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# In Vitro Activity and β-Lactamase Stability of FK-037, a Parenteral Cephalosporin

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**The in vitro activity of FK-037, 5-amino-2-[[(6*R*, 7*R*)-7-[[(*Z*)-2-(2-amino-4-thiazolyl)-2-(methoxyimino) acetyl] amino]-2-carboxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl]methyl]-1-(2-hydroxyethyl)-1H-pyrazolium hydroxide, inner salt, sulfate (1:1), a new parenteral cephem, was compared with those of cefepime, ceftazidime, imipenem, and ciprofloxacin. FK-037 inhibited methicillin-susceptible staphylococci at ≤4 μg/ml. Of 98 isolates of homogenous methicillin-resistant *Staphylococcus aureus*, 55 (56.1%) were inhibited by 8 μg of FK-037 per ml, compared to 3.1% for cefepime. Imipenem was the most active β-lactam tested against staphylococci. The MIC of FK-037 for 90% of the strains tested ($MIC_{90}$) was 0.06 μg/ml for hemolytic streptococci, *Streptococcus pneumoniae*, viridans group streptococci, and *Streptococcus bovis*. The $MIC_{90}$ for many of the members of the family *Enterobacteriaceae* was 1 μg/ml, similar to that of cefepime and lower than those of ceftazidime and imipenem. The $MIC_{90}$ for *Klebsiella pneumoniae* and *Enterobacter cloacae* was 8 μg/ml, similar to that for cefepime, but all isolates were inhibited by 2 μg of imipenem per ml. *K. pneumoniae* isolates with cefotaxime and ceftazidime MICs of >32 μg/ml with Bush type 2b′ β-lactamases were inhibited by 4 μg of FK-037 per ml. *E. cloacae*, *Citrobacter freundii*, and *S. aureus* stably resistant to FK-037 could be selected by repeated transfer in the presence of FK-037. The FK-037 $MIC_{90}$ for *Pseudomonas aeruginosa* was 4 μg/ml, compared to 32 μg/ml for cefepime and ceftazidime and 8 μg/ml for imipenem. *Xanthomonas maltophilia*, *Pseudomonas cepacia*, *Acinetobacter anitratus*, and *Bacteroides* species were resistant to FK-037 (MIC, ≥32 μg/ml). MBCs were identical to or within twofold of the MICs except for a 32-fold greater MBC for *P. aeruginosa*. Inoculum size and acid environment did not lower the activity of FK-037. FK-037 was not appreciably hydrolyzed by Bush group 1, 2a, 2b, and 2e β-lactamases but was hydrolyzed by 2b′ and 2d enzymes at rates comparable to that of ceftazidime. Nonetheless, FK-037 inhibited bacteria possessing TEM-3, -5, and -7 and SHV-5 at ≤8 μg/ml. Overall, FK-037 has lower MICs against staphylococci and *P. aeruginosa* than the currently available iminomethoxy aminothiazolyl cephalosporins and has activity against members of the family *Enterobacteriaceae* comparable to that of cefepime.**

Although a number of parenteral cephalosporins have been synthesized during the past 2 decades, there has been continued interest in finding new cephalosporins with improved activity against gram-positive bacteria, particularly for methicillin-resistant staphylococci, and which retain the excellent activity of the aminothiazolyl cephalosporins against gram-negative organisms. FK-037, an oxime-type cephem, contains a 1-hydroxyethyl-5-amino pyra-zoliomethyl moiety at position 3 of the cephem ring. Preliminary studies showed FK-037 to have a broad spectrum of antibacterial activity against gram-negative and gram-positive bacteria, including methicillin-resistant staphylococci (2, 3). We compared the in vitro activity of FK-037 with those of cefepime, ceftazidime, imipenem, and ciprofloxacin against clinical isolates and determined its stability and the affinity of β-lactamases for FK-037.

## MATERIALS AND METHODS

**Bacterial strains.** The organisms were isolated from patients admitted to the Columbia-Presbyterian Medical Center in New York City during the past 2 years. Staphylococci were collected from blood isolates within the last 2 years. S. Sirot and G. Jacoby donated selected β-lactamase-producing isolates used in determination of activity and β-lactamase stability of FK-037. D. Clark provided *Escherichia coli* isolates UB-1005, DC-1, and DC-3.

**Antimicrobial agents.** FK-037 was a gift of Ortho Pharmaceutical Corp., Raritan, N.J., manufactured by Fujisawa Pharmaceutical Co., Ltd., Osaka, Japan. The other agents were obtained as follows: cefepime was from Bristol Laboratories, Wallingford, Conn.; ceftazidime was from Glaxo Pharmaceuticals Inc., Research Triangle Park, N.C.; cefpirome was from Hoechst AG, Frankfurt, Germany; imipenem was from Merck Sharp & Dohme, Rahway, N.J.; and ciprofloxacin was from Miles, Inc., New Haven, Conn.

**Susceptibility tests.** MICs were determined by an agar dilution method with Mueller-Hinton agar (BBL Microbiology Systems, Cockeysville, Md.) according to the National Committee for Clinical Laboratory Standards guidelines (4). Activity was tested against streptococci by using agar supplemented with 5% sheep blood. MICs of *Haemophilus* and *Moraxella* spp. were determined on *Haemophilus* test medium. Anaerobic species were tested with Wilkins-Chalgren agar (5). All studies with staphylococci were done in the presence of 2% NaCl. Bacteria were inoculated on agar plates by a replicating device with a final inoculum of $10^4$ CFU for aerobes and $10^5$ CFU for anaerobes. The plates were incubated at 35°C for 18 to 20 h for aerobic species and for 48 h in a GasPak jar for anaerobic species. The activity in

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TABLE 1. Comparative activities of FK-037 and other agents against gram-negative bacteria

| Organism (no. of isolates tested) | Agent | MIC (μg/ml) | | |
|---|---|---|---|---|
| | | Range | 50% | 90% |
| *E. coli* (30) | FK-037 | ≤0.015–0.12 | 0.03 | 0.06 |
| | Cefepime | ≤0.015–0.25 | 0.03 | 0.12 |
| | Ceftazidime | 0.03–0.5 | 0.12 | 0.25 |
| | Imipenem | 0.06–1 | 0.12 | 0.25 |
| | Ciprofloxacin | ≤0.008–0.03 | 0.015 | 0.03 |
| *K. pneumoniae* (39) | FK-037 | 0.06–4 | 0.06 | 4 |
| | Cefepime | 0.03–8 | 0.06 | 8 |
| | Ceftazidime | 0.12–>64 | 0.25 | >64 |
| | Imipenem | 0.06–2 | 0.25 | 2 |
| | Ciprofloxacin | ≤0.008–0.25 | 0.03 | 0.25 |
| *Klebsiella oxytoca* (20) | FK-037 | 0.06–4 | 0.06 | 0.5 |
| | Cefepime | ≤0.015–0.25 | 0.03 | 0.12 |
| | Ceftazidime | 0.12–>16 | 0.12 | 0.25 |
| | Imipenem | 0.12–2 | 0.5 | 2 |
| | Ciprofloxacin | 0.015–0.025 | 0.06 | 0.12 |
| *E. cloacae* (46) | FK-037 | 0.03–32 | 0.5 | 8 |
| | Cefepime | 0.06–16 | 0.25 | 8 |
| | Ceftazidime | 0.06–>64 | 0.5 | 64 |
| | Imipenem | 0.12–2 | 0.5 | 2 |
| | Ciprofloxacin | 0.015–0.12 | 0.03 | 0.06 |
| *E. aerogenes* (20) | FK-037 | 0.03–1 | 0.06 | 0.12 |
| | Cefepime | 0.03–0.5 | 0.12 | 0.12 |
| | Ceftazidime | 0.12–>64 | 0.5 | 32 |
| | Imipenem | 0.06–2 | 0.5 | 1 |
| | Ciprofloxacin | 0.03–0.5 | 0.03 | 0.06 |
| *Enterobacter agglomerans* (10) | FK-037 | ≤0.015–0.12 | 0.06 | 0.12 |
| | Cefepime | ≤0.015–0.12 | 0.03 | 0.12 |
| | Ceftazidime | ≤0.015–4 | 0.12 | 2 |
| | Imipenem | 0.12–2 | 0.5 | 1 |
| | Ciprofloxacin | 0.015–0.06 | 0.015 | 0.06 |
| *Hafnia alvei* (11) | FK-037 | 0.03–2 | 0.06 | 0.06 |
| | Cefepime | ≤0.015–0.25 | 0.03 | 0.12 |
| | Ceftazidime | 0.5–32 | 4 | 16 |
| | Imipenem | 0.25–0.5 | 0.5 | 0.5 |
| | Ciprofloxacin | 0.015–0.06 | 0.03 | 0.06 |
| *C. freundii* (40) | FK-037 | 0.03–4 | 0.06 | 0.5 |
| | Cefepime | 0.03–16 | 0.06 | 8 |
| | Ceftazidime | 0.12–>64 | 0.5 | 64 |
| | Imipenem | 0.06–1 | 0.25 | 0.5 |
| | Ciprofloxacin | ≤0.008–0.06 | 0.015 | 0.015 |
| *Citrobacter diversus* (10) | FK-037 | 0.06–0.12 | 0.12 | 0.12 |
| | Cefepime | ≤0.015–0.06 | 0.03 | 0.06 |
| | Ceftazidime | 0.06 | 0.06 | 0.06 |
| | Imipenem | 0.12–0.25 | 0.12 | 0.12 |
| | Ciprofloxacin | ≤0.008–0.06 | 0.015 | 0.015 |
| *P. mirabilis* (30) | FK-037 | 0.015–0.06 | 0.03 | 0.06 |
| | Cefepime | 0.03–0.5 | 0.03 | 0.06 |
| | Ceftazidime | 0.06–0.25 | 0.12 | 0.25 |
| | Imipenem | 0.5–2 | 0.5 | 1 |
| | Ciprofloxacin | 0.015–32 | 0.03 | 0.06 |
| *M. morganii* (20) | FK-037 | 0.015–0.03 | 0.03 | 0.03 |
| | Cefepime | ≤0.015 | ≤0.015 | ≤0.015 |
| | Ceftazidime | 0.015–1 | 0.06 | 0.5 |
| | Imipenem | 0.25–4 | 2 | 4 |
| | Ciprofloxacin | 0.03–0.25 | 0.12 | 0.25 |

*Continued on following page*

TABLE 1—*Continued*

| Organism (no. of isolates tested) | Agent | MIC (μg/ml) | | |
|---|---|---|---|---|
| | | Range | 50% | 90% |
| *P. vulgaris* (20) | FK-037 | 0.03–0.5 | 0.03 | 0.12 |
| | Cefepime | 0.015–0.12 | 0.03 | 0.06 |
| | Ceftazidime | 0.03–0.25 | 0.03 | 0.12 |
| | Imipenem | 0.25–4 | 1 | 2 |
| | Ciprofloxacin | 0.03–0.25 | 0.03 | 0.12 |
| *P. rettgeri* (10) | FK-037 | 0.03–16 | 0.06 | 8 |
| | Cefepime | 0.06–8 | 1 | 8 |
| | Ceftazidime | 0.06–64 | 2 | 8 |
| | Imipenem | 0.5–4 | 1 | 2 |
| | Ciprofloxacin | 0.015–0.5 | 0.12 | 0.5 |
| *Providencia stuartii* (20) | FK-037 | 0.015–8 | 0.12 | 1 |
| | Cefepime | 0.06–4 | 0.06 | 1 |
| | Ceftazidime | 0.03–4 | 0.12 | 1 |
| | Imipenem | 0.06–4 | 1 | 2 |
| | Ciprofloxacin | 0.03–0.5 | 0.12 | 0.25 |
| *Serratia marcescens* (25) | FK-037 | 0.03–0.5 | 0.12 | 0.25 |
| | Cefepime | 0.06–4 | 0.12 | 1 |
| | Ceftazidime | 0.12–8 | 0.5 | 1 |
| | Imipenem | 0.12–2 | 0.5 | 1 |
| | Ciprofloxacin | 0.06–0.5 | 0.12 | 0.25 |
| *P. aeruginosa* (40) | FK-037 | 1–64 | 2 | 4 |
| | Cefepime | 1–>64 | 2 | 32 |
| | Ceftazidime | 1–>64 | 2 | 32 |
| | Imipenem | 0.25–16 | 2 | 8 |
| | Ciprofloxacin | 0.03–16 | 0.25 | 2 |
| *P. cepacia* (16) | FK-037 | 1–>64 | 4 | 64 |
| | Cefepime | 2–>64 | 8 | 64 |
| | Ceftazidime | 2–>64 | 16 | >64 |
| | Imipenem | 1–>32 | 32 | >32 |
| | Ciprofloxacin | 0.12–2 | 0.25 | 0.5 |
| *X. maltophilia* (10) | FK-037 | 8–>64 | 64 | >64 |
| | Cefepime | 2–>64 | 32 | 64 |
| | Ceftazidime | 2–>64 | 32 | 64 |
| | Imipenem | 1–>32 | 32 | >32 |
| | Ciprofloxacin | 0.06–4 | 0.5 | 1 |
| *A. anitratus* (30) | FK-037 | 1–64 | 4 | 32 |
| | Cefepime | 2–64 | 8 | 32 |
| | Ceftazidime | 0.5–>64 | 8 | 32 |
| | Imipenem | 0.06–1 | 0.25 | 0.25 |
| | Ciprofloxacin | 0.12–>16 | 0.5 | 16 |
| *Salmonella* spp. (15) | FK-037 | ≤0.015–0.12 | 0.06 | 0.12 |
| | Cefepime | ≤0.015–0.25 | 0.06 | 0.12 |
| | Ceftazidime | 0.25–1 | 0.5 | 0.5 |
| | Imipenem | 0.12–4 | 0.25 | 0.5 |
| | Ciprofloxacin | 0.03–0.06 | 0.06 | 0.06 |
| *Salmonella typhi* (4) | FK-037 | ≤0.015–0.03 | 0.03 | |
| *Shigella* spp. (12) | FK-037 | ≤0.015–0.12 | 0.03 | 0.06 |
| | Cefepime | ≤0.015–1 | 0.06 | 0.12 |
| | Ceftazidime | 0.06–1 | 0.12 | 0.5 |
| | Imipenem | 0.25–2 | 1 | 2 |
| | Ciprofloxacin | 0.03–0.12 | 0.03 | 0.06 |
| *Aeromonas hydrophila* (10) | FK-037 | ≤0.015–8 | 0.06 | 0.5 |
| | Cefepime | ≤0.015–8 | 0.06 | 0.5 |
| | Ceftazidime | 0.12–1 | 0.25 | 0.5 |
| | Imipenem | 0.25–8 | 0.25 | 2 |
| | Ciprofloxacin | ≤0.008–0.12 | 0.03 | 0.06 |

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TABLE 1—*Continued*

| Organism (no. of isolates tested) | Agent | MIC (μg/ml) | | |
|---|---|---|---|---|
| | | Range | 50% | 90% |
| *Yersinia enterocolitica* (10) | FK-037 | ≤0.015–0.5 | 0.03 | 0.06 |
| | Cefepime | 0.03–8 | 0.06 | 0.5 |
| | Ceftazidime | 0.12–1 | 0.25 | 0.5 |
| | Imipenem | 0.25–8 | 0.25 | 2 |
| | Ciprofloxacin | ≤0.008–0.12 | 0.03 | 0.06 |
| *H. influenzae* (30) | FK-037 | ≤0.015–0.06 | ≤0.015 | ≤0.015 |
| | Cefepime | 0.06–1 | 0.12 | 0.25 |
| | Ceftazidime | 0.03–0.25 | 0.12 | 0.25 |
| | Imipenem | 0.03–2 | 0.5 | 1 |
| | Ciprofloxacin | 0.015 | 0.015 | 0.015 |
| *N. gonorrhoeae* (14) | FK-037 | 0.06–0.12 | 0.06 | 0.06 |
| *M. catarrhalis* (30) | FK-037 | 0.03–0.5 | 0.5 | 0.5 |
| | Cefepime | 0.12–0.5 | 0.5 | 0.5 |
| | Ceftazidime | 0.03–0.25 | 0.03 | 0.06 |
| | Imipenem | 0.03–0.25 | 0.06 | 0.25 |
| | Ciprofloxacin | 0.015–0.25 | 0.03 | 0.06 |

broth was measured by a broth dilution method in 2-ml tubes with an inoculum of $5 \times 10^5$ CFU/ml. MBCs were determined by plating 0.01-ml samples from clear tubes onto an antibiotic-free agar plate and were defined as the concentration showing a 99.9% reduction of the initial inoculum after a 24-h incubation (7).

**Development of resistance.** Development of resistance was determined for two isolates each of *Enterobacter cloacae*, *Citrobacter freundii*, and *Staphylococcus aureus*. Bacteria from the culture tube containing the highest drug concentration that permitted visible growth had repeated exposure at $5 \times 10^5$ CFU to FK-037 for 14 days. The daily MICs were recorded, and the terminal MICs were compared with the initial MICs. To assess whether resistance was a stable mutation or an inducible event, we subcultured the resistant organisms daily in an antibiotic-free broth 10 successive times.

β-Lactamase stability was determined by a spectrophotometric assay by using the change in maximum absorption (6). Cephaloridine was used as a reference compound. Inhibition of hydrolysis of cephaloridine was performed by preincubation of the enzymes with the inhibitors for 10 min prior to adding cephaloridine to the reaction mixture. Enzymes were purified by column separation as described previously (6). The classification of β-lactamases was that of Bush (1).

## RESULTS

The in vitro activities of FK-037 and the other agents tested against gram-negative organisms are shown in Table 1. FK-037 inhibited 50% of the members of the family *Enterobacteriaceae* at ≤0.12 μg/ml, and the MIC for 90% of the strains tested ($MIC_{90}$) was ≤1 μg/ml, except for *E. cloacae* and *Providencia rettgeri*, for which the FK-037 $MIC_{90}$ was 8 μg/ml. In comparison with cefepime, ceftazidime, and imipenem, FK-037 was equal to or 2- to 8-fold more active than cefepime and 4- to 16-fold more active than ceftazidime and imipenem against *E. coli*, *Klebsiella pneumoniae*, *Enterobacter aerogenes*, *C. freundii*, and *Serratia*, *Providencia*, *Salmonella*, and *Shigella* species. Cefepime was twofold more active than FK-037 against *Morganella* spp. and *Proteus mirabilis*. Ciprofloxacin at <2 μg/ml inhibited isolates which were resistant to the β-lactams. *K. pneumoniae* isolates resistant to cefotaxime and ceftazidime were susceptible to FK-037 and cefepime, with $MIC_{90}$s of 4 and 1 μg/ml, respectively. *E. cloacae* was more susceptible to cefepime, imipenem, and ciprofloxacin. Ninety percent of *Pseudomonas aeruginosa* isolates were inhibited at 4 μg of FK-037 per ml compared to $MIC_{90}$s of 32 μg/ml for cefepime and ceftazidime and 8 μg/ml for imipenem. Some isolates resistant to imipenem and ciprofloxacin were inhibited by FK-037. Ninety percent of *Haemophilus influenzae* and *Neisseria gonorrhoeae* isolates were inhibited by 0.06 μg of FK-037 per ml. The FK-037 $MIC_{90}$ was 2 μg/ml for *Moraxella catarrhalis*. Fifty percent of *Pseudomonas cepacia* and *Acinetobacter anitratus* isolates were inhibited by 4 μg/ml, but the FK-037 MICs for *Xanthomonas maltophilia* were ≥64 μg/ml.

FK-037 inhibited most gram-positive organisms (Table 2). Two hundred eighty-eight isolates of staphylococci were tested. FK-037 at ≤16 μg/ml inhibited 50% of the *S. aureus* isolates for which the oxacillin MICs were ≥16 μg/ml. The *S. aureus* isolates for which the oxacillin MICs were 8 μg/ml were inhibited by ≤4 μg/ml compared to ≥32 μg/ml for cefepime. FK-037 inhibited 90% of methicillin-susceptible *S. aureus* isolates at ≤2 μg/ml. FK-037 at 8 μg/ml inhibited 50% of the *Staphylococcus epidermidis* isolates for which the oxacillin MICs were ≥16 μg/ml. The FK-037 $MIC_{90}$s were ≤0.06 μg/ml for beta-hemolytic streptococci, *Streptococcus pneumoniae*, viridans group streptococci, and *Streptococcus bovis*. The activity of FK-037 against these species was similar to that of cefepime and two- to fourfold less than imipenem and ceftazidime. Enterococci, *Listeria* spp., and most anaerobic species were resistant to FK-037, cefepime, and ceftazidime but not to imipenem.

The effect of inoculum size on the activity of FK-037 was determined. MICs for *S. aureus* were not altered by increasing the inoculum from $10^4$ to $10^6$ CFU, geometric mean MICs being 1.14 μg/ml at $10^4$ CFU and 1.15 μg/ml at $10^6$ CFU. The

TABLE 2. In vitro activity of FK-037 against gram-positive and anaerobic bacteria

| Organism (no. of isolates tested) | Agent | MIC (μg/ml) | | |
|---|---|---|---|---|
| | | Range | 50% | 90% |
| High-methicillin-resistant[a] *S. aureus* (41) | FK-037 | 4–64 | 16 | 32 |
| | Cefepime | 8–>64 | 64 | >64 |
| | Ceftazidime | 8–>64 | 32 | >64 |
| | Imipenem | 0.25–>16 | 0.5 | 8 |
| | Ciprofloxacin | 0.12–>16 | 0.5 | >16 |
| Low-methicillin-resistant[b] *S. aureus* (7) | FK-037 | 1–4 | 2 | |
| | Cefepime | 4–32 | 16 | |
| Methicillin-susceptible *S. aureus* (90) | FK-037 | 0.25–4 | 1 | 2 |
| | Cefepime | 1–8 | 4 | 4 |
| | Ceftazidime | 4–16 | 8 | 16 |
| | Imipenem | ≤0.015–0.12 | ≤0.015 | 0.06 |
| | Ciprofloxacin | 0.12–6 | 0.5 | 1 |
| High-methicillin-resistant[a] coagulase-negative staphylococci (57) | FK-037 | 2–64 | 8 | 16 |
| | Cefepime | 8–>64 | 32 | 64 |
| | Ceftazidime | 2–>64 | 64 | >64 |
| | Imipenem | 2–>16 | 8 | >16 |
| | Ciprofloxacin | 0.12–>16 | 0.5 | 16 |
| Low-methicillin-resistant[b] coagulase-negative staphylococci (11) | FK-037 | 1–4 | 2 | 2 |
| | Cefepime | 2–8 | 4 | 8 |
| Methicillin-susceptible coagulase-negative staphylococci (82) | FK-037 | 0.12–2 | 0.5 | 1 |
| | Cefepime | 0.25–16 | 1 | 4 |
| | Ceftazidime | 4–32 | 8 | 32 |
| | Imipenem | 0.03–0.25 | 0.03 | 0.12 |
| | Ciprofloxacin | 0.25–>16 | 0.5 | 2 |
| *Streptococcus pyogenes* (18) | FK-037 | ≤0.015–0.06 | ≤0.015 | 0.06 |
| | Cefepime | ≤0.015–0.12 | ≤0.015 | 0.06 |
| | Ceftazidime | 0.03–0.25 | 0.12 | 0.25 |
| | Imipenem | ≤0.015 | ≤0.015 | ≤0.015 |
| | Ciprofloxacin | 0.25–1 | 0.25 | 0.5 |
| *Streptococcus agalactiae* (11) | FK-037 | 0.03–0.12 | 0.06 | 0.12 |
| | Cefepime | ≤0.015–0.12 | 0.03 | 0.06 |
| | Ceftazidime | 0.12–0.5 | 0.25 | 0.5 |
| | Imipenem | ≤0.015 | ≤0.015 | ≤0.015 |
| | Ciprofloxacin | 0.25–1 | 0.5 | 1 |
| *Streptococcus* groups C and G (22) | FK-037 | ≤0.015–0.06 | ≤0.015 | 0.03 |
| | Cefepime | ≤0.015–0.12 | ≤0.015 | 0.03 |
| | Ceftazidime | 0.06–1 | 0.12 | 0.25 |
| | Imipenem | ≤0.015–0.12 | ≤0.015 | ≤0.015 |
| | Ciprofloxacin | 0.12–4 | 0.5 | 1 |
| Viridans group streptococci (15) | FK-037 | ≤0.015–1 | 0.03 | 0.12 |
| | Cefepime | ≤0.015–0.25 | 0.03 | 0.12 |
| | Ceftazidime | 0.5–8 | 1 | 4 |
| | Imipenem | 0.06–0.25 | 0.06 | 0.25 |
| | Ciprofloxacin | 0.25–4 | 1 | 4 |
| *S. pneumoniae* (24) | FK-037 | ≤0.015–0.5 | 0.03 | 0.12 |
| | Cefepime | ≤0.015–0.5 | 0.03 | 0.25 |
| | Ceftazidime | 0.12–4 | 0.25 | 2 |
| | Imipenem | 0.06–0.25 | 0.06 | 0.25 |
| | Ciprofloxacin | 0.12–4 | 1 | 2 |
| *S. bovis* (10) | FK-037 | ≤0.015–0.12 | 0.03 | 0.06 |
| | Cefepime | ≤0.015–0.25 | 0.03 | 0.12 |
| | Ceftazidime | 0.03–0.5 | 0.25 | 0.5 |
| | Imipenem | ≤0.015–0.06 | 0.03 | 0.06 |
| | Ciprofloxacin | 0.5–2 | 1 | 2 |

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TABLE 2—*Continued*

| Organism (no. of isolates tested) | Agent | MIC (μg/ml) | | |
|---|---|---|---|---|
| | | Range | 50% | 90% |
| *Enterococcus faecalis* (15) | FK-037 | 16–>64 | >64 | >64 |
| | Cefepime | 16–>64 | 32 | 64 |
| | Ceftazidime | 64–>64 | >64 | >64 |
| | Imipenem | 0.25–8 | 2 | 4 |
| | Ciprofloxacin | 0.5–4 | 0.5 | 4 |
| *Listeria monocytogenes* (18) | FK-037 | 8–64 | 32 | 64 |
| | Cefepime | 4–>64 | 32 | 64 |
| | Ceftazidime | 32–>64 | >64 | >64 |
| | Imipenem | 0.06–1 | 0.06 | 0.12 |
| | Ciprofloxacin | 1–4 | 1 | 4 |
| *Bacteroides fragilis* (32) | FK-037 | 32–>64 | 32 | 64 |
| | Cefepime | 32–>64 | 32 | >64 |
| | Ceftazidime | 32–>64 | >64 | >64 |
| | Imipenem | 0.06–1 | 0.25 | 0.5 |
| | Ciprofloxacin | 4–>16 | 8 | 16 |
| Other *Bacteroides* spp. (20) | FK-037 | 64–>64 | >64 | >64 |
| *Clostridium* spp. (20) | FK-037 | 1–64 | 4 | >64 |
| | Cefepime | 1–>64 | 2 | >64 |
| | Ceftazidime | 0.5–>64 | 4 | >64 |
| | Imipenem | 0.25–16 | 0.5 | 16 |
| | Ciprofloxacin | 0.5–2 | 0.5 | 2 |

[a] Oxacillin MIC of ≥16 μg/ml.
[b] Oxacillin MIC of 8 μg/ml.

respective geometric mean MICs at $10^4$ and $10^6$ CFU were 0.06 and 0.56 μg/ml for *E. coli*, 0.05 and 0.32 μg/ml for *K. pneumoniae*, 0.08 and 0.58 μg/ml for *C. freundii*, and 0.19 and 0.76 μg/ml for *E. cloacae*. There was a larger increase in MICs for *P. aeruginosa*—2 μg/ml at $10^4$ CFU and 10.6 μg/ml at $10^6$ CFU—with one of five isolates having an increase in the FK-037 MIC from 4 to 32 μg/ml. The activity of FK-037 was determined at pHs 5.5, 6.5, and 7.5. The geometric mean MICs at pHs 5.5 and 7.5 for five isolates each were as follows: *E. coli*, 0.06 and 0.06 μg/ml; *K. pneumoniae*, 0.09 and 0.07 μg/ml; *C. freundii*, 1.72 and 0.08 μg/ml; *E. cloacae*, 0.31 and 0.45 μg/ml; *P. aeruginosa*, 4.6 and 2 μg/ml; and *S. aureus*, 0.19 and 1.14 μg/ml.

The FK-037 MBCs were identical to or within one dilution of the MICs for *E. coli* and *S. aureus*, but MBCs were 32-fold greater than the MICs for *P. aeruginosa* (Table 3).

FK-037 was not hydrolyzed (relative rate, <0.1%) compared with cephaloridine by TEM-1, TEM-2, SHV-1, K-1, PC-1, P-99, *Morganella morganii*, *Proteus vulgaris*, and *M. catarrhalis* (Bro 1) (Table 4). This was in general similar to what we found for cefotaxime and ceftazidime with similar amounts of these purified enzymes. Cefotaxime was hydrolyzed by the *P. vulgaris* β-lactamase. FK-037 had a relative rate of hydrolysis by TEM-3, TEM-7, and TEM-9 comparable to the relative rates of hydrolysis of cefotaxime and ceftazidime. FK-037 was not tested at low concentrations with group 1 enzymes. We compared the affinities of the *E. cloacae* P99 β-lactamase for FK-037, cefepime, cefotaxime, and ceftazidime at a single concentration of 50 mM. Cefotaxime caused 99% inhibition of the hydrolysis of cephaloridine, 89% inhibition of the hydrolysis of ceftazidime, and 21% inhibition of the hydrolysis of FK-037 and cefepime.

The MICs for isolates containing characterized β-lactamases are shown in Table 5. FK-037 inhibited those isolates at ≤8 μg/ml, with the exception of a strain of *C. freundii* containing TEM-9 (MIC, 32 μg/ml). In general, FK-037 had activity against these isolates similar to those of cefepime and cefpirome. The activity of FK-037 against two permeability mutants of *E. coli* was determined. The FK-037 MIC for the parent strain, UB-1005, was 0.03 μg/ml, and that for mutants DC-1 and DC-3 was 0.015 μg/ml. For the same isolates, the MICs of cefepime and cefpirome were 0.06 and 0.03 μg/ml for UB-1005 and 0.015 μg/ml for DC-1 and DC-3. This indicates that there is a minimal barrier to entry of FK-037 similar to those for cefepime and cefpirome.

The development of progressive resistance was determined for two isolates each of *E. cloacae*, *C. freundii*, and *S. aureus*. The FK-037 MICs for the *S. aureus* isolates rose from 2 to 4 μg/ml and 2 to 8 μg/ml after 14 days. The MICs did not decrease after repeated subculture. The MICs for *E. cloacae* rose from 0.25 to 1 μg/ml to >64 μg/ml. The MICs

TABLE 3. Effect of inoculum size on MICs of FK-037

| Organism[a] | Mean MIC in μg/ml (range) at inoculum of: | |
|---|---|---|
| | $10^4$ CFU | $10^6$ CFU |
| *E. coli* | 0.06 (0.03–0.12) | 0.56 (0.5–2) |
| *K. pneumoniae* | 0.05 (0.03–0.12) | 0.32 (0.12–2) |
| *C. freundii* | 0.08 (0.03–0.12) | 0.58 (0.06–8) |
| *E. cloacae* | 0.19 (0.06–1) | 0.76 (0.25–2) |
| *P. aeruginosa* | 2 (1–4) | 10.6 (4–32) |
| *S. aureus* | 1.14 (0.5–4) | 1.15 (0.5–2) |

[a] Five isolates from each species were tested.

TABLE 4. Relative rate of hydrolysis of FK-037 and other cephems by β-lactamases

| β-Lactamase (Bush classification) | Source | Relative hydrolysis of: | | |
|---|---|---|---|---|
| | | FK-037 | Cefotaxime | Ceftazidime |
| TEM-1 (2b) | *E. coli* | <0.1 | <0.1 | <0.1 |
| TEM-2 (2b) | *E. coli* | <0.1 | <0.1 | <0.1 |
| TEM-3 (2b') | *E. coli* | 57 | 47 | 72 |
| TEM-7 (2b') | *C. freundii* | 26 | 19 | 21 |
| TEM-9 (2b') | *E. coli* | 58 | ND[a] | 59 |
| SHV-1 (2b) | *K. pneumoniae* | <0.1 | <0.1 | <0.1 |
| SHV-2 (2b') | *E. coli* | 7 | 70 | 7 |
| K-1 (2b) | *K. oxytoca* | <0.1 | <0.1 | <0.1 |
| OXA-1 (2d) | *E. coli* | <0.1 | <0.1 | <0.1 |
| OXA-3 (2d) | *E. coli* | 0.6 | <0.1 | 0.1 |
| PSE-1 (2d) | *P. aeruginosa* | 0.2 | 0.3 | <0.1 |
| PSE-2 (2d) | *P. aeruginosa* | 0.8 | 0.4 | 0.2 |
| PC-1 (2a) | *S. aureus* | <0.1 | <0.1 | <0.1 |
| Bro-1 (—) | *M. catarrhalis* | <0.1 | <0.1 | <0.1 |
| Sabath-Abraham (1) | *P. aeruginosa* | <0.1 | <0.1 | <0.1 |
| P99 (1) | *E. cloacae* | <0.1 | <0.1 | <0.1 |
| — (1) | *M. morganii* | <0.1 | 4.8 | <0.1 |
| — (2e) | *P. vulgaris* | <0.1 | 7.6 | <0.1 |

[a] ND, not determined.

for *C. freundii* rose from 0.03 and 0.06 μg/ml to 64 and 4 μg/ml, respectively. These elevated MICs remained after 10 subcultures in the absence of drug.

## DISCUSSION

Although the commercially available cephalosporins in this study inhibit many of the members of the family *Enterobacteriaceae* and streptococci at readily achievable concentrations clinically, their activity against some cephalosporinase-producing organisms is less, and they do not inhibit methicillin-resistant staphylococci. As pointed out by Sanders (8), *Enterobacter* spp. and other species with Bush (1) group 1 β-lactamases have become an increasing problem in hospitals in the United States.

FK-037 is a new synthetic cephalosporin which has aminothiazolyl and iminomethoxyl moieties on the β-acyl side. It differs from cefotaxime and ceftazidime by the presence of a 1-hydroxyethyl-5-amino-pyrazole moiety affixed to position 3 of the cephem nucleus. Cefpirome and cefepime are cephalosporins with analogous charge moieties at C-3, and they have been shown to inhibit some *Enterobacter*, *Morganella*, *Serratia*, and *C. freundii* isolates because of poor affinity for β-lactamases combined with high penicillin-binding protein affinity and excellent penetration across the wall of gram-negative bacteria.

It is probable that the pyrazole moiety provides activity against *P. aeruginosa*, as also occurs with cefpirome and cefepime, which have a bicyclic moiety at C-3, because iminotheoxy cephalosporins such as cefotaxime and ceftriaxone have minimal activity against *P. aeruginosa*.

FK-037 retains the activity of aminothiazolyl cephalosporins against hemolytic streptococci, viridans group streptococci, and organisms such as *Haemophilus*, *Neisseria*, and *Moraxella* spp. Similar to agents with a pyridine, bicylic pyrrole, or pyrazole moiety at C-3, FK-037 lacks activity against *Bacteroides* species and about 50% of *Clostridium* species. The activity of FK-037 against *S. aureus* and against some methicillin-resistant isolates is interesting and is yet to be explained but may be related to binding to penicillin-binding proteins as Yokota and Suzuki suggest (9).

The inoculum size effect and discrepancy of the MBC/MIC ratio for *P. aeruginosa* suggests that resistance will develop, and it is possible to select stably resistant isolates of *E. cloacae* and *C. freundii*.

FK-037 was the most active cephalosporin tested against some methicillin-resistant staphylococci, but whether this will be of clinical importance could only be seen by clinical trials. FK-037 was not hydrolyzed by many β-lactamases. Cefpirome, not tested in this study, also inhibits methicillin-susceptible *S. aureus* at similar concentrations. Cefpirome contains a quaternary ammonium moiety at C-3. The characteristics of FK-037 could provide protection from destruction in vivo. Further animal studies and pharmacological studies with humans are necessary to assess whether this agent can be successfully developed for clinical use.

TABLE 5. Comparative activities of FK-037 against isolates with characterized β-lactamase

| Organism | β-lactamase | MIC (μg/ml) | | | | | |
|---|---|---|---|---|---|---|---|
| | | FK-037 | Ceftazidime | Ceftriaxone | Cefotaxime | Cefepime | Cefpirome |
| *E. cloacae* | P99 | 4 | 32 | 64 | 64 | 1 | 2 |
| *P. vulgaris* | Ic | 0.25 | 0.03 | 8 | 8 | 0.06 | 0.5 |
| *E. coli* | TEM-1 | 0.03 | 0.06 | 0.03 | ≤0.015 | ≤0.015 | 0.03 |
| *E. coli* | TEM-2 | 0.06 | 0.12 | 0.03 | 0.03 | 0.12 | 0.06 |
| *K. pneumoniae* | TEM-3 | 4 | 8 | >64 | >64 | 4 | 4 |
| *K. pneumoniae* | TEM-5 | 0.25 | 8 | 1 | 0.5 | 0.25 | 0.25 |
| *C. freundii* | TEM-7 | 1 | 32 | 1 | 0.5 | 2 | 2 |
| *E. coli* | TEM-9 | 32 | >64 | >64 | >64 | 32 | 32 |
| *K. pneumoniae* | SHV-1 | 0.12 | 1 | 0.25 | 0.5 | 0.25 | 0.5 |
| *E. coli* | SHV-2 | 16 | 4 | 32 | 32 | 4 | 8 |
| *K. pneumoniae* | SHV-5 | 8 | >64 | 64 | 8 | 4 | 8 |
| *K. oxytoca* | K1 | 1 | 0.25 | 0.5 | 0.5 | 0.25 | 0.5 |
| *P. aeruginosa* | PSE-2 | 8 | 0.5 | 32 | 16 | 4 | 8 |
| *P. aeruginosa* | PSE-3 | 4 | 2 | >64 | 32 | 4 | 2 |
| *P. aeruginosa* | PSE-4 | 4 | 0.5 | >64 | >64 | 4 | 4 |
| *P. aeruginosa* | OXA-6 | 4 | 0.5 | >64 | 16 | 2 | 4 |
| *B. catarrhalis* | Bro-1 | 2 | 0.12 | 0.5 | 0.25 | 2 | 0.5 |

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