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Comparative In Vitro Activity and β-Lactamase Stability of FR 17027, a New Orally Active Cephalosporin

HAROLD C. NEU,[1,2]* NAI-XUN CHIN,[1] AND PORNPEN LABTHAVIKUL[1]

Division of Infectious Diseases, Departments of Medicine[1] and Pharmacology,[2] College of Physicians and Surgeons, Columbia University, New York, New York 10032*

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FR 17027, a new orally absorbed cephalosporin ester, inhibited group A and B streptococci and *Streptococcus pneumoniae* at ≤0.1 μg/ml, which is similar to the inhibition concentration of amoxicillin and cefaclor, and was more active than cephalexin. It was less active (MIC, 25 μg/ml) against staphylococci than was cephalexin, and it did not inhibit *Streptococcus faecalis* or *Listeria monocytogenes*. FR 17027 inhibited β-lactamase-producing isolates of *Neisseria gonorrhoeae*, *Haemophilus influenzae*, and *Branhamella catarrhalis* at <0.1 μg/ml and was more active than cefaclor or cephalexin against these bacteria. FR 17027 inhibited *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Klebsiella oxytoca*, *Providencia stuartii*, *Providencia rettgeri*, and *Citrobacter diversus* at ≤1 μg/ml, including isolates resistant to amoxicillin, cephalexin, and cefaclor, but it was less active than ceftizoxime. Some strains of *Enterobacter cloacae*, *Enterobacter agglomerans*, *Citrobacter freundii*, and *Enterobacter aerogenes* were resistant (MIC, >25 μg/ml). FR 17027 did not inhibit *Pseudomonas aeruginosa*, other *Pseudomonas* species, *Acinetobacter* species, or *Bacteroides* species. Activity was minimally affected by growth conditions. FR 17027 was not hydrolyzed by the common β-lactamases present in many of the pathogens causing respiratory and urinary tract infections in outpatients.

Although there has been great progress in the development of parenteral cephalosporins which are stable against β-lactamases and active against a wide spectrum of bacteria, this goal has not been achieved for oral cephalosporins (3, 4). Cephalexin, cephradine, and cefadroxil are only partially stable against β-lactamases, and they have relatively poor activity against *Haemophilus influenzae*. Cefaclor is not completely stable against attack by the plasmid β-lactamases, and it has been associated with a serum-sickness type of illness in some children. FR 17027 (Fig. 1) is a new cephalosporin ester which has stability against β-lactamases and antibacterial activity similar to that of the third generation cephalosporins. FR 17027 is an aminothiazolyl cephalosporin with an ethyl moiety at position three of the dihydrothiazine ring and a carboxyl group affixed to the iminomethoxy portion of the acyl side chain. In studies in our laboratory (D. Brittain, T. Hirose, B. Scully, and H. C. Neu, manuscript in preparation), we have shown that serum levels of 4 μg/ml can be achieved after a 400-mg oral dose. We wished to compare the activity of this new cephalosporin with other oral and parenteral antibiotics against a variety of bacteria for which an oral cephalosporin could be used as initial or follow-up therapy.

MATERIALS AND METHODS

FR 17027 was a gift from Fujisawa SmithKline Corp. All of the other antibiotics were obtained from their respective manufacturers. Fresh dilutions of compounds were prepared daily in either sterile medium or distilled water. The majority of the isolates came from patients seen at The Presbyterian Hospital, New York, N.Y. Some isolates were gifts sent to our laboratory because of the presence of β-lactamases, which were characterized by standard methods (2).

Antimicrobial activity was measured by an agar dilution method with Mueller-Hinton agar unless specified otherwise. A final inoculum of 10^5 CFU, prepared by dilution of a fresh overnight broth culture, was applied to agar with a replicating spot device. Broth dilutions were performed with a final inoculum of 10^5 CFU in tubes with a 1-ml volume. Plates or tubes were incubated at 35°C for 18 h. The MIC was defined as the lowest concentration of antibiotic that inhibited development of visible growth on agar or in broth. MBC was determined by subculture of 0.1 ml of broth in tubes without visible growth and was defined as the concentration which produced ≥99.9% reduction in CFU after 24 h of incubation at 35°C. The susceptibility of streptococci was determined by using Mueller-Hinton agar supplemented with 5% human blood. The susceptibility of *Neisseria* and *Haemophilus* species was determined on lysed human blood–Mueller-Hinton agar in the presence of 5% CO_2. Anaerobic bacterial susceptibility was determined by using brucella agar supplemented with sheep blood and vitamin K. Incubation of anaerobic bacterial cultures was for 48 h in GasPak jars (BBL Microbiology Systems, Cockeysville, Md.).

Susceptibility tests to the various drugs were performed with permeability mutants provided by Clark (1) and Richmond et al. (5). Strains DC2, DC3, and DC13 do not have a barrier to the entry of β-lactam antibiotics.

The bactericidal activity of FR 17027 was determined by incubating clinical isolates of *Escherichia coli*, which contained the TEM-1 β-lactamase, and *Klebsiella pneumoniae*, which contained an SHV-1 β-lactamase. β-Lactamases have been identified by isoelectric focusing (2). Growth was followed by the change in optical density. Sidearm flasks were used with incubation at 37°C in a gyratory shaker.

The presence of β-lactamase in isolates was determined by the nitrocefin assay. β-Lactamases used for the analysis of the stability of the compounds were either purified enzymes or partially purified enzymes as previously described (2). Stability to β-lactamase was determined by a spectrophotometric assay by using the change in absorbance at the absorption maximum of each substrate. Inhibition assays, with nitrocefin as the substrate, were performed with 10^{-4} M

* Corresponding author.

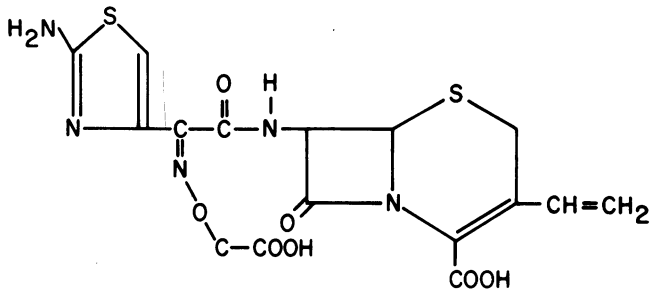

FIG. 1. Structure of FR 17027 [(6*R*, 7*R*)-7-[(*Z*)-2-(2-amino-4-thiazolyl)-2-(carboxymethoxyamino) acetamido]-8-oxo-3 vinyl-5-thia-1-azabicyclo-[4,2,0]-oct-2-ene-2-carboxylic acid].

nitrocefin or cephaloridine in a final volume of 3 ml. Enzyme and FR 17027 at 10^{-4} or 10^{-5} M were incubated at 30°C for 10 min, and then nitrocefin or cephaloridine was added. Change in absorbance at 482 nm for nitrocefin and at 265 nm for cephaloridine was followed over 10 min with a temperature-controlled recording spectrophotometer. As a control, the change in the absorbance of nitrocefin plus enzyme or of cephaloridine plus enzyme was followed.

Destruction of FR 17027 was determined by incubation of FR 17027 with 10^5 organisms in Mueller-Hinton broth for 24 h. Bacteria were removed by centrifugation and filtration with Millipore filters. The filtrate was assayed to determine the concentration of active drug remaining in the solution. An agar plate assay with an *E. coli* specimen from our collection was used to assay the drug concentrations by regression line analysis with five standards. A sample of FR 17027 was incubated in the same medium and processed similarly to be used as a control of nonenzymatic decay of the compound.

RESULTS

The activity of FR 17027 against gram-positive bacteria is shown in Table 1. Unlike cephalexin and cefaclor, FR 17027 had poor activity against *Staphylococcus aureus* and *Staphylococcus epidermidis*, with 90% MICs of 25 and 50 μg/ml, respectively. However, FR 17027 inhibited important streptococcal species, with the exception of *Streptococcus faecalis*, *Streptococcus bovis*, and viridans group streptococci at concentrations of ≤0.01 to 0.2, and was as active or more active than cephalexin and cefaclor but less active than ceftizoxime. FR 17027 did not inhibit *Listeria monocytogenes* or *Corynebacterium* JK group organisms.

FR 17027 inhibited *Branhamella catarrhalis*, *H. influenzae*, *Neisseria meningitidis*, and *Neisseria gonorrhoeae* at concentrations below 0.1 μg/ml and had activity comparable to ceftizoxime (Table 2). It inhibited organisms such as *E. coli*, *K. pneumoniae*, *Citrobacter diversus*, *Klebsiella oxytoca*, *Proteus mirabilis*, *Providencia stuartii*, *Providencia rettgeri*, *Salmonella* sp., *Shigella* sp., and *Aeromonas hydrophila* at concentrations of <1 μg/ml. In general, FR 17027 was less active than ceftizoxime but more active than cephalexin, cefaclor, or amoxicillin. Most of the isolates of the species mentioned above were inhibited by the combination of trimethoprim-sulfamethoxazole. FR 17027 was more active than cephalexin and cefaclor against *Citrobacter freundii*, *Enterobacter aerogenes*, *Enterobacter cloacae*, *Serratia marcescens*, *Morganella morganii*, *Yersinia* sp., and *Proteus vulgaris* with 50% MICs of ≤0.01 to 3.1 μg/ml compared with 50% MICs of >25 μg/ml for the other orally active cephalosporins. But FR 17027 did not inhibit some *C. freundii*, *Enterobacter agglomerans*, and *E. cloacae* (MIC, >100 μg/ml). In general, FR 17027 was less active than ceftizoxime against *Enterobacter* sp., *C. freundii*, and *S. marcescens*. FR 17027 did not inhibit *Acinetobacter* sp. or *Pseudomonas* sp. The activity of FR 17027 against anaerobic bacteria is shown in Table 3. It did not inhibit *Bacteroides* sp. or most clostridia, with the exception of *Clostridium perfringens*, at concentrations readily achieved with doses of 50 to 400 mg. Furthermore, MICs against the few anaerobic cocci were also high.

Overall, FR 17027 inhibited 73% of amoxicillin-resistant (MIC, ≥25 μg/ml) members of the family *Enterobacteriaceae*, *H. influenzae*, and *B. catarrhalis* at ≤0.1 μg/ml. Only *C. freundii*, *E. cloacae*, and *S. marcescens* required higher concentrations of FR 17027 for inhibition, and 50% of these isolates were inhibited by 6.3 μg/ml. Similarly, FR 17027 inhibited 71% of cephalexin-resistant *Enterobacteriaceae* and *H. influenzae* at ≤0.1 μg/ml% and 60% of cefaclor-resistant *Enterobacteriaceae* at <0.1 μg/ml%.

The effect of various assay conditions upon the activity of FR 17027 was examined. The type of medium used to determine MICs (Mueller-Hinton, brain heart infusion, tryptic digest, nutrient, Columbia) did not yield more than a twofold difference in activity of five strains of *Staphylococcus aureus*, *Escherichia coli*, *K. pneumoniae*, *M. morganii*, *C. freundii*, *Proteus mirabilis*, *Enterobacter cloacae*, and *Pseudomonas aeruginosa*. Similarly, the MICs and MBCs were not changed more than twofold when the pH of the Mueller-Hinton medium varied from 6 to 8. Serum and urine did not alter the activity of FR 17027 (Table 4). *P. aeruginosa* and *S. marcescens* isolates resistant to FR 17027 were not inhibited in urine at concentrations of 100 μg/ml.

Increase of the inoculum size from 10^5 to 10^7 CFUs caused an increase in MICs for all of the organisms tested. However, at 10^5 CFUs there was no discrepancy between the MICs and MBCs (Table 5). The isolates are representative of the five isolates tested for each species. At 10^7 CFUs, there was an increase in MBCs for *Escherichia coli*, *K. pneumoniae*, *Proteus mirabilis*, and *S. marcescens*.

The activity of FR 17027, cephalexin, cefaclor, and amoxicillin was tested against *E. coli* isolates which were shown to contain TEM-1 and TEM-2 β-lactamases, as determined by isoelectric focusing. Only FR 17027 had an MIC of <0.4 μg/ml, whereas the other agents had MICs of ≥25 μg/ml.

The β-lactamase stability of FR 17027, cephalexin, cephradine, and cefaclor is shown in Table 6. FR 17027 was the most stable compound, and cefaclor was the least stable. Cefaclor was hydrolyzed by plasmid β-lactamases of the TEM type isolated from *H. influenzae* and was very readily hydrolyzed by a *B. catarrhalis* β-lactamase. Cephalexin and cephradine were hydrolyzed by the *B. catarrhalis* β-lactamase, but at a 10-fold lower rate than was cefaclor.

There was a good correlation between MICs and the destruction of FR 17027 when intact bacteria were incubated with FR 17027 (Table 7). However, *Pseudomonas aeruginosa*, which did not destroy FR 17027, was resistant, and *Pseudomonas cepacia*, which was not resistant, destroyed FR 17027. The stability of FR 17027 was also demonstrated by the lack of growth of *E. coli* containing the TEM-1 and TEM-2 enzymes and of *K. pneumoniae* containing the SHV-1 enzyme when incubated in the presence of 5 μg of FR 17027 per ml.

Inhibition studies of the hydrolysis of cephaloridine with three β-lactamases were performed as outlined above. With the P99 type Ia enzymes, FR 17027 at 10^{-4} M produced a 95% inhibition of the β-lactamase activity of *Enterobacter cloacae*. However, FR 17027 failed to inhibit the activity of

TABLE 1. Comparative activity of FR 17027 against gram-positive bacteria

Organism (no. of isolates tested)	Antibiotic	MIC (μg/ml)		
		Range	50%	90%
Staphylococcus aureus (22)	FR 17027	3.1–50	12.5	25
	Ceftizoxime	0.4–12.5	1.6	3.1
	Cephalexin	0.8–12.5	3.1	12.5
	Cefaclor	0.8–12.5	6.3	6.3
	Amoxicillin	0.1–>100	12.5	>100
	Cloxacillin	0.1–1.6	0.4	1.6
Staphylococcus aureus (methicillin-resistant) (12)	FR 17027	12.5–>100	50	>100
	Vancomycin	0.2–1.6	0.8	1.6
Staphylococcus epidermidis (17)	FR 17027	3.1–>100	12.5	50
	Ceftizoxime	0.4–>100	0.4	25
	Cephalexin	0.1–>100	1.6	12.5
	Cefaclor	0.1–>100	0.8	12.5
Streptococcus pyogenes (24)	FR 17027	≤0.01–0.2	0.05	0.2
	Ceftizoxime	<0.01	≤0.01	0.01
	Cephalexin	0.2–0.8	0.2	0.8
	Cefaclor	≤0.01–0.1	0.05	0.1
	Amoxicillin	≤0.01–0.1	≤0.01	0.01
Streptococcus agalactiae (17)	FR 17027	0.02–0.2	0.1	0.2
	Ceftizoxime	0.02–0.2	0.05	0.2
	Cephalexin	1.6–6.3	1.6	3.1
	Cefaclor	0.2–0.8	0.4	0.8
	Amoxicillin	≤0.01–0.5	≤0.01	0.02
Streptococcus bovis (13)	FR 17027	1.6–6.3	1.6	6.3
	Ceftizoxime	0.1–6.3	0.2	6.3
	Cephalexin	1.6–>100	1.6	100
	Cefaclor	0.2–25	0.2	25
	Amoxicillin	<0.1–0.4	<0.1	0.4
Streptococcus faecalis (15)	FR 17027	>100	>100	>100
	Amoxicillin	0.1–0.4	0.2	0.4
Streptococcus pneumoniae (15)	FR 17027	<0.01–0.4	0.05	0.2
	Ceftizoxime	0.01–0.4	0.05	0.2
	Cephalexin	0.8–6.3	0.8	3.1
	Cefaclor	0.2–3.1	0.2	3.1
	Amoxicillin	<0.01–0.4	0.05	0.1
Viridans group streptococci (19)	FR 17027	≤0.01–>100	0.4	12.5
	Ceftizoxime	≤0.01–>100	0.1	0.8
	Cephalexin	0.1–>100	1.6	25
	Cefaclor	0.1–>100	0.4	6.3
	Amoxicillin	≤0.01–0.4	≤0.01	0.2
Streptococcus group C (8)	FR 17027	≤0.01–0.05		
Streptococcus group F (8)	FR 17027	0.4–3.1		
Streptococcus group G (5)	FR 17027	0.02–1.6		
Listeria monocytogenes (7)	FR 17027	3.1–>100		
	Ceftizoxime	3.1–>100		
	Cephalexin	3.1–>100		
	Cefaclor	3.1–>100		
	Amoxicillin	0.1–0.2		
Corynebacterium JK species (9)	FR 17027	>100		
	Ceftizoxime	>100		
	Cephalexin	>100		
	Cefaclor	>100		
	Amoxicillin	>100		

TABLE 2. Comparative activity of FR 17027 against gram-negative bacteria

Organism (no. of isolates tested)	Antibiotic	MIC (μg/ml)		
		Range	50%	90%
Acinetobacter antitratus (18)	FR 17027	0.8–>100	50	50
	Ceftizoxime	25–>100	50	100
	Cephalexin	>100	>100	>100
	Cefaclor	>100	>100	>100
	Amoxicillin	>100	>100	>100
Acinetobacter lwoffii (10)	FR 17027	0.8–3.1	0.8	3.1
	Ceftizoxime	0.05–3.1	0.1	0.8
	Cephalexin	>100	>100	>100
	Cefaclor	>100	>100	>100
Achromobacter xylosoxidans (2)	FR 17027	25,50		
Aeromonas hydrophila (9)	FR 17027	≤0.01–0.8		
	Ceftizoxime	≤0.01–0.1		
	Cephalexin	>100		
	Cefaclor	>100		
	Amoxicillin	>100		
Bordetella bronchiseptica (2)	FR 17027	>100		
	Ceftizoxime	>100		
	Cephalexin	>100		
	Cefaclor	50		
	Amoxicillin	100		
Branhamella catarrhalis (13)	FR 17027	≤0.01–0.05	≤0.01	0.05
	Ceftizoxime	≤0.01–0.05	≤0.01	0.05
	Cephalexin	3.1–6.3	3.1	6.3
	Cefaclor	0.02–3.1	0.4	1.6
	Amoxicillin	12.5–>100	>100	>100
Campylobacter jejuni (10)	FR 17027	0.4–1.6	1.6	1.6
	Cephalexin	>25	>25	>25
	Cefaclor	>25	>25	>25
	Amoxicillin	>25	>25	>25
Citrobacter diversus (13)	FR 17027	0.05–3.1	0.1	0.4
	Ceftizoxime	≤0.01–0.1	0.1	0.05
	Cephalexin	3.1–>100	3.1	50
	Cefaclor	0.4–12.5	0.8	12.5
	Amoxicillin	12.5–>100	>100	>100
	TMP/SMX[a]	0.16–0.3	0.16	0.31
Citrobacter freundii (19)	FR 17027	0.025–100	1.6	>100
	Ceftizoxime	≤0.01–50	0.2	25
	Cephalexin	>100	>100	>100
	Cefaclor	0.1–>100	50	>100
	Amoxicillin	6.3–>100	25	>100
Enterobacter aerogenes (21)	FR 17027	0.4–>100	0.4	12.5
	Ceftizoxime	≤0.01–50	0.05	0.8
	Cephalexin	>100	>100	>100
	Cefaclor	>100	>100	>100
	Amoxicillin	>100	>100	>100
Enterobacter agglomerans (7)	FR 17027	0.05–>100		
	Cetizoxime	≤0.01–>100		
	Cephalexin	6.3–>100		
	Cefaclor	6.3–>100		
	Amoxicillin	6.3–>100		
Enterobacter cloacae (30)	FR 17027	0.05–>100	0.1	>100
	Ceftizoxime	≤0.01–>100	0.4	12.5
	Cephalexin	>100	>100	>100
	Cefaclor	>100	>100	>100
	Amoxicillin	>100	>100	>100
	TMP/SMX	0.16–5	0.16	2.5
Enterobacter hafniae (4)	FR 17027	0.05–0.2		
	Ceftizoxime	0.05–0.2		
	Cephalexin	>100		
	Cefaclor	>100		
	Amoxicillin	50–100		
	TMP/SMX	0.16–5		
Escherichia coli (32)	FR 17027	≤0.01–3.1	0.1	1.6
	Ceftizoxime	≤0.01–3.1	0.02	0.2
	Cephalexin	6.3–>100	6.3	50
	Cefaclor	3.1–>100	6.3	50
	Amoxicillin	1.6–>100	>100	>100
Flavobacterium meningosepticum (1)	FR 17027	>100		
Haemophilus influenzae (15)	FR 17027	0.1–0.2	0.1	0.2
	Ceftizoxime	≤0.01–0.1	≤0.05	0.1
	Cephalexin	0.8–25	6.3	12.5
	Cefaclor	0.4–12.5	1.6	6.3
	Amoxicillin	0.1–>100	>100	>100
	TMP/SMX	0.16–0.31	0.16	0.31
Haemophilus parainfluenzae (2)	FR 17027	<0.1		
	Ceftizoxime	<0.1		
Klebsiella oxytoca (15)	FR 17027	0.02–0.4	0.1	0.2
	Ceftizoxime	≤0.01–0.01	≤0.01	0.01
	Cephalexin	3.1–100	3.1	100
	Cefaclor	0.1–100	0.4	100
	Amoxicillin	>100	>100	>100
Klebsiella ozaenae (2)	FR 17027	0.01–0.8		
Klebsiella pneumoniae (30)	FR 17027	≤0.01–0.8	0.05	0.2
	Ceftizoxime	≤0.01–0.2	≤0.01	0.05
	Cephalexin	3.1–>100	3.1	12.5
	Cefaclor	3.1–>100	3.1	12.5
	Amoxicillin	1.6–>100	>100	>100
	TMP/SMX	0.16–0.31	0.16	0.31
Klebsiella rhinoscleromatis (1)	FR 17027	≤0.01		
Morganella morganii (19)	FR 17027	≤0.01–25	1.6	25
	Ceftizoxime	≤0.01–12.5	1.6	6.3
	Cephalexin	100–>100	>100	>100
	Cefaclor	6.3–>100	>100	>100
	Amoxicillin	>100	>100	>100
	TMP/SMX	0.16–1.25	0.16	1.25
Neisseria meningitidis (8)	FR 17027	≤0.01–0.05		

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TABLE 2.—*Continued*

Organism (no. of isolates tested)	Antibiotic	MIC (μg/ml)		
		Range	50%	90%
Neisseria lactamica (3)	FR 17027	<0.1–0.05		
Neisseria gonorrhoeae (15)	FR 17027	≤0.01–0.1	0.02	0.05
	Ceftizoxime	≤0.01–0.1	0.02	0.05
	Cephalexin	0.8–12.5	6.3	12.5
	Cefaclor	0.2–12.5	1.6	3.1
Proteus mirabilis (29)	FR 17027	≤0.01–0.05	<0.01	0.01
	Ceftizoxime	≤0.01–0.002	<0.01	0.01
	Cephalexin	12.5–>100	12.5	50
	Cefaclor	0.8–>100	1.6	50
	Amoxicillin	0.8–>100	1.6	100
	TMP/SMX	0.31–5	0.31	1.25
Proteus vulgaris (16)	FR 17027	≤0.01–6.3	≤0.01	1.6
	Ceftizoxime	≤0.01–6.3	≤0.01	0.8
	Cephalexin	12.5–>100	100	>100
	Cefaclor	3.1–>100	25	>100
	Amoxicillin	12.5–>100	100	>100
Providencia rettgeri (11)	FR 17027	≤0.01–12.5	≤0.01	0.8
	Ceftizoxime	≤0.01–12.5	≤0.01	0.2
	Cephalexin	100–>100	>100	>100
	Cefaclor	100–>100	>100	>100
	Amoxicillin	12.5–>100	100	>100
Providencia stuartii (20)	FR 17027	<0.02–0.8	<0.02	0.4
	Ceftizoxime	<0.02–0.1	<0.02	0.05
	Cephalexin	25–>100	100	>100
	Cefaclor	25–>100	100	>100
	Amoxicillin	12.5–>100	100	>100
	TMP/SMX	0.31–1.25	0.31	1.25
Pseudomonas aeruginosa (30)	FR 17027	0.8–>10	50	>100
	Ceftizoxime	0.2–>100	50	>100
Pseudomonas cepacia (15)	FR 17027	0.04–>100	6.3	>100
	Ceftizoxime	0.4–>100	1.6	>100
	Cephalexin	>100	>100	>100
	Cefaclor	6.3–100	6.3	>100
Pseudomonas maltophilia (9)	FR 17027	100–>100		
	Ceftizoxime	50–>100		
	Cephalexin	>100		
	Cefaclor	>100		
Pseudomonas, other[b] (8)	FR 17027	12.5–>100		
	Ceftizoxime	3.1–>100		
	Cefaclor	>100		
Salmonella sp. (29)	FR 17027	0.02–0.4	0.05	0.2
	Ceftizoxime	0.02–0.8	0.02	0.1
	Cephalexin	6.3–25	6.3	25
	Cefaclor	1.6–25	6.3	25
Salmonella typhi (5)	FR 17027	0.02–0.4		
	Amoxicillin	0.8–>100		
Serratia liquefaciens (4)	FR 17027	0.05–0.2		
	Ceftizoxime	0.05–0.2		
	Cephalexin	>100		
	Cefaclor	>100		
	Amoxicillin	>100		

TABLE 2.—*Continued*

Organism (no. of isolates tested)	Antibiotic	MIC (μg/ml)		
		Range	50%	90%
Serratia marcescens (31)	FR 17027	0.02–>100	1.6	100
	Ceftizoxime	≤0.1–>100	0.8	25
	Cephalexin	>100	>100	>100
	Cefaclor	>100	>100	>100
	Amoxicillin	>100	>100	>100
	TMP/SMX	0.16–>5	0.63	5
Shigella sp. (29)	FR 17027	0.1–0.1	0.2	0.4
	Ceftizoxime	≤0.01–0.05	0.02	0.05
	Cephalexin	3.1–12.5	6.3	12.5
	Cefaclor	1.6–25	3.1	12.5
Yersinia enterocolitica (10)	FR 17027	0.02–6.3	0.8	3.1
	Ceftizoxime	≤0.01–1.6	0.05	0.8
	Cephalexin	3.1–>100	12.5	>100
	Cefaclor	0.4–>100	25	>100

[a] TMP/SMX, Trimethoprim/sulfamethoxazole present at 1:20 ratio; hence, 0.16 μg/ml equals 0.16 μg of trimethoprim plus 3.1 μg of sulfamethoxazole per ml.

[b] Includes *Pseudomonas fluorescens*, *Pseudomonas putida*, *Pseudomonas stutzeri*, and *Pseudomonas alcaligenes*.

the *Escherichia coli* TEM β-lactamase and produced only a 16.5% inhibition of the *Proteus vulgaris* type Ic β-lactamase.

The activity of FR 17027 against permeability mutants of *E. coli* was determined. FR 17027 was excluded from *E. coli* since the MICs and MBCs were two- to eightfold lower for the permeability mutants, whereas the MICs for cephalexin and cefaclor were only twofold lower or identical for the parent strain and the mutants. However, FR 17027 was considerably more active than the other agents against permeable strains, with the MIC of FR 17027 for *E. coli* DC2 being 0.1 μg/ml, as compared with the MIC of cephalexin of 6.3 μg/ml and the MIC of cefaclor of 1.6 μg/ml.

DISCUSSION

Marked progress in the development of cephalosporin antibiotics has occurred in the past decade (3, 4). Those cephalosporins which contain an aminothiazolyl group on the acyl side chain have extremely good activity against members of the family *Enterobacteriaceae*, *H. influenzae*, and streptococci, including *Streptococcus pneumoniae* (4). There have not been any orally absorbed cephalosporins which possess excellent β-lactamase stability against plasmid and chromosomal β-lactamases. FR 17027 is orally absorbed, producing serum levels of 4 μg/ml and urine levels in excess of 100 μg/ml after ingestion of a 400-mg dose (Brittain et al., manuscript in preparation). FR 17027 had excellent activity against most β-hemolytic streptococci and *S. pneumoniae*, but it did not inhibit staphylococcal species at achievable concentrations (MICs, ≥25 μg/ml). FR 17027 did not inhibit most anaerobic species or *Pseudomonas aeruginosa*. FR 17027 inhibited *H. influenzae*, *N. gonorrhoeae*, *Neisseria meningitidis*, and *B. catarrhalis*. FR 17027 inhibited *E. coli*, *K. pneumoniae*, and many other *Enterobacteriaceae* at concentrations of <1 μg/ml, including isolates resistant to cephalexin, cefaclor, and amoxicillin. FR 17027 was not as active as ceftizoxime, which was used in this study as a prototype of the aminothiazolyl iminomethoxy cephalosporins, but was more active than cephalexin and cefaclor, the two most widely used oral cephalosporins. Furthermore, FR 17027 was not hydrolyzed by the β-lactamases that hydrolyze cefaclor.

TABLE 3. Activity of FR 17027 against anaerobic bacteria

Organism (no. of isolates tested)	Antibiotic	MIC (μg/ml)		
		Range	50%	90%
Bacteroides fragilis (17)	FR 17027	6.3->100	12.5	>100
	Ceftizoxime	1.6->100	1.6	100
	Cephalexin	>100		
	Cefaclor	>100		
	Clindamycin	0.1–1.6	0.1	1.6
Bacteroides sp. (17)[a]	FR 17027	6.3->100	12.5	>100
	Ceftizoxime	1.6–100	1.6	100
	Clindamycin	0.1–1.6	0.1	1.6
	Metronidazole	3.1–25	0.2	0.4
Clostridium botulinum type E (1)	FR 17027	>100		
Clostridium difficile (2)	FR 17027	100		
Clostridium novyi (1)	FR 17027	>100		
Clostridium perfringens (4)	FR 17027	1.6		
	Ceftizoxime	0.02–0.2		
Clostridium septicum (1)	FR 17027	100		
	Ceftizoxime	12.5		
Clostridium subterminale (1)	FR 17027	>100		
Eubacterium lentum (1)	FR 17027	>100		
Peptococcus asacchrolyticus (1)	FR 17027	12.5		
Peptococcus sp. (1)	FR 17027	12.5		
Peptococcus magnus (1)	FR 17027	12.5		

[a] *Bacteroides melaninogenicus*, *Bacteroides ovatus*, *Bacteroides distasonis*, *Bacteroides disiens*, *Bacteroides bivius*.

TABLE 4. Activity of FR 17027 in normal human serum and urine

Organism	Concn (μg/ml) in:					
	Serum[a]		Urine[b]		Mueller-Hinton broth	
	MIC	MBC	MIC	MBC	MIC	MBC
Enterobacter cloacae	0.025	0.025	0.1	0.2	0.05	0.1
Escherichia coli	0.025	1.6	0.2	0.4	0.2	0.2
Klebsiella pneumoniae	0.025	0.025	0.2	0.2	0.2	0.4
Morganella morganii	0.025	0.025	0.2	3.1	0.05	0.2
Proteus mirabilis	0.025	0.025	>0.05	0.8	>0.05	0.05
Pseudomonas aeruginosa	>100	>100	100	>100	100	>100
Serratia marcescens	>100	>100	100	>100	100	100

[a] Normal human serum which was not heat inactivated.
[b] pH 5.6; pooled from four normal male volunteers and sterilized by filtration with Millipore filters; inoculum size, 10^5 CFU.

TABLE 5. Effect of inoculum size on MICs and MBCs of FR 17027 against β-lactamase-producing bacteria

Organism	Concn (μg/ml) with the following no. of CFU:					
	10^3		10^5		10^7	
	MIC	MBC	MIC	MBC	MIC	MBC
Enterobacter cloacae	<0.05	<0.05	<0.05	<0.05	3.1	3.1
Escherichia coli	0.2	0.2	0.2	0.2	3.1	12.5
Klebsiella pneumoniae	<0.05	<0.05	<0.05	<0.05	3.1	100
Morganella morganii	<0.05	<0.05	<0.05	0.2	25	25
Proteus mirabilis	<0.05	0.1	0.2	0.4	3.1	100
Pseudomonas aeruginosa	50	100	100	>100	>400	>400
Serratia marcescens	<0.05	<0.05	0.1	0.1	25	>100

TABLE 6. Stability of FR 17027 to hydrolysis by β-lactamases

Enzyme	Richmond Sykes type	DNA type[a]	Source	Relative rate of hydrolysis for the following antibiotics[b]:			
				FR 17027	Cefaclor	Cephalexin	Cephradine
TEM-1	III	P	*Haemophilus influenzae*	0	10	2	0
TEM-2	III	P	*Escherichia coli*	0	27	0	0
OXA-2	V	P	*Escherichia coli*	0	104	0	0
OXA-3	V	P	*Escherichia coli*	0	126	0	0
PSE-1	V	P	*Pseudomonas aeruginosa*	0	26	0	2
PSE-4	V	P	*Pseudomonas aeruginosa*	0	23	0	0
		P	*Staphylococcus aureus*	0	35	20	26
SHV-1	III	P	*Klebsiella* sp.	0	12	0	0
P99	Ia	C	*Enterobacter* sp.	0	46	31	13
		C	*Branhamella* sp.	0	225	23	36
K-1	IV	C	*Klebsiella* sp.	0	55	36	20

[a] P, Plasmid; C, chromosome.
[b] Based on a rate of 100 for cephaloridine.

TABLE 7. Inhibitory concentrations against bacteria containing various β-lactamases

Organism	Enzyme or classification[a]	MIC (μg/ml)	% FR 17027 destroyed in 24 h
Acinetobacter calcoaceticus		25	95
Bacteroides fragilis		>100	95
Citrobacter freundii	Ia	25	95
Enterobacter cloacae[b]	Ia	>100	95
Escherichia coli	IIIa	0.4	<5
Escherichia coli	IIIa	0.2	<5
Klebsiella pneumoniae	SHV-1	0.1	<5
Klebsiella pneumoniae	IV	0.8	<5
Pseudomonas aeruginosa	Id	>100	0
Pseudomonas cepacia	I	0.8	95

[a] Based on Richmond-Sykes classification.
[b] Constitutive production of β-lactamase.

Furthermore, FR 17027 was not hydrolyzed by the β-lactamases that hydrolyze cefaclor.

These observations indicate that FR 17027 may prove to be a useful agent for treating selected upper respiratory tract infections in children and adults, and it may also be effective in some urinary tract infections. Current studies in our laboratory indicate that concentrations well above MBCs for most of the common organisms producing respiratory and urinary infections can be obtained with 200- and 400-mg doses.

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