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Cefmenoxime (SCE-1365), a Novel Broad-Spectrum Cephalosporin: In Vitro and In Vivo Antibacterial Activities

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The activity of cefmenoxime (SCE-1365), 7β-[2-(2-aminothiazol-4-yl)-(*Z*)-2-methoxyiminoacetamido]-3-[(1-methyl-1H-tetrazol-5-yl)thiomethyl]ceph-3-em-4-carboxylic acid, was compared with that of other cephalosporins. Cefmenoxime exhibited high activity against a wide variety of gram-positive and gram-negative bacteria. The in vitro activity of cefmenoxime against *Streptococcus pyogenes*, *Haemophilus influenzae*, and *Enterobacteriaceae*, including indole-positive *Proteus*, *Serratia marcescens*, *Enterobacter cloacae*, and *Citrobacter freundii*, was 10 to 1,000 times greater than that of several other cephalosporins. Against *Pseudomonas aeruginosa*, cefmenoxime showed activity two to four times that of sulbenicillin and carbenicillin but less than that of cefsulodin. Variation in pH, addition of horse serum, and type of growth medium had definite effects on the activity of cefmenoxime, and the inoculum size affected the activity against bacterial species. In *Escherichia coli* cefmenoxime showed marked affinity for penicillin-binding protein 3 (PBP-3), followed by PBP-1 (1A and 1B). This affinity profile was well correlated with its filamentous cell-forming activity under extremely low drug concentrations and with its bactericidal activity against microorganisms. The high in vitro activity of cefmenoxime was reflected in the degree of protection observed in mice infected intraperitoneally with a wide variety of gram-positive and gram-negative bacteria. Furthermore, cefmenoxime showed good therapeutic activity against infection models in mice such as respiratory tract infection caused by *Klebsiella pneumoniae* and urinary tract infection caused by *Proteus mirabilis*.

In recent years, with the increasing use of cephalosporins, an increasing number of infections caused by cephalosporin-resistant bacteria have been observed. The present report concerns the activity of cefmenoxime (SCE-1365), 7β-[2-(2-aminothiazol-4-yl)-(*Z*)-2-methoxyiminoacetamido]-3-[(1-methyl-1H-tetrazol-5-yl)-thiomethyl]ceph-3-em-4-carboxylic acid (Fig. 1) (10).

Cefotiam, 7β-[2-(2-aminothiazol-4-yl)acetamido]-3-[[1-(2-dimethylaminoethyl)-1H-tetrazol-5-yl]thiomethyl]-ceph-3-em-4-carboxylic acid (18), is a cephalosporin with potent antibacterial activity, originally discovered and developed by Takeda Chemical Industries, Ltd., Osaka, Japan. It appears that the high antibacterial activity of cefotiam depends mainly on the aminothiazole acyl side chain at the 7-position of the cephalosporin (9). Introduction of various substituents into the α-carbon of the acyl side chain at the 7-position of the cephalosporin resulted in cephalosporins with various properties (1, 14, 20). Various other chemical modifications have also been made in the 3-position of the cephalosporins (M. Ochiai, A. Morimoto, T. Okada, Y. Matsusita, O. Aki, M. Kida, and K. Okonogi, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 18th, Atlanta, Ga., Abstr. no. 150, 1978). Consequently, cefmenoxime was selected from these cephalosporins for further study.

MATERIALS AND METHODS

Antibiotics. Cefmenoxime, cefotaxime, deacetylcefotaxime, cefuroxime, cefoxitin, cefmetazole, cefotiam, and cefsulodin were prepared in Takeda Chemical Industries, Ltd., Osaka, Japan. Cefazolin (Fujisawa Pharmaceutical Co., Ltd.), sulbenicillin (Takeda Chemical Industries, Ltd.), and carbenicillin (Fujisawa Pharmaceutical Co., Ltd.) were obtained from commercial sources. The sodium salt of these antibiotics was used in this study.

Organisms. Laboratory strains were maintained on Trypticase soy agar (TSA; BBL Microbiology Systems), TSA supplemented with 10% bovine blood (blood-TSA), or chocolate agar (beef extract agar supplemented with 10% horse blood). Clinical isolates of bacteria were kindly supplied from several clinical laboratories. Strains of *Streptococcus pyogenes* and *Haemophilus influenzae* were maintained on blood-TSA and chocolate agar, respectively, and other clin-

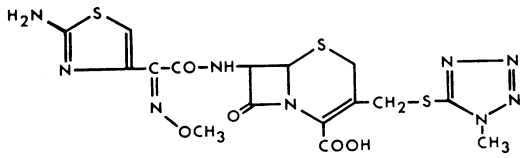

FIG. 1. *Chemical structure of cefmenoxime.*

ical isolates were maintained on Dorset egg medium (Nissui). Strains of *Escherichia coli* and *Klebsiella pneumoniae*, not inhibited by cefazolin at a concentration of 50 μg/ml, and strains of *Serratia marcescens*, not inhibited by gentamicin at a concentration of 25 μg/ml, were classified arbitrarily as resistant strains when the inoculum was 10^8 colony-forming units (CFU)/ml.

Determination of the minimal inhibitory concentration (MIC). Organisms were transferred to slants of TSA, blood-TSA, chocolate agar, or Trypticase soy broth (TSB; BBL) and cultivated at 37°C overnight. Bacterial suspensions were prepared in a concentration of about 10^6 or 10^8 CFU/ml. In the agar dilution method, one loopful (2 mm in diameter) of bacterial suspension was streaked for a length of about 2 cm on TSA, blood-TSA, MacConkey agar (Eiken), or chocolate agar containing twofold serial dilutions of each cephalosporin. With the broth dilution method, 0.1 ml of a suspension containing about 10^7 CFU/ml was inoculated into tubes containing 5 ml of TSB containing twofold serial dilutions of each cephalosporin. The MIC was defined as the lowest concentration of cephalosporin that prevented visible growth of the organism after overnight incubation at 37°C.

Determination of minimal bactericidal concentration (MBC). After the MIC was determined by the broth dilution method, a volume of 2 μl (by calibrated loop) from each tube was subcultured onto an antibiotic-free TSA plate. The MBC was defined as the lowest concentration of antibiotic which prevented visual growth of the organism on subculture after overnight incubation at 37°C.

Bactericidal activity. All experiments to determine the killing kinetics of cephalosporins were performed in TSB containing twofold serial dilutions of antibiotic with incubation at 37°C. Overnight cultures in TSB were diluted to a concentration of about 10^6 CFU/ml. Portions were removed at various time intervals, and the number of CFU was determined by the plate count method. Cephalosporin-free control cultures were inoculated and treated similarly to the cephalosporin-containing test cultures.

Bacteriolytic activity. Growth of the cultures was followed by measuring the absorbance in an automatic continuous recording photometer (Bio-Log II, Jasco Juan-Quentin S.A.), using a 3-cm path-length cell. Cells were seeded with an overnight culture in TSB to give a concentration of about 10^6 CFU/ml. Twofold serial dilutions of each cephalosporin were added to actively growing cultures in TSB.

Development of resistance in vitro. To generate resistance, the bacteria were grown in TSB in the presence of twofold serial dilutions of each cephalosporin. From the tube containing the highest concentration of the cephalosporin that allowed normal or nearly normal growth, successive transfers were made every 48 h into other series of tubes containing the same or higher concentrations.

Morphological test. Overnight cultures grown in TSB at 37°C were diluted 20 times with TSB and cultivated with shaking at 37°C for 1.5 h. T tubes containing 9 ml of the culture and 1 ml of various concentrations of cefmenoxime were incubated with shaking at 37°C for 4 h, and a loopful of bacterial suspension from each tube was smeared on a glass slide. Drying and fixation were performed according to the conventional method, and the cells were stained with methylene blue. About 100 to 200 cells were observed, and the morphological responses were distinguished as follows: cells having a similar size and a degree of staining comparable to those of the control cells were defined as normal, cells more than four times as long as normal were defined as filaments, and cell debris or cells with their bacillary structure retained but the cytoplasm not stained were defined as lysed cell debris.

Assay of PBPs. Competitive binding affinities of cephalosporins for penicillin-binding proteins (PBPs) in *E. coli* KN126 were measured by the method reported by Nozaki et al. (8). The binding affinities of cephalosporins for each PBP were expressed in terms of IC_{50}, which was the concentration (in micrograms per milliliter) required to prevent [^{14}C]benzylpenicillin binding by 50%.

Protective test. *Streptococcus pyogenes* and *S. pneumoniae* were cultured overnight on blood-TSA slants, and other organisms were cultured in brain heart infusion (Difco Laboratories) overnight at 37°C. *S. pneumoniae* was suspended in TSB, and other organisms were suspended in 5% mucin (Difco). Four-week-old male Slc:ICR mice, weighing 19 to 23 g, were infected intraperitoneally with the organism suspended in 0.5 ml of the medium. The challenge doses of organisms were about 30 to 100 times the number of bacteria required to kill 50% of the control mice. Groups of five mice at each dose level were given subcutaneously 0.2 ml of cephalosporin solutions. Mice infected with *S. pneumoniae* and *Pseudomonas aeruginosa* were given cephalosporins at 0 and 4 h and at 0, 2, and 4 h after infection, respectively, and mice infected with other bacteria were given cephalosporins at 0 h after infection. All experiments were repeated at least five times. The 50% effective dose (ED_{50}; in milligrams per kilogram) was calculated by the probit method from the survival rates recorded 5 days after infection (6).

Therapeutic test. *Klebsiella* pneumonia in mice was induced by the method of Nishi and Tsuchiya (7). Four-week-old male Slc:ICR mice, weighing 20 to 24 g, were infected with *K. pneumoniae* DT-S by the aerosol method, resulting in deposition of about 10^4 CFU of bacteria per lung. The number of bacteria in the lung increased to about 10^8 CFU by the time chemotherapy was started (30 h after infection). Urinary tract infection caused by *Proteus mirabilis* was produced by the method of Iwahi and Tsuchiya (5). Four-week-old female CF1/b mice (Takeda Chemical Industries, Ltd.), weighing 19 to 23 g, were infected with *P. mirabilis* IFO3849 by the ascending route,

resulting in a deposition of about 10 CFU of bacteria per kidney; the number of bacteria in the kidney reached about 10^4 CFU at the time of chemotherapy initiation (3 days after infection). Cephalosporins were administered subcutaneously to groups of 10 mice at each dose level in two dosing series a day. Each dosing series consisted of four treatments at 30-min intervals. Treatment was started at 30 h after infection and continued for 10 days in the case of *K. pneumoniae* infection; it was started at 3 days after infection and continued for 5 days in the case of *P. mirabilis* infection. The number of bacteria in the lung, bladder wall, kidney, and urine was determined by the method previously described (5, 7).

RESULTS

Antibacterial spectrum. Cefmenoxime exhibited a broad spectrum of antibacterial activity against various pathogenic bacterial species, including *Haemophilus influenzae*, indole-positive *Proteus*, *Serratia marcescens*, *Citrobacter freundii*, and *Enterobacter cloacae*. The spectrum of cefmenoxime was similar to that of cefotaxime and was wider than that of other cephalosporins.

Activity against clinical isolates. The activity of cefmenoxime against clinical isolates was compared with that of cefotaxime, cefuroxime, cefoxitin, cefmetazole, cefotiam, and cefazolin with an inoculum of 10^6 CFU/ml (Table 1). Against clinical isolates of *Staphylococcus aureus*, the activity of cefmenoxime, resembling that of cefuroxime and cefmetazole, was greater than that of cefotaxime and cefoxitin, but the drug was less active than cefotiam and cefazolin. Against *Staphylococcus epidermidis*, cefmenoxime was as active as cefotaxime, more active than cefoxitin and cefmetazole, and less active than cefotiam and cefazolin. The activity of cefmenoxime against *S. pyogenes* and several gram-negative bacteria was similar to that of cefotaxime and was much greater than that of other cephalosporins. The MIC of cefmenoxime required to inhibit the growth of most strains was ≤ 0.025 μg/ml for *H. influenzae*, ≤ 0.39 μg/ml for cefazolin-susceptible *E. coli* and *K. pneumoniae*, *Proteus mirabilis*, *P. vulgaris*, *P. morganii*, *P. rettgeri*, and *P. inconstans*, ≤ 1.56 μg/ml for cefazolin-resistant *E. coli* and *K. pneumoniae* and gentamicin-susceptible *S. marcescens*, 6.25 μg/ml for gentamicin-resistant *S. marcescens* and *E. cloacae*, and 12.5 μg/ml for *C. freundii*. The activity of cefmenoxime against these gram-negative rods was similar to that of cefotaxime and was 10 to 1,000 times greater than that of the other cephalosporins used for reference. The growth of most strains of *Pseudomonas aeruginosa*, *P. cepacia*, and *P. maltophilia* was inhibited at 50 μg or less of cefmenoxime per ml, and the growth of most strains of *Acinetobacter calcoaceticus* was inhibited by 100 μg/ml. The activity of cefmenoxime against *P. aeruginosa* was about twice that of sulbenicillin, four times that of carbenicillin, and one-eighth that of cefsulodin (data not shown).

Effect of culture medium, pH, horse serum content, and inoculum size. The MICs of cefmenoxime against some laboratory strains of gram-positive and gram-negative bacteria were determined under various conditions, and the effect of inoculum size was also studied with clinical isolates. The MICs of cefmenoxime were distinctly unaffected by variations in culture medium or its pH and were only slightly affected by the addition of 10 to 50% horse serum to the medium. Changes in inoculum size had no significant effect on the MICs of cefmenoxime against *Staphylococcus aureus* FDA209P, *E. coli* NIHJ JC-2, *E. coli* T7, *Proteus mirabilis* IFO3849, and *P. vulgaris* IFO3988, but the MICs against *K. pneumoniae* DT, *P. morganii* IFO3168, *P. inconstans* IFO12930, *Serratia marcescens* IFO12648, *C. freundii* IFO12681, and *E. cloacae* IFO13535 increased with an increase of the inoculum size from 10^4 to 10^9 CFU/ml. The MICs of cefmenoxime and cefotaxime against cefazolin-resistant clinical isolates of *K. pneumoniae*, *Proteus vulgaris*, *P. morganii*, *P. inconstans*, and *Serratia marcescens* changed with increase in the inoculum size.

Development of resistance in vitro. Resistance of *S. aureus* FDA209P and *E. coli* NIHJ JC-2 to cefmenoxime was acquired gradually, and after the 25th transfer the MIC of cefmenoxime was greater than 100 μg/ml for *Staphylococcus aureus* and 25 μg/ml for *E. coli*.

Cross-resistance. The resistant mutants of *S. aureus* FDA209P and *E. coli* NIHJ JC-2, which developed in vitro against cefmenoxime, cefotiam, cefazolin, cephaloridine, and cephalothin, showed cross-resistance to each of the other cephalosporins.

Correlation of MIC and MBC. The MBCs of cefmenoxime against clinical isolates of *E. coli*, *K. pneumoniae*, and *Serratia marcescens* were similar to or twice the MICs.

Bactericidal activity. The bactericidal activity of cefmenoxime against *S. aureus* FDA209P, *E. coli* NIHJ JC-2, and *S. marcescens* IFO12648 was similar to or slightly greater than that of cefotaxime. A dose-dependent decrease in the CFU of *S. aureus* was observed at a concentration less than 1.56 μg of cefmenoxime per ml, but at concentrations of 3.13 and 6.25 μg/ml, an "Eagle effect" (4) was observed. Against *E. coli* NIHJ JC-2, cefmenoxime showed distinct bactericidal activity at concen-

TABLE 1. *Comparative activities of cefmenoxime and other cephalosporins against 90% of clinical isolates*

Organism (no. of strains)	MIC (μg/ml)[a]						
	Cefmenoxime	Cefotaxime	Cefuroxime	Cefoxitin	Cefmetazole	Cefotiam	Cefazolin
Staphylococcus aureus (105)[b]	1.56	3.13	3.13	3.13	1.56	1.56	0.78
S. epidermidis (52)[b]	1.56	1.56	0.78	3.13	1.56	0.78	0.39
Streptococcus pyogenes (37)[c]	0.013	0.013	0.013	0.78	0.39	0.05	0.1
Escherichia coli, cefazolin susceptible (104)[b]	0.2	0.1	6.25	6.25	1.56	0.2	3.13
E. coli, cefazolin resistant (25)[b]	0.78	1.56	100	50	6.25	6.25	>100
Klebsiella pneumoniae, cefazolin susceptible (75)[b]	0.2	0.2	6.25	6.25	1.56	0.39	3.13
K. pneumoniae, cefazolin resistant (91)[b]	1.56	1.56	100	100	50	25	>100
Haemophilus influenzae (69)[d]	0.025	0.025	0.78	12.5	6.25	1.56	50
Proteus mirabilis (107)[b]	0.1	0.05	3.13	6.25	1.56	0.39	6.25
P. vulgaris (78)[b]	0.39	1.56	>100	12.5	6.25	100	>100
P. morganii (81)[b]	0.39	1.56	50	25	12.5	12.5	100
P. rettgeri (40)[b]	0.2	0.1	3.13	25	12.5	0.78	50
P. inconstans (32)[b]	0.39	0.78	50	12.5	25	12.5	>100
Serratia marcescens, gentamicin susceptible (105)[b]	1.56	6.25	>100	100	100	>100	>100
S. marcescens, gentamicin resistant (79)[b]	6.25	12.5	>100	>100	>100	>100	>100
Citrobacter freundii (80)[b]	12.5	50	>100	>100	>100	100	>100
Enterobacter cloacae (78)[b]	6.25	25	>100	>100	>100	>100	>100
Pseudomonas aeruginosa (108)[b]	25	25	>100	>100	>100	>100	>100
P. cepacia (27)[b]	12.5	12.5	25	100	50	>100	>100
P. maltophilia (16)[b]	50	>100	>100	>100	>100	>100	>100
Acinetobacter calcoaceticus (36)[b]	100	50	100	100	100	>100	>100

[a] Inoculum size was one loopful of bacterial suspension (10^6 CFU/ml).
[b] Medium was TSA.
[c] Medium was blood-TSA.
[d] Medium was chocolate agar (beef extract agar supplemented with 10% horse blood).

trations higher than 0.1 μg/ml. The CFU, however, increased 6 h after incubation at 0.1 μg/ml and 8 h after at 0.2 μg/ml, and no regrowth was observed at concentrations higher than 0.39 μg/ml for 8 h. The CFU of *S. marcescens* decreased at cefmenoxime concentrations of 0.05 and 0.1 μg/ml for the first 4 h, but a distinct increase in the CFU was observed 8 h after incubation. At concentrations higher than 0.2 μg/ml, no regrowth of bacteria was observed (Fig. 2).

Bacteriolytic activity. The bacteriolytic activities of cefmenoxime and cefotaxime were markedly influenced by the growth phase. Cefmenoxime was added at concentrations of 1.56 μg/ml for *S. aureus* FDA209P, 0.39 μg/ml for *E. coli* NIHJ JC-2, and 0.2 μg/ml for *S. marcescens* IFO12648 at various times during growth. The bacteriolytic activity of cefmenoxime against *S. aureus* and *E. coli* decreased as the time of incubation increased, and no bacteriolysis was observed on addition of cefmenoxime at the mid-exponential growth phase (opacity 40 and 50%). Even though cefmenoxime was added at an early exponential growth phase (opacity 20%), no bacteriolysis of *S. marcescens* was observed. Various concentrations of cefmenoxime were added at the early exponential growth phase of *S. aureus* (opacity 20%), *E. coli* (opacity 20%), and *S. marcescens* (opacity 10%) (Fig. 3). Bacteriolysis of *S. aureus* and *E. coli* was observed at cefmenoxime concentrations higher than 0.78 μg/ml and higher than 0.2 μg/ml, respectively. After the addition of cefmenoxime, the increase in opacity of the *S. marcescens* culture was suppressed dose dependently, but no decrease in opacity was observed. Although the MICs of

cefmenoxime and cefotaxime were the same against *S. marcescens* IFO12648, no effect on suppression of opacity of the culture of *S. marcescens* was observed after the addition of cefotaxime.

Morphological change. The MICs of cefmenoxime against *E. coli* NIHJ JC-2, *K. pneumoniae* DT, *P. rettgeri* IFO13501, and *S. marcescens* IFO12648 by the agar dilution method were 0.2, 0.1, 0.0016, and 0.2 μg/ml, respectively, with an inoculum size of 10^8 CFU/ml. When these bacterial strains were exposed to a wide range of cefmenoxime concentrations from much below to well above the MICs, filamentation of organisms was observed. Lysis of the cells occurred at concentrations considerably higher than the MICs (Fig. 4).

Affinity for PBPs. The competition of increasing concentrations of cefmenoxime for [^{14}C]benzylpenicillin binding to PBPs of *E. coli* KN126 was examined, and the quantitative data obtained by densitometry are summarized in Table 2. Cefmenoxime had the highest affinity for PBP-3 and high affinities, in descending order, for PBP-1A, -1B, and -2. The affinity of cefmenoxime for PBP-4, -5, and -6 was much lower.

Protective effect. The protective effects of cefmenoxime in mice infected intraperitoneally with several strains of gram-positive and gram-negative bacteria were compared with those of cefotaxime, cefuroxime, cefoxitin, cefmetazole, cefotiam, and cefazolin (Table 3). In mice infected with *S. aureus* 308 A-1, the protective activity of cefmenoxime was greater than that of

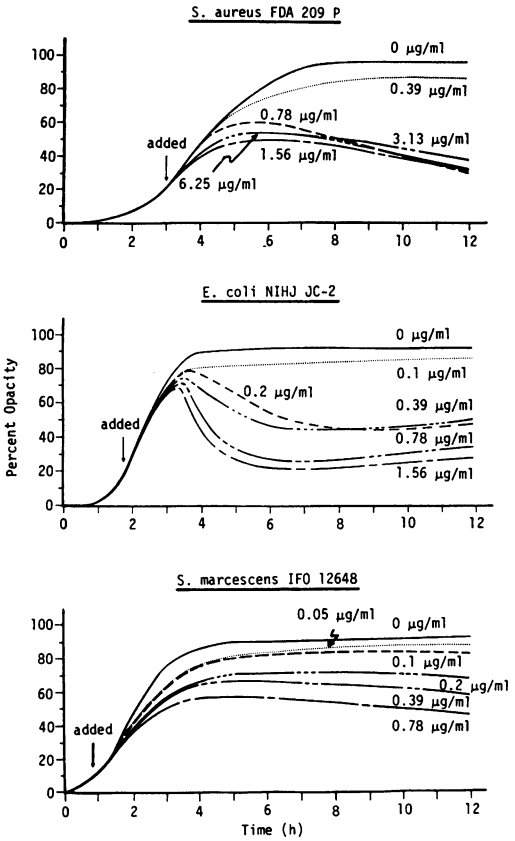

FIG. 3. *Bacteriolytic effect of cefmenoxime on S. aureus FDA209P, E. coli NIHJ JC-2, and S. marcescens IFO12648.*

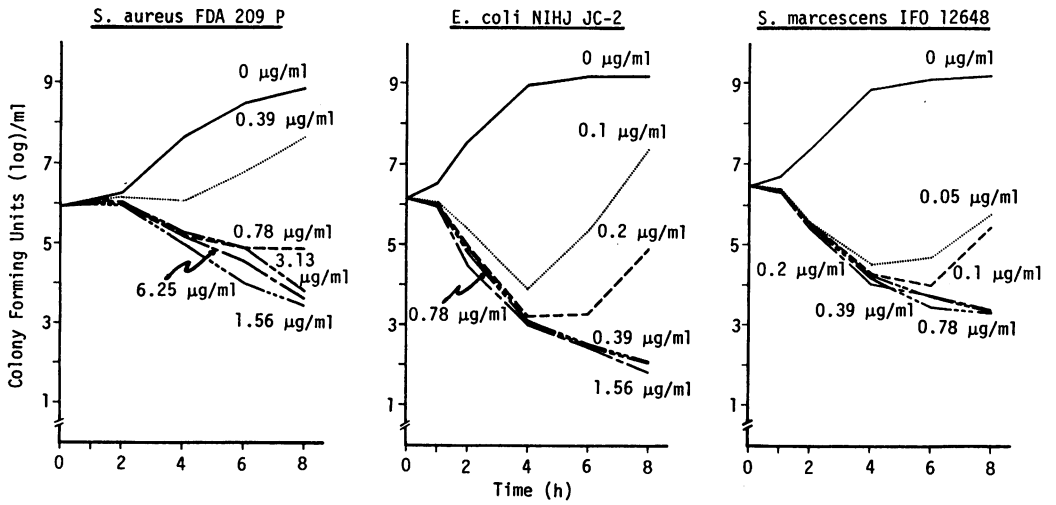

FIG. 2. *Bactericidal effect of cefmenoxime on S. aureus FDA209P, E. coli NIHJ JC-2, and S. marcescens IFO12648.*

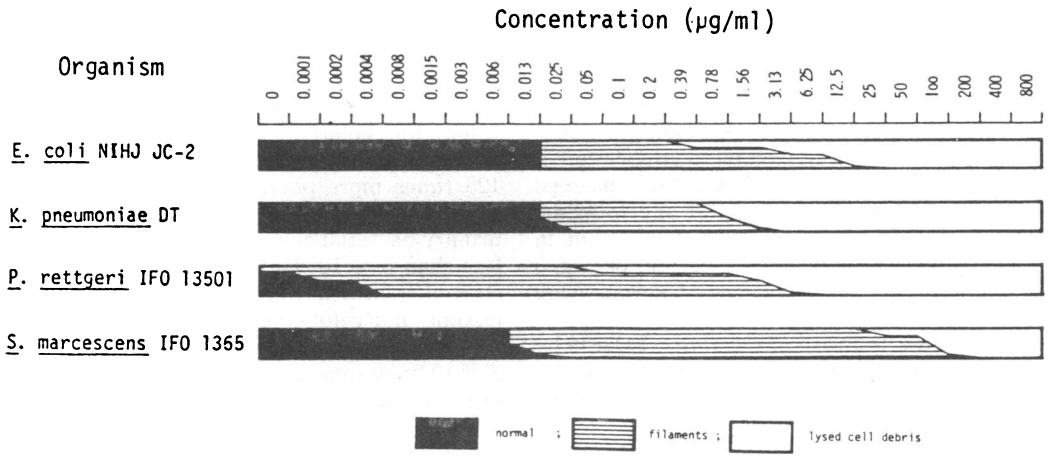

FIG. 4. *Morphological response profile of gram-negative organisms after 4 h of exposure to cefmenoxime.*

TABLE 2. *Binding affinities of cefmenoxime, cefotiam, and cefuroxime for each PBP of E. coli* KN126 *and their MICs against the same organism*

Cephalosporin	MIC (μg/ml)	I_{50} (μg/ml)[a]						
		1A	1B	2	3	4	5	6
Cefmenoxime	0.1	0.135	0.35	2.05	0.08	20.5	>500	ND[b]
Cefotiam	0.2	0.075	0.70	2.15/41.5[c]	0.105	21.0	>500	>500
Cefuroxime	6.25	0.105	3.65	24.0	1.20	120	>500	ND[b]

[a] Concentration required to prevent [^{14}C]benzylpenicillin binding by 50%.
[b] Cefmenoxime and cefuroxime have binding affinities for PBP 6, but the ID_{50} value was not quantitated.
[c] Competition was biphasic.

cefoxitin and similar to that of other cephalosporins. In mice infected with *S. pyogenes* or *S. pneumoniae,* cefmenoxime was much more active than other cephalosporins. In mice infected with several gram-negative bacteria, especially cefazolin-resistant strains of *E. coli* and *K. pneumoniae, Proteus* spp., *S. marcescens, C. freundii,* and *E. cloacae,* cefmenoxime was much more active than other cephalosporins. Although the MICs of cefmenoxime and cefotaxime were the same for *P. morganii* TN373, *P. morganii* GN5278, and *P. rettgeri* TN338, the protective effect of cefmenoxime in mice infected with these strains was much greater than that of cefotaxime. In mice infected with other strains, cefmenoxime showed activity similar to that of cefotaxime. Although cefmenoxime showed protective activity against *Pseudomonas aeruginosa* and *A. calcoaceticus* infections, its activity was inferior to that in mice infected with other microorganisms.

Effect of challenge dose. The protective effect of cefmenoxime in mice infected with various challenge doses was studied (Table 4). The properties of the test organisms were as follows. *E. coli* O111 and *K. pneumoniae* DT were cefazolin-susceptible strains, and *E. coli* T7 and *K. pneumoniae* S22 were cefazolin-resistant strains. The susceptibility of *P. vulgaris* GN4712 to cefmenoxime changes with increase in the inoculum size. *S. marcescens* TN66 was not lysed by treatment with cefmenoxime. The ED_{50} increased slightly in the dose range from 10^5 to 10^7 CFU/mouse for *E. coli* O111, from 10^3 to 10^6 CFU/mouse for *E. coli* T7, from 10^2 to 10^4 CFU/mouse for *K. pneumoniae* DT, from 10^5 to 10^6 CFU/mouse for *K. pneumoniae* S22, from 10^2 to 10^4 CFU/mouse for *P. vulgaris* GN4712, and from 10^1 to 10^5 CFU/mouse for *S. marcescens* TN66. At higher challenge doses of these strains, the ED_{50} increased markedly.

Effect of administration route. Cefmenoxime was equally effective when administered by the subcutaneous, intravenous, and intraperitoneal routes in mice infected with several strains of gram-positive and gram-negative bacteria. The protective activity of cefmenoxime after oral administration was inferior to that after parenteral administration.

Therapeutic effect. Against the pneumonia

caused by *K. pneumoniae* DT-S in mice, cefmenoxime was about 2, 4, and 50 times more potent than cefotaxime, cefotiam, and cefazolin, respectively, as judged by comparative SD_{50} (50% survival dose) and CD_{50} (50% clearance dose) values (Table 5). Complete eradication of bacteria from the lungs of all mice was observed at a dose of 40 mg of cefmenoxime or cefotaxime per kg and 160 mg of cefotiam per kg, but in mice treated with 640 mg of cefazolin per kg, 3 of 10 mice died and challenge organisms were found in 4 of 7 surviving mice.

In mice infected with *P. mirabilis* IFO3849 by transurethral infection, cefmenoxime showed a similar effect on both vesical and renal infections, but other cephalosporins exhibited a more potent effect against the vesical infection than the renal infection. On vesical infection, cefmenoxime was 2, 4, and 8 times more potent than cefotaxime, cefotiam, and cefazolin, respectively; on renal infection, cefmenoxime was 4, 8, and 128 times more potent than cefotaxime, cefotiam, and cefazolin, respectively (Table 6). The urinary bacterial counts in all mice decreased to less than 5×10^3 CFU/ml within 3 days after start of treatment at doses of 3.13 mg of cefmenoxime and cefotaxime per kg and 12.5 mg of cefotiam per kg, but bacteriuria was observed in 3 of 10 mice treated with 50 mg of cefazolin per kg on the day after the completion of treatment.

TABLE 3. *Comparative protective activities of cefmenoxime and other cephalosporins against intraperitoneal infection by gram-positive and gram-negative bacteria in mice*

Organism (challenge dose, CFU/mouse)[a]	ED_{50} (mg/kg)[b]						
	Cefmenoxime	Cefotaxime	Cefuroxime	Cefoxitin	Cefmetazole	Cefotiam	Cefazolin
Staphylococcus aureus 308 A-1 (10^8)	2.85 (0.78)	4.13 (1.56)	1.85 (0.78)	7.66 (1.56)	2.23 (0.78)	2.01 (0.39)	1.23 (0.2)
Streptococcus pyogenes E-14 (10^4)	0.038 (0.013)	0.025 (0.013)	0.005 (0.013)	0.257 (0.78)	0.115 (0.39)	0.103 (0.05)	0.091 (0.2)
S. pneumoniae type I (10^2)	0.488 (0.006)	0.913 (0.013)	0.461 (0.013)	24.1 (1.56)	9.70 (0.39)	5.07 (0.05)	0.854 (0.1)
Escherichia coli O111 (10^6)	0.016 (0.013)	0.017 (0.025)	0.550 (1.56)	1.86 (1.56)	0.699 (0.78)	0.066 (0.025)	1.12 (0.78)
E. coli T7 (10^4)	0.490 (0.2)	0.259 (0.2)	10.3 (12.5)	13.1 (12.5)	2.59 (1.56)	0.952 (0.39)	31.7 (12.5)
Klebsiella pneumoniae DT (10^3)	0.185 (0.025)	0.445 (0.025)	12.2 (0.78)	17.1 (1.56)	13.0 (0.78)	5.25 (0.1)	10.1 (1.56)
K. pneumoniae S22 (10^6)	0.322 (0.1)	0.349 (0.1)	14.8 (3.13)	17.4 (3.13)	6.82 (3.13)	5.62 (0.78)	48.1 (12.5)
Proteus mirabilis GN4336 (10^6)	0.049 (0.1)	0.079 (0.05)	7.11 (3.13)	12.6 (6.25)	15.7 (1.56)	3.61 (0.78)	8.07 (6.25)
P. vulgaris GN4712 (10^2)	0.202 (0.1)	0.534 (0.05)	368 (>100)	8.43 (3.13)	8.15 (1.56)	51.9 (6.25)	461 (>100)
P. morganii TN373 (10^6)	0.097 (0.025)	1.17 (0.025)	20.9 (25)	11.0 (12.5)	9.77 (12.5)	58.8 (0.39)	138 (>100)
P. rettgeri TN338 (10^7)	0.256 (0.006)	1.43 (0.006)	32.2 (0.2)	16.6 (0.78)	25.0 (0.39)	9.72 (0.025)	42.5 (0.39)
Serratia marcescens TN66 (10^3)	0.158 (0.2)	0.241 (0.2)	33.7 (50)	13.1 (12.5)	11.2 (6.25)	6.12 (3.13)	186 (>100)
Citrobacter freundii TN549 (10^5)	0.271 (0.2)	0.278 (0.2)	3.72 (6.25)	74.0 (>100)	19.2 (100)	1.34 (0.78)	80.7 (12.5)
Enterobacter cloacae TN603 (10^4)	0.139 (0.2)	0.109 (0.2)	10.6 (12.5)	39.8 (50)	6.54 (12.5)	0.277 (0.39)	10.3 (12.5)
Pseudomonas aeruginosa U31 (10^6)	88.6 (25)	629 (50)	>800 (>100)	>800 (>100)	>800 (>100)	>800 (>100)	>800 (>100)
Acinetobacter calcoaceticus TN1035 (10^3)	67.0 (25)	31.5 (12.5)	54.4 (25)	171 (100)	298 (50)	349 (50)	461 (>100)

[a] *S. pneumoniae* type I was suspended in TSB, and other organisms were suspended in 5% mucin. Mice were infected intraperitoneally with test organisms in 0.5 ml of medium.

[b] Cephalosporins were administered subcutaneously at 0 and 4 h after infection in mice infected with *S. pneumoniae* type I and at 0 h after infection in mice infected with other organisms. ED_{50} values were calculated by the probit method. MIC in micrograms per milliliter; given in parentheses was determined with a bacterial suspension of 10^6 CFU/ml.

TABLE 4. *Effect of challenge dose on ED_{50} of cefmenoxime against experimental infection in mice*[a]

Challenge dose (CFU/mouse)	ED_{50} (mg/kg)[b]					
	E. coli O111	*E. coli* T7[c]	*K. pneumoniae* DT	*K. pneumoniae* S22	*P. vulgaris* GN4712	*S. marcescens* TN66
10^1						0.100
10^2			0.033		0.108	0.133
10^3		0.229	0.101		0.254	0.166
10^4		0.287	0.241		0.339	0.413
10^5	0.006	0.427	1.85	0.135	2.89	0.939
10^6	0.016	0.520	9.90	0.302	20.2	8.60
10^7	0.034	2.44	51.9	2.68	159	227
10^8	10.3	51.5	271	52.3	>800	>800

[a] Organisms were suspended in 5% mucin. Mice were infected intraperitoneally with test organism in 0.5 ml of medium.

[b] Cefmenoxime was administered at 0 h after infection. ED_{50} values were calculated by the probit method.

[c] Cefazolin-resistant strain.

TABLE 5. *Therapeutic effects of cefmenoxime, cefuroxime, cefotiam, and cefazolin against pneumonia caused by K. pneumoniae in mice*[a]

Cephalosporin	SD_{50}[b] (mg/kg)	CD_{50}[c] (mg/kg)	MIC[d] (μg/ml)
Cefmenoxime	7.6 (4.8–12.1)	16.1 (11.2–22.6)	0.013
Cefotaxime	19.9 (14.6–27.3)	24.1 (17.9–33.1)	0.013
Cefotiam	36.7 (20.8–51.1)	53.0 (38.3–73.0)	0.1
Cefazolin	400 (274–695)	>640	1.56

[a] Four-week-old Slc:ICR mice weighing 20 to 24 g were used. Bacterial suspensions (10^9 CFU/ml) were nebulized at a pressure of 1 kg/cm^2 for 40 min. Therapy was started at 30 h after infection and continued for 10 days. Two dosing series of cephalosporins were given daily, starting at 8 a.m. and 4 p.m., respectively. Each dosing series consisted of four subcutaneous injections at 30-min intervals.

[b] Calculated by survival rate at 11 days after infection and by the probit method. Number in parentheses indicates 95% confidence limits.

[c] Calculated by rate of negative bacterial recovery at 11 days after infection and by the probit method. Number in parentheses indicates 95% confidence limits.

[d] Determined with a bacterial suspension of 10^6 CFU/ml.

TABLE 6. *Therapeutic effects of cefmenoxime, cefotaxime, cefotiam, and cefazolin against urinary tract infection caused by P. mirabilis in mice*[a]

Cephalosporin	CD_{50} (mg/kg)[b]		MIC (μg/ml)
	Bladder wall	Kidney	
Cefmenoxime	0.40 (0.029–0.89)	0.75 (0.23–1.61)	0.2
Cefotaxime	0.53 (0.30–0.81)	0.84 (0.43–1.56)	0.1
Cefotiam	2.41 (1.62–3.57)	4.79 (2.57–10.5)	1.56
Cefazolin	4.39 (1.06–8.97)	27.5 (14.4–126)	25

[a] Four-week-old CF1/b mice weighing 19 to 23 g were used. Bacterial suspensions (10^3 CFU/0.05 ml) were inoculated into the bladder. Therapy was started at 3 days after infection and continued for 5 days. Two dosing series of cephalosporins were given daily, starting at 8 a.m. and 4 p.m., respectively. Each dosing series consisted of four subcutaneous injections at 30-min intervals.

[b] Bladder walls and kidneys were examined bacteriologically at 8 days after infection. Values were calculated by rate of negative bacterial recovery and by the probit method. Number in parentheses indicates 95% confidence limits.

DISCUSSION

The spectrum of antibacterial activity is widened in cefmenoxime to include species which are resistant to many cephalosporins, and its potency against gram-negative bacteria is more satisfactory. The antibacterial activity of β-lactam antibiotics against gram-negative rods has been attributed to (i) the permeability of the drug through the bacterial outer membrane, (ii) the stability to hydrolysis by β-lactamases, and (iii) the inhibitory activity of the drug against

the peptidoglycan-synthesizing enzymes (2, 3, 11–13, 15–17). Most species of cephalosporin-resistant bacteria are β-lactamase producers, and cefmenoxime is stable to hydrolysis by β-lactamase from these bacteria, except *P. vulgaris* β-lactamase (Ochiai et al., Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 18th, Atlanta, Ga., Abstr. no. 150, 1978). This factor, resistance to hydrolysis by β-lactamases, may be related to the potent antibacterial activity of cefmenoxime against various β-lactamase-producing organisms and to a minor effect of the inoculum size on the MICs of cefazolin-resistant strains. Although the susceptibility of clinical isolates of indole-positive *Proteus* to cefmenoxime showed a wider variation than those of other species of bacteria, it is known that *Proteus* spp. produce a few kinds of β-lactamases (13). Therefore, the relationship between the susceptibility to cefmenoxime and β-lactamase production needs further study.

Recently, the functions of PBP-1, -2, and -3 as the lethal targets in *E. coli* have been elucidated; they are shown to be involved in peripheral cell wall expansion, cell shape determination, and septum formation, respectively (15–17). Cefmenoxime shows remarkable affinity for these PBPs, with highest affinity for PBP-3, followed by PBP-1 (1A and 1B) and PBP-2 in *E. coli* KN126. This affinity profile is well reflected in the strong filamentous cell-forming activity under extremely low drug concentrations and in the comparatively low bacteriolytic activity for the drug's strong bactericidal activity. In addition, the difference in MICs of cefmenoxime, cefotiam, and cefuroxime against *E. coli* KN126 seems to depend upon the affinities for PBP-1, -2, and -3 in the same organism. Against *S. marcescens*, cefmenoxime showed bactericidal activity, but no bacteriolytic activity was observed even at very high concentrations. Study of the affinity of cefmenoxime for PBPs of *S. marcescens* appears to be interesting.

The protective activity of cefmenoxime and various cephalosporins in mice infected intraperitoneally with various bacterial strains was well reflected in the in vitro antibacterial activity. In some cases, however, the protective activity did not appear to be correlated with in vitro antibacterial activity. Cefmenoxime and cefotaxime had the same MICs against *P. morganii* TN373 and GN5278 and *P. rettgeri* TN338, but the protective activity of cefmenoxime in mice infected with these organisms was greater than that of cefotaxime. Protective activities of antibiotics in mice may be influenced by the growth site of challenge organisms and by the tissue distribution and metabolism of antibiotics. In mice subcutaneously administered a single dose of 20 mg/kg, cefmenoxime and cefotaxime concentrations in plasma were almost equal but the cefmenoxime concentrations in the liver and kidney were much higher than those of cefotaxime. Furthermore, cefmenoxime was not metabolized in mice, but the major part of cefotaxime was metabolized to deacetylcefotaxime, a less active metabolite (19). These findings suggest that *P. morganii* and *P. rettgeri* might have grown not only in the peritoneum but also in the liver and kidney.

The difference in the fate of cefmenoxime and cefotaxime in mice was also reflected in the therapeutic effect of the two cephalosporins in infection models resembling the clinical picture in humans, such as pneumonia caused by *K. pneumoniae* and urinary tract infection caused by *P. mirabilis*. The MICs of cefmenoxime and cefotaxime against *K. pneumoniae* DT-S were the same, whereas the cefmenoxime level in the lung was about two times higher. The therapeutic activity of cefmenoxime in *Klebsiella* pneumonia was about two times more potent than that of cefotaxime. In the kidney of mice given cefotaxime, only deacetylcefotaxime, not cefotaxime, was observed and the deacetylcefotaxime levels in the kidney were about one-half the cefmenoxime level. The in vitro activity of cefmenoxime against *P. mirabilis* IFO3849 was one-half that of cefotaxime and deacetylcefotaxime. The similar therapeutic activity of the two cephalosporins in urinary tract infection caused by *P. mirabilis* IFO3849 may be a reflection of these findings.

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