100 | >100 |
| | AMPC | 6.25~>100 | >100 | >100 |
| *Pseudomonas aeruginosa* (20) | FK482 | >100 | >100 | >100 |
| | CFIX | 25~>100 | 100 | >100 |
| | CCL | >100 | >100 | >100 |
| | CEX | >100 | >100 | >100 |
| | AMPC | >100 | >100 | >100 |
| *Bacteroides fragilis* (31)[c] | FK482 | 1.56~>100 | 25 | >100 |
| | CFIX | 3.13~>100 | 25 | >100 |
| | CCL | 25~>100 | >100 | >100 |
| | CEX | 12.5~>100 | 50 | >100 |
| | AMPC | 0.78~>100 | 12.5 | >100 |

Agar dilution method (stamp method): Mueller-Hinton agar, $10^3$ cfu/spot, 37°C, 18~20 hours.

[a] Supplemented with 10% horse blood, [b] supplemented with 5% horse blood, [c] agar dilution method (stamp method): GAM agar, GasPak, $10^3$ cfu/spot, 37°C, 24 hours.

Abbreviations: See footnote in Table 1.

resistance to FK482 (Table 4). Strains of *S. aureus* which were moderately resistant to methicillin were moderately susceptible to FK482, and strains of *E. coli* which were highly resistant to CCL and CEX were moderately susceptible to FK482. Strains of *K. pneumoniae* resistant to CCL and CEX were highly susceptible to FK482 and CFIX, and FK482, like CFIX, was highly active against AMPC-resistant *E. coli*, *P. mirabilis*, *H. influenzae*, *B. catarrhalis* and *N. gonorrhoeae*.

Table 4. Antibacterial activity of FK482 and reference antibiotics against β-lactam-resistant strains.

| Organism (No. of strains) | Mean MIC (μg/ml) | | | | |
|---|---|---|---|---|---|
| | FK482 | CFIX | CCL | CEX | AMPC |
| Methicillin-resistant: | | | | | |
| *Staphylococcus aureus* (24)[a] | 7.7 | >100 | >100 | >100 | 38.6 |
| CCL-resistant: | | | | | |
| *Escherichia coli* (7)[b] | 13.8 | 18.6 | 82.2 | 100 | >100 |
| *Klebsiella pneumoniae* (5)[c] | 0.4 | 0.2 | 7.2 | 12.5 | >100 |
| CEX-resistant: | | | | | |
| *E. coli* (10)[b] | 4.7 | 6.7 | 38.0 | 57.5 | >100 |
| *K. pneumoniae* (9)[b] | 0.5 | 0.2 | 3.7 | 15.8 | >100 |
| AMPC-resistant: | | | | | |
| *S. aureus* (40)[b] | 0.3 | ND | 3.3 | ND | 23.3 |
| *E. coli* (14)[b] | 0.2 | 0.3 | 1.8 | 6.6 | >100 |
| *Proteus mirabilis* (5)[b] | 0.1 | ≦0.025 | 3.6 | 16.5 | >100 |
| *Haemophilus influenzae* (20)[d] | 0.7 | 0.06 | 5.1 | 30.8 | 9.8 |
| *Branhamella catarrhalis* (30)[e] | 0.3 | 0.3 | 1.8 | 3.8 | 2.7 |
| *Neisseria gonorrhoeae* (3)[d] | ≦0.025 | ≦0.025 | 0.5 | 7.9 | 6.3 |

[a] 6.25～50 μg/ml, [b] ≧12.5 μg/ml, [c] ≧6.25 μg/ml, [d] ≧3.13 μg/ml, [e] ≧0.78 μg/ml.
ND: Not done.
Abbreviations: See footnote in Table 1.

Table 5. Influence of introduced ampicillin-resistance plasmids on the antibacterial activity of FK482 and reference antibiotics against *Escherichia coli* CSH2.

| Antibiotic | | MIC distribution (μg/ml) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0.2 | 0.39 | 0.78 | 1.56 | 3.13 | 6.25 | 12.5 | 25 | 50 | 100 | 200 | ≧400 |
| FK482 | Parent | | 1 | | | | | | | | | | |
| | $R^+$ | 3 | 10 | | | | | | | | | | |
| CFIX | Parent | | 1 | | | | | | | | | | |
| | $R^+$ | 1 | 11 | 1 | | | | | | | | | |
| CCL | Parent | | | | | 1 | | | | | | | |
| | $R^+$ | | | | 3 | 4 | 3 | 2 | 1 | | | | |
| CEX | Parent | | | | | | 1 | | | | | | |
| | $R^+$ | | | | | | 8 | 5 | | | | | |
| AMPC | Parent | | | | | | 1 | | | | | | |
| | $R^+$ | | | | | | | | | | | | 13 |

Agar dilution method (stamp method): Mueller-Hinton agar, $10^3$ cfu/spot, 37°C, 20 hours.
Abbreviations: See footnote in Table 1.

## Effect of Introduced Ampicillin-resistance Plasmids on Antibacterial Activity of FK482 against *E. coli* CSH2

To evaluate the effect of plasmids conferring ampicillin-resistance on the antibacterial activity of FK482, the MICs of FK482 for *E. coli* CSH2 were compared with those for strains of *E. coli* CSH2 possessing these plasmids. The antibacterial activities of FK482 and CFIX was not affected in contrast to a 4-fold or greater increase in MIC of CCL for 3 of 13 strains and ≧64 fold increase in MIC of ampicillin for all 13 strains (Table 5).

## Comparison of Broth Dilution MICs and MBCs of FK482

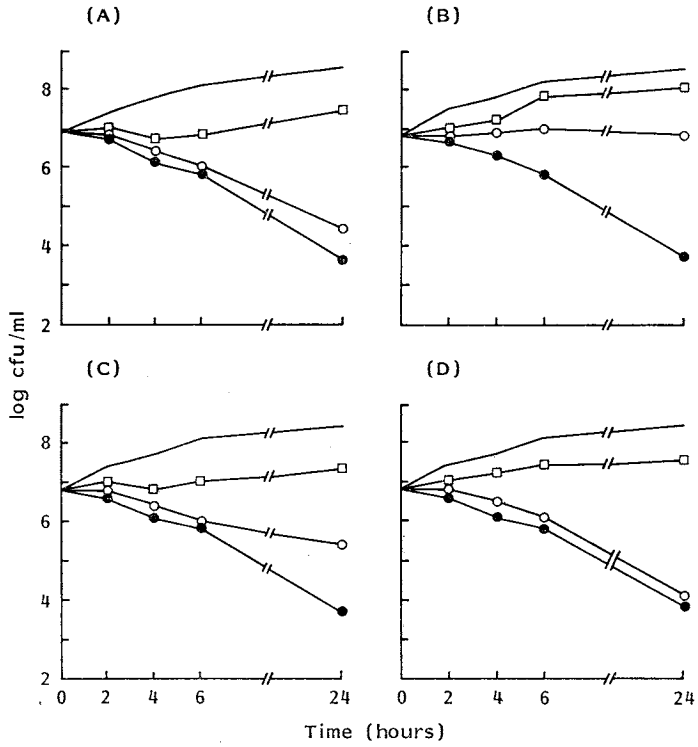
Broth dilution MICs and MBCs for 5 to 8 isolates of *S. aureus*, *E. coli*, *K. pneumoniae* and *P.*

Table 6. Comparison of broth dilution MICs and MBCs of FK482 and reference antibiotics.

| Organism (No. of strains) | Antibiotic | MIC (μg/ml) | | MBC[a] (μg/ml) | |
|---|---|---|---|---|---|
| | | Mean | Range | Mean | Range |
| *Staphylococcus aureus* (8) | FK482 | 0.39 | 0.1~1.56 | 17.7 | 12.5~25 |
| | CCL | 2.63 | 1.56~6.25 | >100 | >100 |
| | CEX | 5.73 | 3.13~12.5 | >100 | >100 |
| | AMPC | 0.60 | 0.2~50 | 22.9 | 12.5~>100 |
| *Escherichia coli* (8) | FK482 | 0.33 | 0.2~0.78 | 0.66 | 0.2~3.13 |
| | CFIX | 0.55 | 0.2~1.56 | 0.85 | 0.2~3.13 |
| | CCL | 2.20 | 0.78~6.25 | 25 | 1.56~>100 |
| | CEX | 8.10 | 6.25~12.5 | 50 | 12.5~>100 |
| | AMPC | 3.70 | 0.78~6.25 | 4.4 | 1.56~6.25 |
| *Klebsiella pneumoniae* (6) | FK482 | 0.35 | 0.2~0.78 | 8.85 | 6.25~12.5 |
| | CFIX | 0.16 | 0.1~0.2 | 3.13 | 1.56~6.25 |
| | CCL | 1.11 | 0.78~1.56 | >100 | >100 |
| | CEX | 8.85 | 6.25~12.5 | >100 | >100 |
| *Proteus mirabilis* (5) | FK482 | 0.23 | 0.2~0.39 | 7.19 | 3.13~12.5 |
| | CFIX | 0.04 | ≦0.025~0.1 | 1.36 | 0.78~1.56 |
| | CCL | 2.37 | 1.56~3.13 | >100 | 50~>100 |
| | CEX | 19.0 | 12.5~25 | >100 | >100 |
| | AMPC | 3.13 | 0.78~>100 | 75.9 | 25~>100 |

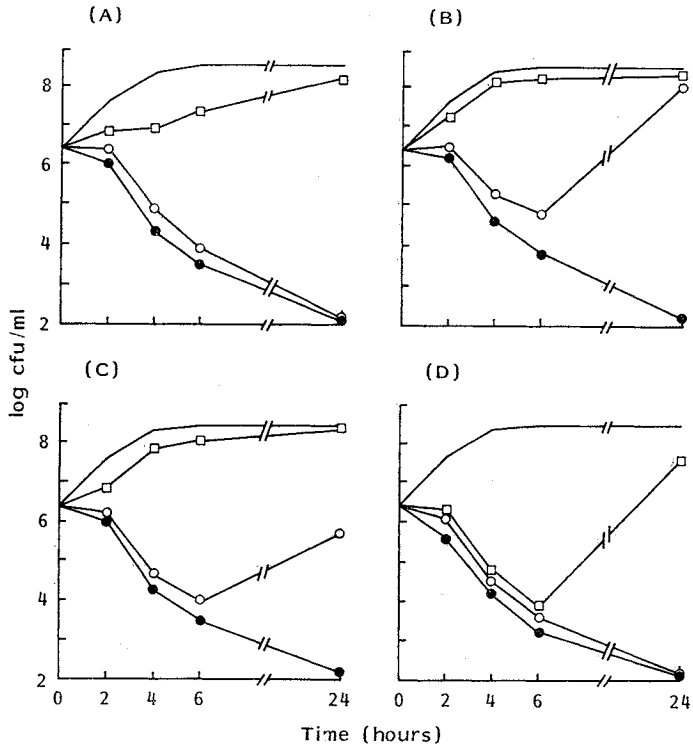
Mueller-Hinton broth, $10^5$ cfu/ml, 37°C, 18 hours.
[a] Killing 99.9% of the inoculum.
Abbreviations: See footnote in Table 1.

Fig. 3. Bactericidal activity of FK482 and reference antibiotics against *Staphylococcus aureus* 2558.
— Control, □ 1/4 MIC, ○ 1 MIC, ● 4 MIC.




(A) FK482 (MIC: 0.39 μg/ml), (B) cephalexin (MIC: 3.13 μg/ml), (C) cefaclor (MIC: 3.13 μg/ml), (D) amoxicillin (MIC: 0.39 μg/ml).

Fig. 4. Bactericidal activity of FK482 and reference antibiotics against *Escherichia coli* 3147.
— Control, □ 1/4 MIC, ○ 1 MIC, ● 4 MIC.




(A) FK482 (MIC: 0.2 μg/ml), (B) cephalexin (MIC: 6.25 μg/ml), (C) cefaclor (MIC: 0.78 μg/ml), (D) cefixime (MIC: 0.2 μg/ml).

*mirabilis* are shown in Table 6. Against *S. aureus*, the mean MIC and MBC of FK482 were 0.39 and 17.7 μg/ml, respectively, lower than the corresponding values for the other test drugs. Against *E. coli*, the mean MIC and MBC of FK482 were 0.33 and 0.66 μg/ml, respectively, which were almost the same as those of CFIX, and lower than those of CCL, CEX and AMPC. Against *K. pneumoniae* and *P. mirabilis*, the mean MICs and MBCs of FK482 ranged from 0.23 to 0.35 μg/ml and from 7.19 to 8.85 μg/ml, respectively. Although FK482 was slightly less active than CFIX, it was more potent than CCL, CEX or AMPC against these two organisms.

### Bactericidal Activity of FK482 against *S. aureus* and *E. coli*

The bactericidal activity of FK482 against *S. aureus* 2558 (Fig. 3) and *E. coli* 3147 (Fig. 4) was compared with that of the reference drugs. The bactericidal activity of FK482 against *S. aureus* was closed to that of AMPC. That is, FK482 was bactericidal at the MIC, 0.39 μg/ml, and more. On the other hand, the bactericidal activity of CCL at the MIC, 3.13 μg/ml, was weaker than that of FK482. In addition, CEX was only bacteriostatic at the MIC, 3.13 μg/ml. Against *E. coli*, FK482 was bactericidal at the MIC, 0.2 μg/ml. CCL and CEX at the MIC were weaker than FK482 in bactericidal activity and the regrowth of *E. coli* was observed 6 hours after incubation. On the other hand, CFIX had the most potent bactericidal activity of the test drugs; that is, at 1/4 the MIC the drug was bactericidal up to 6 hours after incubation.

Table 7. Stability of FK482 and reference antibiotics to β-lactamases.

| Type | | Source | Relative rate of hydrolysis[a] | | | |
|---|---|---|---|---|---|---|
| | | | FK482 | CFIX | CCL | CEX |
| CSase | Ia (1) | *Serratia marcescens* FP1184 | 3.2 | 4.4 | 140 | 41 |
| | Ia (2) | *Enterobacter cloacae* FP1185 | 0.5 | 0.3 | 45 | 45 |
| | Ib | *Escherichia coli* FP1186 | 3.5 | 1.0 | 102 | 46 |
| | Ic | *Proteus vulgaris* FP1187 | 26.5 | 2.6 | 292 | 37 |
| | Id | *Pseudomonas aeruginosa* FP1380 | 17.8 | 0.9 | 31 | 35 |
| | CXase | *Bacteroides fragilis* FP786 | 319.6 | 12.0 | 46 | 69 |
| PCase | II | *Proteus mirabilis* FP240 | <0.1 | <0.1 | 0.1 | <0.1 |
| | III | *Escherichia coli* FP1189 | 0.1 | 0.4 | 2.7 | 0.6 |
| | IV | *Klebsiella pneumoniae* FP239 | 0.1 | <0.1 | 3.2 | 0.3 |
| | V | *Pseudomonas aeruginosa* FP1190 | 1.0 | 0.1 | 0.3 | 0.4 |
| | | *Staphylococcus aureus* FP1191 | <0.1 | <0.1 | 2.2 | 0.3 |

Substrate concentration: 50 μg/ml.

[a] Relative initial velocity: Cephaloridine; 100 for cephalosporinase (CSase), ampicillin; 100 for penicillinase (PCase).

CXase: Cefuroximase.

Abbreviations: See footnote in Table 1.

Table 8. Affinity of FK482 and reference antibiotics for the penicillin-binding proteins (PBPs).

| | PBPs | $ID_{50}$[a] | | | |
|---|---|---|---|---|---|
| | | FK482 | CFIX | CCL | CEX |
| *Staphylococcus aureus* 209P JC-1 | 1 | 0.58 | 2.9 | 0.2 | 0.2 |
| | 2 | 0.17 | <0.2 | 125 | 1.7 |
| | 3 | 0.12 | 6.2 | <0.2 | 0.2 |
| | 4 | 28 | 25 | >125 | 24 |
| MIC (μg/ml) | | 0.20 | 25 | 0.78 | 1.56 |
| *Enterococcus faecalis* FP183 | 1 | 7.9 | | 0.66 | |
| | 2 | <0.2 | | <0.2 | |
| | 3 | <0.2 | | 6.1 | |
| | 4 | >125 | | 26 | |
| | 5 | 74 | | 97 | |
| MIC (μg/ml) | | 12.5 | | 100 | |
| *Escherichia coli* NIHJ JC-2 | 1a | 0.09 | <0.2 | 1.6 | 0.8 |
| | 1bs | 2.3 | ≦0.2 | 7.2 | >125 |
| | 2 | 1.6 | 16 | 27 | ≧125 |
| | 3 | 0.07 | 0.2 | 1.6 | 8.7 |
| | 4 | 1.1 | >125 | 1.6 | 3.0 |
| | 5 | >125 | >125 | >25 | >125 |
| | 6 | >125 | 13 | >25 | >125 |
| MIC (μg/ml) | | 0.2 | 0.2 | 3.13 | 12.5 |

[a] Concentrations (μg/ml) of drug required to reduce $^{14}$C-benzylpenicillin binding by 50%.

Abbreviations: See footnote in Table 1.

## Stability of FK482 to β-Lactamases

FK482 had better stability than CCL and CEX to the cephalosporinases tested except the enzyme from *B. fragilis* and penicillinases (Table 7). The excellent stability of FK482 to Ia and Ib types of cephalosporinases was almost the same as that of CFIX, but its stability to Ic and Id types and enzyme from *B. fragilis* was lower than that of CFIX.

### Affinity of FK482 for the PBPs of *S. aureus*, *E. faecalis* and *E. coli*

The affinities of FK482 and the reference drugs to PBPs of *S. aureus* 209P JC-1, *E. faecalis* FP183 and *E. coli* NIHJ JC-2 are expressed as concentrations of drugs required to reduce $^{14}$C-benzylpenicillin binding by 50% (Table 8). For the main PBPs (3, 2 and 1) of *S. aureus*, the $ID_{50}$ of FK482 was 0.58 μg/ml or below, FK482 showed higher average binding affinities than the reference drugs for these PBPs. Both FK482 and the reference drugs showed low affinity for PBP 4. Against *E. faecalis* PBPs, FK482 showed very high affinity for PBP 2 ($ID_{50}$, <0.2 μg/ml) and PBP 3 (<0.2 μg/ml), moderate affinity for PBP 1 (7.9 μg/ml), and low affinity for PBPs 5 (74 μg/ml) and 4 (>125 μg/ml), while CCL showed very high affinity for PBPs 2 (<0.2 μg/ml) and 1 (0.66 μg/ml), moderate affinity for PBP 3 (6.1 μg/ml), and low affinity for PBPs 4 (26 μg/ml) and 5 (97 μg/ml). For all of the main PBPs (3, 1a and 1bs) of *E. coli*, the $ID_{50}$ of FK482 was 2.3 μg/ml or below, whereas the $ID_{50}$s of CCL and CEX were more than 3-fold higher than that of FK482. The binding affinity of FK482 was 3 times higher than that of CFIX for PBP 3, but 10-fold lower than that of CFIX for PBP 1bs.

Table 9. Ability of FK482 and reference antibiotics to penetrate the outer membrane of *Escherichia coli*.

| Antibiotic | Relative permeability[a] |
|---|---|
| FK482 | 0.024 |
| CFIX | 0.012[11] |
| CCL | 0.49 |
| CEX | 0.25 |
| Cephaloridine | 1.00 |

[a] Permeability coefficients were expressed as a ratio to that of cephaloridine.

Abbreviations: See footnote in Table 1.

### Ability of FK482 to Penetrate the Outer Membrane of *E. coli*

The ability of FK482 to penetrate the outer membrane of *E. coli* was one order of magnitude lower than that of CCL and CEX (Table 9), but twice that of CFIX[11].

## Discussion

FK482 is a cephalosporanic acid derivative with a hydroxyimino-aminothiazole side chain at the 7-position and a vinyl group at the 3-position. Introduction of the hydroxyimino instead of carboxymethoxyimino at 7-position (*e.g.*, CFIX) results in a significant enhancement of antibacterial activity against Gram-positive organisms. *In vitro*, FK482 exhibited good antibacterial activity against Staphylococci, Streptococci and *E. faecalis* in combination with broad spectrum, potent antibacterial and bactericidal activities against Gram-negative bacteria and excellent stability to most β-lactamases tested. The good antibacterial activity of FK482 against Gram-positive bacteria can be explained by its high affinity for PBPs (Table 8) and its high stability to β-lactamases (Table 7). That is, the MICs of FK482, CFIX, CCL and CEX against *S. aureus* 209P JC-1 were 0.2, 25, 0.78 and 1.56 μg/ml, respectively. The affinities of FK482 for the PBPs (1, 2 and 3) of *S. aureus*, three of the essential target enzymes, were higher than those of the reference drugs. In particular, for PBPs (1 and 3), FK482 had binding affinities 5 and 50 times higher, respectively, than those of CFIX and for PBP 2, 700 and 10 times higher than those of CCL and CEX, respectively. This suggests that the high average binding affinities of FK482 for the main PBPs of *S. aureus* as well as its high stability to penicillinases are responsible for its potent antibacterial activity against *S. aureus*. FK482 also exhibited moderate activity against *E. faecalis* resistant to commercially available cephems. The MICs of FK482 and CCL against *E. faecalis* FP183 were 12.5 and 100 μg/ml, respectively. FK482 showed a very high affinity for PBPs (2 and 3) of *E. faecalis*, while CCL showed a similarly high affinity for PBPs (1 and 2). In other words, the PBP-affinity of FK482 differs from that of CCL for only PBP 3 of *E. faecalis*. Although the role of the PBPs of *E. faecalis* in antibacterial activity is thus far less defined than those of

*S. aureus*, the potent antibacterial activity of FK482 against this organism may be related to its excellent affinity for PBPs (2 and 3). Against Gram-negative bacteria, FK482 had excellent *in vitro* activity similar or slightly inferior to that of CFIX and superior to that of CCL and CEX.

In comparison with CFIX, FK482 activity might be explained by its higher affinity for PBP 3 (Table 8) and higher ability to penetrate the outer membrane of *E. coli* (Table 9) than CFIX, although FK482 had relatively lower stability to β-lactamases (Table 7) and lower affinity for PBP 1bs of *E. coli* (Table 8) than CFIX.

In comparison with CCL and CEX, FK482 activity might be explained by its higher affinity for PBPs (1a, 1bs and 3), three of the essential target enzymes of *E. coli* (Table 8) as well as its higher stability to β-lactamases (Table 7), even though the ability of FK482 to penetrate the outer membrane of *E. coli* was weaker than that of CCL and CEX (Table 9). The susceptibility of *E. faecalis* to cephalosporins having an oxyimino group in the 7-position is known to vary with the media used for the susceptibility test[12,13], and the activity of FK482, which has a hydroxyimino group in the 7-position, was also clearly affected by the test medium. That is, its activity in Mueller-Hinton agar was moderate, but in sensitivity test agar, its activity was almost the same as that of AMPC. In experimental pyelonephritis induced by *E. faecalis* in rabbits, FK482 was as effective as AMPC and more effective than CCL[14]. FK482 clearly merits further *in vivo* evaluation.

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