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Comparative In Vitro Activities of Cefotaxime and Ceftizoxime (FK749): New Cephalosporins with Exceptional Potency

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Cefotaxime and its desacetoxymethyl derivative, ceftizoxime (previously known as FK749), are both extremely active against a wide spectrum of bacteria. In the present comparative study, the activity of ceftizoxime exceeded that of cefotaxime by a factor of four or more for strains of *Klebsiella*, *Enterobacter*, *Providencia*, *Serratia*, and *Bacteroides*; the only species for which the activity of cefotaxime exceeded that of ceftizoxime by a factor of four was *Vibrio cholerae*. Against other species, the activity of the two drugs was roughly comparable. Both showed outstanding activity against *Haemophilus influenzae* and *Neisseria gonorrhoeae*. Comparative turbidimetric and morphological studies revealed that ceftizoxime was able to induce spheroplast formation and rapid lysis in *Escherichia coli* strains at lower concentrations than cefotaxime. This difference was not found, however, when *E. coli* strains resistant to ampicillin by an intrinsic (nonenzymic) mechanism were tested.

During the last few years, so-called "new-generation" injectable cephalosporins have emerged which are characterized by outstanding resistance to most β-lactamases. Prominent among these are cefuroxime (10) and the cephamycin antibiotic, cefoxitin (11), both of which are now in clinical use. The resistance of these new agents to enzymic destruction considerably broadens their antibacterial spectrum, but their intrinsic antibacterial activity is, in itself, relatively modest. More recently, a new cephalosporin, cefotaxime, has been described (1, 6, 7) which combines enzyme stability with high intrinsic activity against all but a few bacterial species. Attempts to further improve on this activity have led to the development in Japan of the desacetoxymethyl derivative of cefotaxime, ceftizoxime (Fig. 1), previously known by manufacturer code FK749.

MATERIALS AND METHODS

Antibiotics. Ceftizoxime (sodium salt) was provided by Fujisawa Pharmaceutical Co., Ltd.; cefotaxime sodium was provided by Roussel Laboratories Ltd. Suitable concentrations were freshly prepared, as required, in sterile distilled water.

Titrations. Minimum inhibitory concentrations (MICs) of ceftizoxime and cefotaxime were estimated by the agar-incorporation method, using serial doubling dilutions of antibiotic in Oxoid DST agar, supplemented with 5% horse blood or 5% heated horse blood where necessary. Bacteria were inoculated onto the plates by using an automatic multipoint inoculator (Denley-Tech Ltd.), the prongs of which deposited 50 to 100 colony-forming units from overnight broth cultures diluted $1:10^4$.

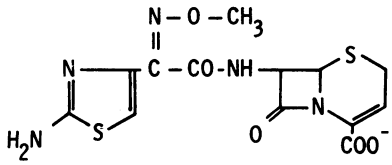
Bacterial strains. Five hundred ninety-five recent clinical isolates of bacteria were examined. Most were isolated in this laboratory; the *Vibrio cholerae* strains were randomly selected from those sent from various geographical areas as part of a global survey of antibiotic resistance in this species.

Turbidimetric studies. Four strains of *Escherichia coli* were investigated. These were chosen from laboratory stock cultures to represent (i) an ampicillin-sensitive strain, (ii) a strain resistant to ampicillin by virtue of an R-TEM-type β-lactamase, (iii) two strains resistant to β-lactam antibiotics by an intrinsic (nonenzymic) mechanism.

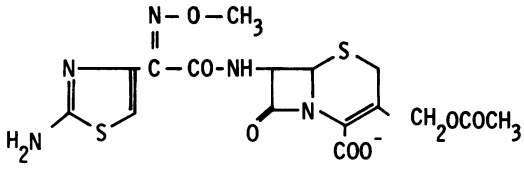
Cultures were grown from small inocula in "complete" broth (3) in a modified version (C. Aldridge, M.Sc. thesis, University of London, London, England, 1975) of the multichannel bacterial growth monitoring device described by Mackintosh et al. (8), in which the opacity of 12 independent bacterial cultures could be continuously recorded. Antibiotic was added at a standard point in the logarithmic growth phase (30% maximum opacity) equivalent to a viable count of ca. 5×10^7 bacteria per ml.

Microscopy. Observations of antibiotic-induced morphological changes in broth cultures of bacteria were made after a 1-h exposure to antibiotic by interference-contrast microscopy.

Bladder model. The design and operation of the in vitro bladder model have been described in detail elsewhere (5). In the present experiments, 20 ml of an overnight broth culture of *E. coli* was diluted with fresh broth at a rate of 1 ml per min (the normal diurnal ureteric urine flow rate) and at 1-h intervals the bladder model was emptied (simulating micturition) leaving a residual volume of 20 ml. By using a gradient-forming device (Mixograd; Gilson Medical Electronics), a dose of 125 mg of cephalosporin was infused into the system with the broth inflow over a 12-h period, commencing immediately after the fourth



FK 749



cefotaxime

FIG. 1. *Chemical structures of ceftizoxime and cefotaxime.*

hourly micturition. The profile of drug exposure is illustrated in Fig. 2.

RESULTS

MICs. The MICs of ceftizoxime and cefotaxime for 386 gram-negative bacilli are shown in Table 1. Both antibiotics were extremely active against this group of strains, none of which yielded an MIC greater than 8 μg/ml. The modal MIC did not exceed 0.06 μg of ceftizoxime per ml or 0.12 μg of cefotaxime per ml for any of the nine genera studied. In general, the activity of ceftizoxime exceeded that of cefotaxime by a factor of two or four, and the newer cephalosporin was at least eight times more active against *Providencia* strains. The only species of this group against which cefotaxime appeared consistently superior was *V. cholerae*.

The MICs of ceftizoxime and cefotaxime for *Haemophilus influenzae*, *Neisseria gonorrhoeae*, *Streptococcus pneumoniae*, various he-

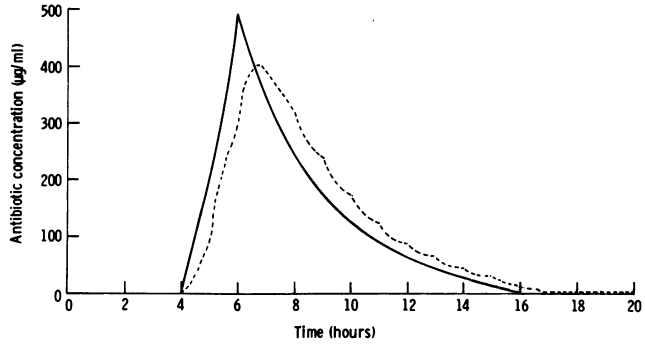


FIG. 2. *Profile of drug exposure used in the bladder model. The solid line shows the concentration of drug in the broth inflow; the broken line shows the actual concentration of antibiotic achieved in the bladder.*

TABLE 1. *Comparative activity of ceftizoxime and cefotaxime against 9 genera of gram-negative bacilli*

Organism (no. tested)	Antibiotic	MIC (μg/ml)		
		Range	Mode	MIC_{90}[a]
Escherichia coli (116)	Ceftizoxime	0.015 to 2.0	0.03	0.25
	Cefotaxime	0.03 to 2.0	0.06	0.25
Klebsiella aerogenes (93)	Ceftizoxime	<0.007 to 0.12	0.007	0.03
	Cefotaxime	0.007 to 0.25	0.03	0.12
Enterobacter spp. (31)	Ceftizoxime	0.007 to 0.25	0.03	0.12
	Cefotaxime	0.015 to 0.5	0.12	0.25
Proteus spp. (44)	Ceftizoxime	<0.007 to 8.0	0.007	0.06
	Cefotaxime	<0.007 to 4.0	0.03	0.06
Providencia spp. (18)	Ceftizoxime	<0.007 to 0.03	0.007	0.015
	Cefotaxime	0.03 to 0.25	0.06	0.12
Citrobacter spp. (10)	Ceftizoxime	0.015 to 0.12	0.06	0.12
	Cefotaxime	0.06 to 0.25	0.12	0.12
Serratia marcescens (25)	Ceftizoxime	0.03 to 0.25	0.03	0.12
	Cefotaxime	0.06 to 1.0	0.12	0.25
Salmonella spp. (25)	Ceftizoxime	0.007 to 2.0	0.03	0.03
	Cefotaxime	0.03 to 1.0	0.06	0.12
Vibrio cholerae (24)	Ceftizoxime	0.015 to 0.06	0.03	0.03
	Cefotaxime	0.007	0.007	0.007

[a] MIC_{90}, Concentration inhibiting 90% of the strains tested.

molytic streptococci, and *Staphylococcus aureus* are shown in Table 2. The two cephalosporins displayed outstanding and similar activity against most of these strains with modal MICs of 0.015 μg or less per ml. Exceptions occurred among *Staphylococcus aureus* strains (modal MIC, 0.5 μg/ml) and among streptococci belonging to Lancefield groups B and D; for group B streptococci the modal MIC of ceftizoxime was 0.12 μg/ml and that of cefotaxime was 0.06 μg/ml. Group D streptococci were uniformly resistant to 16 μg of either drug per milliliter.

Table 3 lists the MICs of some other relatively resistant species. Against *Pseudomonas aeruginosa*, *Acinetobacter*, and *Alcaligenes* strains, cefotaxime appeared slightly more active; against *Bacteroides fragilis* and *Listeria monocytogenes*, ceftizoxime was the more active. For *Bacteroides*, the modal MIC of ceftizoxime was 2 μg/ml compared with 16 μg of cefotaxime per ml.

Turbidimetric response profiles. Figure 3 shows the effect of ceftizoxime and cefotaxime on dense populations of two strains of *E. coli*, expressed in terms of the time taken for lysis of the culture to occur at various antibiotic concentrations. One of these strains (ECSA 1) was ampicillin-susceptible; the other (*Hos*) was resistant to ampicillin by virtue of an R-TEM-type β-lactamase. Strains possessing this type of resistance generally appear susceptible to cephalosporins when light inocula are used, but exhibit a very marked inoculum effect (3). Ceftizoxime induced rapid lysis of both these strains at lower concentrations than did cefotaxime. However, when the two cephalosporins were tested against strains of *E. coli* possessing intrinsic (nonenzymic) resistance to ampicillin, ceftizoxime appeared somewhat less active than cefotaxime (Fig. 4).

Morphological response profiles. The morphological response to ceftizoxime and

TABLE 2. *Comparative activity of ceftizoxime and cefotaxime against H. influenzae, N. gonorrhoeae, streptococci, and Staphylococcus aureus*

Organism (no. tested)	Antibiotic	MIC (μg/ml)		
		Range	Mode	MIC_{90}[a]
H. influenzae (17[b])	Ceftizoxime	<0.007 to 0.06	0.007	0.03
	Cefotaxime	<0.007 to 0.06	0.015	0.03
N. gonorrhoeae (21)	Ceftizoxime	<0.007 to 0.03	<0.007	0.007
	Cefotaxime	<0.007 to 0.03	<0.007	0.015
Streptococcus pneumoniae (5)	Ceftizoxime	0.007 to 0.015	0.015	0.015
	Cefotaxime	0.015	0.015	0.015
Other streptococci:				
Group A, C, G (15)	Ceftizoxime	0.007 to 0.015	0.007	0.015
	Cefotaxime	0.007 to 0.015	0.015	0.015
Group B (7)	Ceftizoxime	0.12	0.12	0.12
	Cefotaxime	0.03 to 0.06	0.06	0.06
Group D (12)	Ceftizoxime	>16.0	>16.0	>16.0
	Cefotaxime	>16.0	>16.0	>16.0
Staphylococcus aureus (56)	Ceftizoxime	0.12 to 1.0	0.5	0.5
	Cefotaxime	0.12 to 1.0	0.5	0.5

[a] MIC_{90}, Concentration inhibiting 90% of the strains tested.
[b] Including six β-lactamase-producing strains.

TABLE 3. *Comparative activity of ceftizoxime and cefotaxime against less susceptible species*

Organism (no. tested)	Antibiotic	MIC (μg/ml)		
		Range	Mode	MIC_{90}[a]
P. aeruginosa (39)	Ceftizoxime	0.25 to 32.0	16	16
	Cefotaxime	0.25 to 32.0	8	16
Acinetobacter-Alcaligenes (7)	Ceftizoxime	0.5 to 32.0	4	4
	Cefotaxime	0.5 to 32.0	2 to 4	4
B. fragilis (27)	Ceftizoxime	0.12 to 8.0	2	4
	Cefotaxime	0.5 to 32.0	16	16
L. monocytogenes (5)	Ceftizoxime	1.0 to 2.0	1	2
	Cefotaxime	4.0 to 8.0	4	8

[a] MIC_{90}, Concentration inhibiting 90% of the strains tested.

cefotaxime of an ampicillin-sensitive *E. coli* strain (ECSA 1) is shown in Fig. 5. Ceftizoxime induced spheroplast formation at lower concentrations than did cefotaxime. The range of concentrations of cefotaxime which inhibited division only (causing filamentation of the bacteria) was particularly broad.

Bladder model. When dense cultures of *E. coli* ECSA 1 were exposed to a 125-mg dose of ceftizoxime or cefotaxime in the dynamic conditions of the bladder model, growth was suppressed for a period of about 23 h from the commencement of the dose in each case.

DISCUSSION

Amid the plethora of new β-lactam antibiotics that have been described in recent years, cefotaxime and its desacetoxymethyl derivative, ceftizoxime, exhibit outstanding properties in terms of β-lactamase stability, antibacterial spectrum, and intrinsic activity. The activity of these agents against gram-negative organisms is particularly remarkable, with modal MICs of 0.007 μg/ml for some species—even less for *N. gonorrhoeae*. Against the gram-positive organisms we tested, the new compounds also displayed high potency, but (with the exception of penicillinase-producing staphylococci) this was no greater than that of benzylpenicillin. Curiously, penicillin-susceptible enterococci were found to be resistant to both cefotaxime and ceftizoxime in keeping with the general resistance of this species to cephalosporins.

Cefotaxime and ceftizoxime showed some dif-

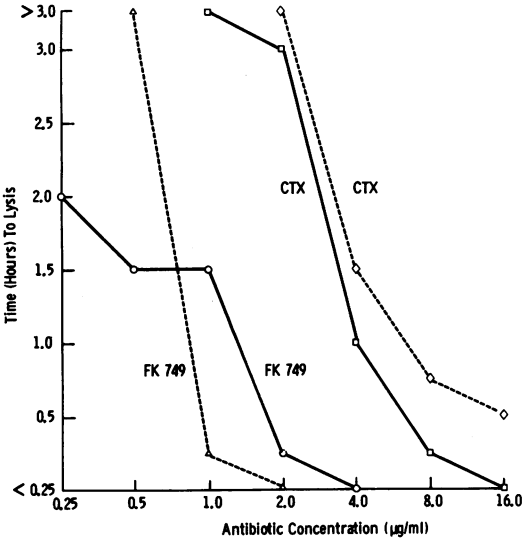


FIG. 3. *Turbidimetric response profiles of two strains of E. coli showing the time taken, after exposure to various drug concentrations, for bacterial lysis to occur. —, E. coli ECSA 1 (ampicillin-sensitive); - - -, E. coli Hos (R-TEM-type β-lactamase-producing strain).*

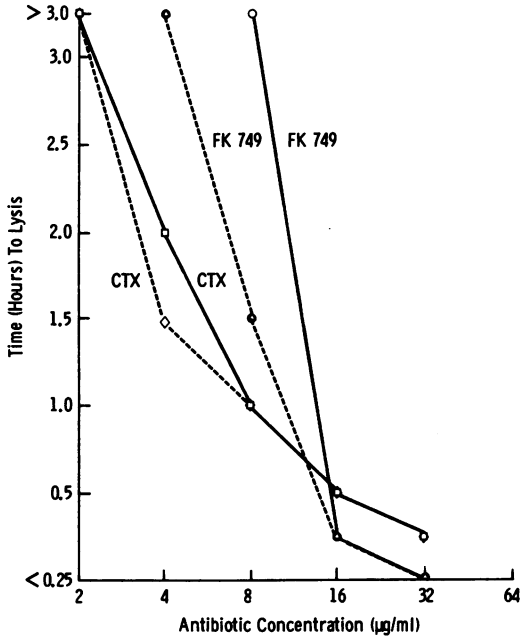


FIG. 4. *Turbidimetric response profiles of two strains of E. coli intrinsically resistant to β-lactam antibiotics showing the time taken, after exposure to various antibiotic concentrations, for bacterial lysis to occur. —, E. coli Hil; - - -, E. coli Box.*

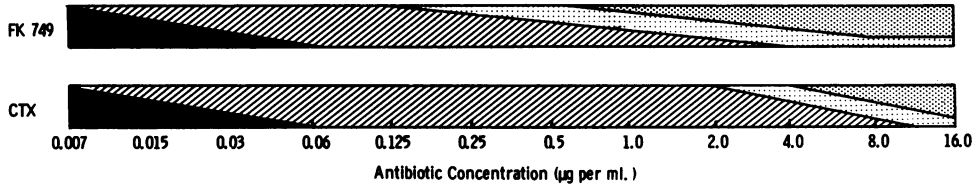


FIG. 5. *Morphological response profiles of a strain of E. coli (ECSA 1), showing the morphological changes induced after a 1-h exposure to various concentrations of ceftizoxime or cefotaxime. Black, normal morphology; hatching, filaments; light stippling, emergent spheroplasts; dense stippling, lysed cell debris.*

ferential activity, particularly against gram-negative bacteria. In most cases, this was in favor of ceftizoxime, and against some species, including *Klebsiella*, *Enterobacter*, *Providencia*, *Serratia*, and *Bacteroides*, the activity of ceftizoxime exceeded that of cefotaxime by a factor of 4 or more. Cefotaxime appeared to be the more active drug against *V. cholerae*, *P. aeruginosa*, and *Acinetobacter* spp.

Comparative turbidimetric and morphological studies of *E. coli* exposed to the two compounds showed that ceftizoxime was able to evoke spheroplast formation, and hence rapid lysis, at lower concentrations than was cefotaxime. This was true of ampicillin-susceptible and R-TEM-type β-lactamase-producing strains, the two groups that represent the majority of clinical isolates of *E. coli*. However, the superior lytic activity of ceftizoxime did not extend to *E. coli* strains displaying intrinsic resistance to β-lactam antibiotics, a group that formed about 20% of ampicillin-resistant *E. coli* isolates encountered in a previous study (4). Such strains are generally very resistant to cephalosporins (4).

Cefotaxime possesses an acetoxy grouping at position 3 of the cephem nucleus and, by analogy with other cephalosporins incorporating this feature, would be susceptible to modification by mammalian deacetylating enzymes to produce, in vivo, the corresponding hydroxymethyl derivative which generally results in much reduced antibacterial activity (2). Ceftizoxime lacks this feature, so that the problem of in vivo instability should not arise.

The appearance of cefotaxime and ceftizoxime undoubtedly represents a significant advance in the development of injectable cephalosporins. Assuming that these new compounds exhibit the expected lack of toxicity in clinical use, they should prove of great value in the treatment of severe infection. Because of its generally higher intrinsic activity and its potentially greater in vivo stability, ceftizoxime appears to have some advantages over cefotaxime.

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