CEFODIZIME, AN AMINOTHIAZOLYLCEPHALOSPORIN

I. *IN VITRO* ACTIVITY

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Cefodizime possesses a broad antibacterial spectrum including staphylococci, streptococci and *Enterobacteriaceae*. *Neisseria gonorrhoeae* and *Haemophilus influenzae* are also highly susceptible to cefodizime. Because of its β-lactamase stability cefodizime is active against bacterial strains producing especially plasmid-coded enzymes. The MICs of cefodizime are slightly higher than those of cefotaxime, but with most Gram-negative bacteria they are lower than those of cefazolin, cefotiam and piperacillin. The *in vitro* activity of cefodizime is not dependent on inoculum size, or on the pH and composition of the test medium. Cefodizime did not induce *in vitro* resistance of *Staphylococcus aureus* or *Escherichia coli*. Because of its binding properties to PBPs 1A/B and 3, cefodizime leads to filamentation of Gram-negative rods and, at only slightly higher concentrations, to bacteriolysis.

In recent years a number of β-lactamase stable, highly active broad-spectrum cephalosporins have been developed and introduced into therapy. The first representative of these modern cephalosporins was cefotaxime (CTX). This compound possesses an aminothiazolyl side-chain with a *syn*-methoximino group in the 7-position. This side-chain is responsible for the outstanding microbiological properties of CTX[1,2]. Subsequently, the laboratories of Hoechst AG synthesized cefodizime (HR 221), a derivative of CTX, which contains a mercaptothiazolyl radical instead of the 3-acetoxy group of the parent compound (Fig. 1).

The introduction of structurally similar side-chains produced cephalosporins with prolonged serum half-lives in animals and man. This improvement in the pharmacokinetic profile was also found with cefodizime[3,4].

In this study we investigated the *in vitro* properties of cefodizime and compared it with other β-lactam antibiotics.

Materials and Methods

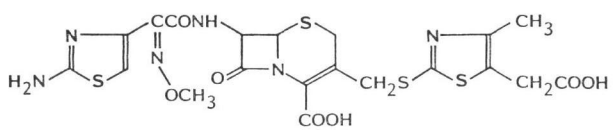
Test Strains

The bacterial strains used were clinical isolates collected between 1978 and 1983 from various hospitals in Europe together with laboratory strains maintained in our department. All bacterial strains were stored on agar slants at room temperature or in liquid nitrogen.

Antibiotics

Cefodizime and cefotaxime (CTX) were synthesized by Hoechst AG, Frankfurt, FRG. Cefotiam

Fig. 1. Cefodizime (HR 221).



(CTM), cefazolin (CEZ) and piperacillin (PIPC) are commercially available.

Susceptibility Testing

The susceptibility of aerobic and anaerobic bacteria was tested as previously described using an agar dilution method with Mueller-Hinton agar[5].

In the case of *Neisseria gonorrhoeae,* the test medium used was GC-medium supplemented with liver autolysate[6] as specified by Statens Seruminstitut, Copenhagen, Denmark. The inoculum was adjusted to 1×10^9 cfu/ml. The inoculated plates were incubated for 18 hours at 35°C under a humidified atmosphere enriched with 5% CO_2.

Influence of Inoculum Size

The influence of the inoculum size on the activity of cefodizime was examined in serial dilution tests in Mueller-Hinton medium (Difco). Five simultaneously prepared geometrical dilution series of the compound were inoculated with suspensions containing different densities of the test organism. The initial counts were approximately 1×10^2, 10^3, 10^4, 10^5 and 10^6 cfu/ml of nutrient medium.

Influence of Nutrient Medium

The effect of the composition of the culture medium was examined in the serial dilution test. For this purpose, the MICs of cefodizime for the test organisms were determined in the following commercial bacterial culture media: Mueller-Hinton medium (Difco), ABM III (Difco), standard I (Merck), heart infusion medium (Difco), brain heart infusion medium (Difco), standard bouillon (Oxoid), Bacto Tryptone 1% (Difco), Isosensitest broth (Oxoid).

The effect of the pH on the activity of cefodizime was determined in heart infusion medium (Difco) which had been adjusted to values between pH 5.5 and pH 9. In this medium, the MICs of cefodizime for the test strains were determined by the serial dilution method.

Killing Curves

Mueller-Hinton medium was inoculated with the individual test strains and incubated at 37°C on a rotary shaker. After two hours cefodizime was added to the cultures at different concentrations. Samples were withdrawn at intervals, diluted with saline and spread on agar plates. After 18 hours at 37°C the number of cfu/ml were counted.

Induction of Resistance *In Vitro*

A dilution series of cefodizime in Mueller-Hinton medium was inoculated with one of the test strains (*Staphylococcus aureus* SG 511, *Salmonella typhimurium*) and incubated at 37°C. After 18 hours, bacteria from the test tube, containing cefodizime at a subinhibitory concentration and showing a sufficiently dense growth were used to inoculate a fresh dilution series. The two test strains were passaged 30 times in this manner.

β-Lactamase Stability

The enzymes used were released from the bacteria by ultra-sonication and partially purified by chromatography on Sephacryl S200 superfine (Pharmacia). For the spectrophotometric UV-test, 2 ml of a 10^{-3} M solution of cefodizime and 50 μl of enzyme solution were mixed. The absorbance at 255 nm was monitored for 10 minutes at room temperature. The relative rates of hydrolysis were compared with the decay of cephaloridine.

Affinity for Penicillin-binding Proteins (PBPs)

The binding of cefodizime to PBPs of *Escherichia coli* K12 was determined as previously described using [^{125}I]ampicillin[7].

Results

Activity against Aerobic and Facultative Anaerobic Bacteria

Cefodizime possesses a broad antibacterial spectrum (Table 1), which includes both staphylococci and streptococci (except enterococci). The MIC_{90} values determined were 10.22 μg/ml and 0.11 μg/ml respectively. The *in vitro* activity of cefodizime against staphylococci came close to that of CTX.

Table 1. *In vitro* activity of cefodizime and other cephalosporins against aerobic and facultative anaerobic bacteria.

Organism (No. of strains)	Antibiotic	MIC (μg/ml)		
		50%	90%	Range
Staphylococcus aureus (25)	CDZ	4.39	10.22	2.0 ~ 16.0
	CTX	1.12	3.0	0.25 ~ 4.0
	CTM	0.26	0.66	0.125~ 1.0
	CEZ	0.17	0.76	0.031~ 1.0
	PIPC	3.03	45.59	0.25 ~ 128.0
Streptococcus sp. (A, B, C) (25)	CDZ	0.03	0.11	0.004~ 0.25
	CTX	0.002	0.04	≤0.002~ 0.125
	CTM	0.05	0.23	0.008~ 0.5
	CEZ	0.04	0.17	0.004~ 0.25
	PIPC	0.03	0.14	0.004~ 0.25
Pseudomonas aeruginosa (24)	CDZ	30.64	>128.0	0.25 ~>128.0
	CTX	8.94	63.12	0.125~ 64.0
	CTM	1.0	>128.0	1.0 ~>128.0
	CEZ	>128.0	>128.0	>128.0
	PIPC	3.15	27.9	0.062~ 32.0
Escherichia coli (27)	CDZ	0.03	0.13	0.015~ 0.25
	CTX	0.02	0.07	0.008~ 0.125
	CTM	0.06	0.17	0.031~ 0.25
	CEZ	1.1	5.76	0.5 ~ 16.0
	PIPC	1.0	23.2	0.062~>128.0
Citrobacter sp. (21)	CDZ	2.52	126.0	0.125~>128.0
	CTX	0.11	5.4	0.062~ 32.0
	CTM	1.08	25.49	0.062~ 128.0
	CEZ	>128.0	>128.0	1.0 ~>128.0
	PIPC	1.96	24.88	1.0 ~ 32.0
Salmonella sp. (26)	CDZ	0.05	0.46	≤0.002~ 0.5
	CTX	0.02	0.07	≤0.002~ 0.125
	CTM	0.05	0.27	0.008~ 0.5
	CEZ	0.88	2.68	1.0 ~ 4.0
	PIPC	0.29	6.18	0.004~ 8.0
Klebsiella sp. (25)	CDZ	0.07	0.33	≤0.002~>128.0
	CTX	0.03	0.19	0.004~ 8.0
	CTM	0.09	0.61	0.015~>128.0
	CEZ	0.13	14.19	1.0 ~>128.0
	PIPC	4.59	74.92	0.5 ~>128.0
Enterobacter sp. (25)	CDZ	0.44	19.24	≤0.002~>128.0
	CTX	0.12	4.42	≤0.002~>128.0
	CTM	0.96	61.57	0.062~>128.0
	CEZ	35.44	>128.0	2.0 ~>128.0
	PIPC	1.96	24.88	0.062~>128.0
Serratia marcescens (24)	CDZ	1.92	15.22	0.5 ~ 64.0
	CTX	0.37	1.33	0.125~ 4.0
	CTM	53.59	>128.0	4.0 ~>128.0
	CEZ	>128.0	>128.0	>128.0
	PIPC	3.01	8.39	2.0 ~>128.0
Proteus mirabilis (23)	CDZ	0.004	0.02	≤0.002~ 0.031
	CTX	0.01	0.03	0.008~ 0.062
	CTM	0.27	3.22	0.125~ 4.0
	CEZ	0.25	80.63	2.0 ~>128.0
	PIPC	0.24	0.67	0.062~ 128.0

Table 1. (Continued).

Organism (No. of strains)	Antibiotic	MIC (μg/ml)		
		50%	90%	Range
Proteus rettgeri (12)	CDZ	0.004	0.62	≤0.002~ 2.0
	CTX	0.005	0.22	≤0.008~ 0.5
	CTM	0.16	14.81	0.062~ 64.0
	CEZ	1.8	>128.0	0.062~>128.0
	PIPC	1.8	64.0	0.031~ 64.0
Proteus vulgaris (12)	CDZ	0.03	8.97	0.008~ 64.0
	CTX	0.01	0.27	0.015~ 1.0
	CTM	6.86	>128.0	0.125~>128.0
	CEZ	>128.0	>128.0	4.0 ~>128.0
	PIPC	0.32	4.93	0.25 ~>128.0
Morganella morganii (22)	CDZ	0.13	3.4	0.031~ 8.0
	CTX	0.04	2.06	≤0.002~ 8.0
	CTM	0.39	14.49	0.008~>128.0
	CEZ	>128.0	>128.0	>128.0
	PIPC	1.2	34.4	0.031~ 128.0
Haemophilus influenzae (11)	CDZ	≤0.002	<0.004	≤0.002~ 0.004
	CTX	≤0.002	<0.008	≤0.002~ 0.008
	CTM	0.01	0.65	0.008~ 2.0
	CEZ	1.12	4.8	0.5 ~ 8.0
	PIPC	0.1	1.0	0.25 ~ 2.0

CDZ: cefodizime.

CTM and CEZ possess higher activity against this species. Against *Pseudomonas aeruginosa* cefodizime showed only very limited activity. The *Enterobacteriaceae* were highly susceptible to cefodizime. The 90% of the strains of *E. coli, Salmonella* sp., *Klebsiella* sp., *Proteus mirabilis* and *Proteus rettgeri* were inhibited by cefodizime below 0.5 μg/ml. The vast majority of strains of *Enterobacter* sp., *Serratia marcescens, Proteus vulgaris* and *Morganella morganii* were also susceptible to cefodizime. At therapeutically relevant concentrations of 8~16 μg/ml cefodizime inhibited more than 60% of the strains of *Citrobacter* sp.

Comparison of the MIC_{50} and MIC_{90} of cefodizime with those of the other compounds revealed that cefodizime possesses an *in vitro* activity against *Enterobacteriaceae* which is comparable to CTX, thus surpassing CEZ, CTM and PIPC for most of the species tested.

Haemophilus influenzae was extremely susceptible to cefodizime. The MIC_{90} was calculated to be <0.004 μg/ml. Cefodizime was the most active β-lactam antibiotic in this test series.

Activity against Anaerobes

Table 2 shows the MICs of cefodizime and the reference compounds against Gram-positive and Gram-negative anaerobes. It can be seen that cefodizime has virtually the same *in vitro* activity as CTX against Gram-positive anaerobes (peptostreptococci, *Propionibacterium,* clostridia). Moreover no significant differences were detected between cefodizime and CTX as regards their activity against sensitive representatives of the *Bacteroidaceae.* For these organisms the MICs of cefodizime were below 5 μg/ml, sometimes even below 1 μg/ml, thus surpassing CTM, CEZ and PIPC for these strains. CTX-resistant strains are also resistant to cefodizime.

Table 2. *In vitro* activity against obligate anaerobes.

Organism	MIC (μg/ml)				
	CDZ	CTX	CTM	CEZ	PIPC
Bacteroides fragilis 312	>100.0	>100.0	>100.0	>100.0	>100.0
B. fragilis 960	>100.0	>100.0	100.0	100.0	100.0
B. fragilis 1313	5.0	2.5	50.0	12.5	12.5
B. fragilis 17390	2.5	2.5	50.0	12.5	6.25
B. fragilis 18125	5.0	10.0	50.0	25.0	6.25
B. fragilis 19016	1.25	5.0	25.0	50.0	50.0
B. ovatus 103	>100.0	50.0	>100.0	50.0	50.0
B. vulgatus 1446	>100.0	>100.0	100.0	50.0	50.0
B. thetaiotaomicron 123	>100.0	25.0	100.0	50.0	50.0
B. thetaiotaomicron 1428	>100.0	25.0	100.0	50.0	50.0
B. thetaiotaomicron 1445	>100.0	25.0	100.0	50.0	50.0
B. distasonis 1366	0.039	0.039	12.5	6.25	25.0
Sphaerophorus varius 5262	0.156	0.156	100.0	50.0	0.391
S. varius 3085	>100.0	25.0	12.5	25.0	50.0
S. freundii 1369	100.0	>100.0	3.125	0.781	6.25
Peptostreptococcus anaerobius 932	1.25	0.313	6.25	0.195	6.25
Propionibacterium acnes 6919	0.019	0.078	0.195	0.195	0.781
P. acnes 6922	0.019	0.078	0.195	0.195	0.781
Clostridium tetani 19406	2.5	10.0	1.563	0.195	0.781
C. perfringens 194	0.039	0.625	3.125	0.195	0.391

Activity against *N. gonorrhoeae*

Cefodizime was the most active compound against *N. gonorrhoeae* (Table 3). Cefodizime inhibited 90% of the strains at 0.21 μg/ml. All 28 test strains (including 22 benzylpenicillin resistant isolates) were inhibited by cefodizime at 0.250 μg/ml. CEZ had only a weak activity against these selected strains of *N. gonorrhoeae*.

Table 3. *In vitro* activity against 28 strains of *N. gonorrhoeae* (6 benzylpenicillin-sensitive and 22 benzylpenicillin-resistant strains).

Antibiotic	MIC (μg/ml)		
	50%	90%	Range
CDZ	0.07	0.21	<0.016~ 0.25
CTX	0.022	0.52	<0.016~ 1.0
CTM	0.09	0.831	<0.016~ 4.0
CEZ	5.66	26.6	0.125~>32.0

Influence of the Inoculum Size on the MIC

The MICs of cefodizime determined with different sizes of inocula of various test organisms are listed in Table 4. From these values it can be deduced that the inoculum size has little or no effect on the *in vitro* activity of cefodizime against β-lactamase free organisms (*S. aureus* SG 511, *E. coli* O 55). Furthermore, even very large inocula of bacteria which form the clinically important plasmid-coded β-lactamases only slightly impair the activity of cefodizime (*E. coli* TEM, Rms 212). However, a marked influence on the *in vitro* activity of cefodizime was detectable with organisms whose ability to produce a β-lactamase is chromosomally coded, especially β-lactamases of RICHMOND types I and IVc.

Effect of the Composition and pH of the Culture Medium on the MIC

The composition of the test medium had no effect on the *in vitro* activity of cefodizime (Table 5). In eight commercial culture media, there was only a slight difference between the MICs of cefodizime for *S. aureus* SG 511, *E. coli* TEM, *E. coli* O 55 and *E. coli* 1507E. Bacto Tryptone 1% was the only test medium for which a slight increase in MICs was detected. The pH of the culture medium also

Table 4. Influence of the inoculum size on the *in vitro* activity of cefodizime.

Organism	MIC (μg/ml) at an initial count of				
	1×10^8 (cfu/ml)	1×10^6 (cfu/ml)	1×10^4 (cfu/ml)	1×10^3 (cfu/ml)	1×10^2 (cfu/ml)
Staphylococcus aureus SG 511	6.25	6.25	3.125	3.125	3.125
Escherichia coli O 55	0.156	0.078	0.078	0.078	0.078
E. coli TEM	0.313	0.313	0.313	0.313	0.156
E. coli Rms 212	0.5	0.5	0.5	0.5	0.25
Klebsiella aerogenes 1082E	>100.0	100.0	50.0	25.0	25.0
Enterobacter cloacae P99	1,000.0	1,000.0	500.0	500.0	250.0
E. freundii GN 346	500.0	125.0	125.0	62.5	31.25
Proteus vulgaris GN 76	1.0	0.062	0.062	0.008	0.008
Citrobacter sp. J20	1,000.0	31.25	7.813	3.906	0.976

Table 5. Dependence of the *in vitro* antibacterial activity of cefodizime on the culture medium.

Test medium		MIC (μg/ml)			
		S. aureus SG 511	*E. coli* O 55	*E. coli* TEM	*E. coli* 1507E
Mueller-Hinton medium	(Difco)	6.25	0.313	0.625	0.156
ABM III	(Difco)	6.25	0.156	0.625	0.313
Standard I	(Merck)	6.25	0.156	0.625	0.313
Heart infusion	(Difco)	6.25	0.156	0.625	0.313
Brain heart infusion	(Difco)	12.5	0.313	0.625	0.313
Standard bouillon	(Oxoid)	12.5	0.156	0.625	0.313
Bacto Tryptone 1%	(Difco)	25.0	0.625	2.5	1.25
Isosensitest broth	(Difco)	6.25	0.156	0.625	0.313

Table 6. Dependence of the *in vitro* antibacterial activity of cefodizime on pH.

Heart infusion broth with pH	MIC (μg/ml)	
	S. aureus SG 511	*E. coli* O 55
5.5	0.195	0.156
6.0	0.781	0.156
6.5	1.563	0.156
7.0	3.125	0.156
7.5	3.125	0.156
8.0	3.125	0.156
8.5	12.5	0.156
9.0	25.0	0.156

Table 7. *In vitro* induction of resistance by cefodizime.

Organism	MIC (μg/ml)	
	At the beginning	After 30 passages
Staphylococcus aureus SG 511	6.25	6.25
Salmonella typhimurium	0.313	0.625

had no effect on the *in vitro* activity of cefodizime against *E. coli* O 55 (Table 6). Against *S. aureus* SG 511, however, there was a marked difference between the inhibitory concentrations determined at pH 5.5 (0.195 μg/ml) and those determined at pH 9 (25.0 μg/ml).

Bactericidal Activity

Figs. 2 and 3 show the bactericidal activity of cefodizime against *E. coli* 1507E and *S. aureus* SG 511. The slope of the curves in Fig. 2 demonstrates that the number of surviving *E. coli* 1507E cells decreased steadily after the addition of cefodizime in concentrations corresponding to the MIC and multiples thereof. At 1/2 MIC, cefodizime induced a 2-hour retardation of bacterial growth. With

Fig. 2. Bactericidal activity of cefodizime against *E. coli* 1507E (MIC 0.313 μg/ml).

□ Control, ▲ 1.25 μg/ml, ● 0.625 μg/ml, △ 0.313 μg/ml, ○ 0.156 μg/ml.

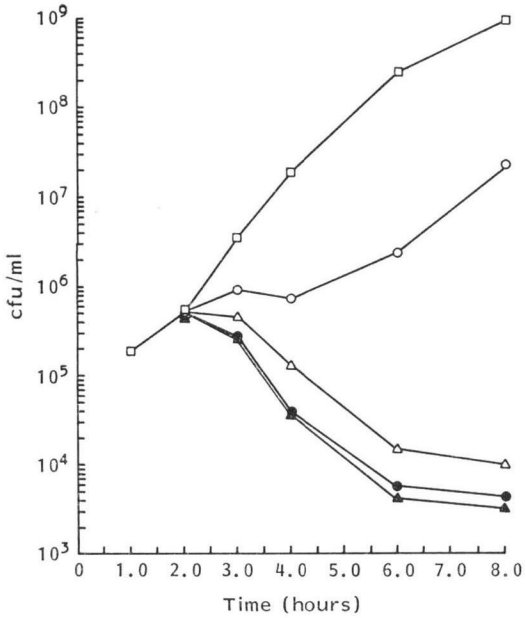


Fig. 3. Bactericidal activity of cefodizime against *S. aureus* SG 511 (MIC 3.125 μg/ml).

□ Control, ▲ 12.5 μg/ml, ● 6.25 μg/ml, △ 3.125 μg/ml, ○ 1.563 μg/ml.

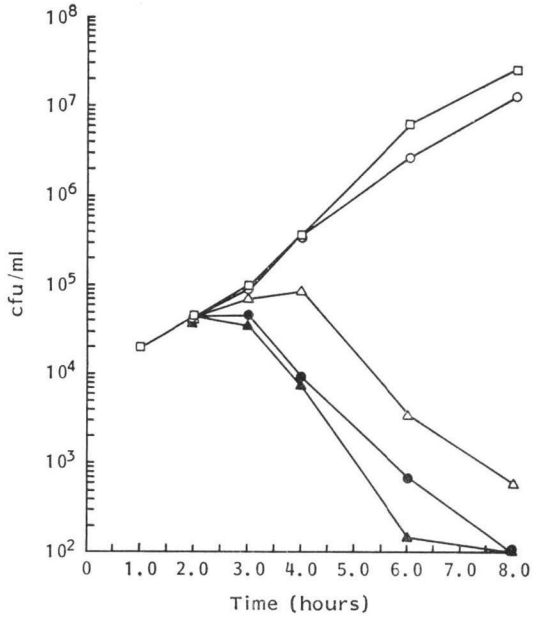


Table 8. β-Lactamase stability of cefodizime, cefotaxime and cefazolin.

Enzyme (RICHMOND class)	Relative rates of hydrolysis*		
	CDZ	CTX	CEZ
Enterobacter cloacae P99 (I)	0.79	0.16	23.0
E. freundii GN 346 (Ia)	3.1	0	83.7
Pseudomonas aeruginosa 18 HS (Ia)	9.8	1.9	57.8
Proteus vulgaris GN 16 (Ic)	32.7	15.6	N.D.
Escherichia coli TEM (IIIa)**	0	0	16.7
E. coli Rms 212$^+$ (IIIb)**	2.8	0	N.D.
Klebsiella aerogenes 1082E (IVc)	16.0	5.9	101.9
Citrobacter freundii J 20	25.1	17.7	79.4
Bacteroides fragilis 620	50.0	37.5	N.D.
B. ovatus 103	64.0	22.3	57.3

* Cephaloridine=100.

** Plasmid-mediated β-lactamase.

S. aureus SG 511 (Fig. 3), addition of cefodizime at twice and four times the MIC led after 1 hour to a reduction in cfu/ml. After 8 hours, the cfu/ml were less than 10^2. At the MIC, the number of cfu in the culture increased slightly for 2 hours, but subsequently decreased rapidly. At subinhibitory concentrations (1/2 MIC), *S. aureus* SG 511 was not inhibited by cefodizime.

Induction of Resistance *In Vitro*

Thirty passages of both *S. aureus* SG 511 and *S. typhimurium* under subinhibitory concentrations of cefodizime did not lead to an increase in the MIC (Table 7). The MIC of cefodizime for *S. aureus* SG 511 remained constant at 6.25 μg/ml and that for *S. typhimurium* increased by only 1 dilution step

Table 9. Affinity for the penicillin-binding proteins of *E. coli* K12.

	ID_{50} (μg/ml)			
	PBP 1A/B	PBP 2	PBP 3	PBP 5/6
CDZ	0.1	0.07	<0.005	> 1.0
CTX	0.02	3.8	0.002	10.0
CEZ	0.9	0.7	1.0	>10.0

(0.313 vs. 0.625 μg/ml).

β-Lactamase Stability

Table 8 shows the relative rates of hydrolysis of cefodizime, CTX and CEZ. The data indicate that cefodizime was cleaved by all the β-lactamases which hydrolyze CTX. The rate of decay of cefodizime was approximately 2 to 3 times higher than that of CTX. Cefodizime showed a high degree of stability against the β-lactamases produced by *Enterobacter* and especially against the plasmid-coded enzymes. All β-lactamases hydrolyzed CEZ faster than cefodizime.

Affinity for Penicillin-binding Proteins (PBPs)

Cefodizime like CTX showed high affinity for PBP 3 of *E. coli* K12 (Table 9). Its affinity for PBP 1A/B was more than 20 times lower. The rate of binding of CEZ to these important proteins is lower than that of cefodizime.

At concentrations below the MIC, the binding pattern of both cefodizime and CTX can also be seen in formation of filaments. At MIC and higher concentrations, protoplasts are formed. Osmotic differences cause the protoplasts to burst, thereby leading to bacteriolysis.

Discussion

The antibacterial spectrum of cefodizime is typical of an aminothiazolyl cephalosporin. As shown in Table 1, the spectrum of cefodizime included nearly all the test strains. The 90% of the test strains of *Staphylococcus* sp. and *Streptococcus* sp. (serological groups A, B and C) were inhibited at 10.22 and 0.11 μg/ml respectively.

The MIC_{90} values determined for *E. coli, Klebsiella* sp., *P. mirabilis* and *P. rettgeri* were lower than 1 μg/ml. *P. vulgaris* and *M. morganii* were also susceptible to cefodizime (MIC_{90} 3.4 and 8.9 μg/ml respectively). The majority of the strains of *S. marcescens, Enterobacter* sp. and *Citrobacter* sp. were inhibited by cefodizime at concentrations easily achievable *in vivo*.

H. influenzae was highly susceptible to cefodizime (MIC_{90} 0.004 μg/ml).

The activity of cefodizime against anaerobic bacteria is very similar to that of CTX (Table 2). Sensitive strains of *Bacteroides fragilis* were inhibited by cefodizime at concentrations of 5 μg/ml and below. Like the reference compounds, cefodizime was inactive against CTX-resistant strains.

N. gonorrhoeae was inhibited by cefodizime at concentrations below 0.25 μg/ml (Table 3). This was true not only for the benzylpenicillin sensitive strains but also for benzylpenicillin resistant isolates. Our data agree with results published recently[3,8,9]. On the basis of the study it can be said that the antibacterial spectrum and degree of *in vitro* activity of cefodizime is somewhat inferior to that of CTX. However, it possesses a lower MIC than CEZ, CTM or PIPC for the most genera in its spectrum.

The *in vitro* antibacterial activity of cefodizime against *E. coli* O 55 was not influenced significantly by the pH or composition of the test medium (Tables 5 and 6). The higher MIC at pH 9.0 against *S. aureus* SG 511 in comparison to lower pH values is obviously due to different susceptibilities of this strain at various pH values as we find similar results with this strain also with other cephalosporin derivatives. The MICs of cefodizime for β-lactamase negative bacteria or test strains producing a plasmid-mediated β-lactamase were not dependent on the density of inoculum used (Table 4). Continuous cultivation (30 passages) of one strain of each of *S. aureus* and *E. coli* under subinhibitory concentrations of cefodizime did not induce an increase in MIC against these strains (Table 7) such as has been reported for other cephalosporins including CEZ[10].

The high *in vitro* activity of cefodizime against *Enterobacteriaceae* and *N. gonorrhoeae* resistant

to the so-called second-generation cephalosporins or penicillins together with the inoculum-independence of its *in vitro* activity can be attributed to the compound's β-lactamase stability (Table 8). We found that only chromosomally coded β-lactamases and enzymes from *Bacteroides* sp. hydrolyze cefodizime. Virtually no cleavage was measured with the plasmid-mediated β-lactamases, which are the enzymes of greatest clinical importance.

Because of its high affinity for PBPs 1A/B and 3 (Table 9), cefodizime exhibits a rapid bactericidal activity. It efficiently killed both *E. coli* and *S. aureus* at concentrations corresponding to the MIC and above (Figs. 2 and 3).

In conclusion, cefodizime is a new aminothiazolyl cephalosporin with a broad range and high degree of antibacterial activity. It acts predominantly against streptococci (except enterococci), *Enterobacteriaceae, N. gonorrhoeae* and *H. influenzae*. Because of its high stability to β-lactamases, cefodizime inhibits bacteria resistant to penicillins and/or older cephalosporins. Like other cephalosporins cefodizime is a bactericidal antibiotic.

Finally, it must be emphasized that the chemotherapeutic assessment of an antibiotic should not be based solely on examination of *in vitro* properties. The potential value of cefodizime should also be established by investigations of its pharmacokinetic behavior and, in particular, of its efficacy in experimental infections in animals.

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References

1) BUCOURT, R.; D. BORMANN, R. HEYMES & M. PERRONNET: Chemistry of cefotaxime. J. Antimicrob. Chemother. 6, Suppl. A: 63～67, 1980
2) SCHRINNER, E.; M. LIMBERT, R. HEYMES & W. DÜRCKHEIMER: Cefotaxime. *In* Beta-lactam Antibiotics. pp. 120～127, Japan Sci. Soc. Press, Tokyo; Springer Verlag; Berlin; Heidelberg; New York, 1981
3) JONES, R. N.; A. L. BARRY, C. THORNSBERRY & H. W. WILSON: *In vitro* antimicrobial activity evaluation of cefodizime (HR 221), a new semisynthetic cephalosporin. Antimicrob. Agents Chemother. 20: 760～768, 1981
4) KLESEL, N.; M. LIMBERT, K. SEEGER, G. SEIBERT, I. WINKLER & E. SCHRINNER: Cefodizime, an aminothiazolylcephalosporin. II. Comparative studies on the pharmacokinetic behavior in laboratory animals. J. Antibiotics 37: 901～909, 1984
5) SEIBERT, G.; M. LIMBERT & N. KLESEL: Comparison of the antibacterial in *in vitro* and *in vivo* activity of ofloxacin (HOE 280, DL 8280) and nalidixic acid analogues. Eur. J. Clin. Microbiol. 2: 548～553, 1983
6) REYN, A.: Laboratory diagnosis of gonococcal infections. Bull. Wld. Hlth. Org. 32: 449～469, 1965
7) SCHWARZ, U.; K. SEEGER, F. WENGENMAYER & H. STRECKER: Penicillin-binding proteins of *Escherichia coli* identified with a ^{125}I-derivative of ampicillin. FEMS Microbiol. Lett. 10: 107～109, 1981
8) AHONKHAI, V. I.; C. E. CHERUBIN & M. A. SHULMAN: *In vitro* activity of cefodizime (HR 221). Antimicrob. Agents Chemother. 22: 715～718, 1982
9) SCULLY, B. E.; K. JULES & H. C. NEU: *In vitro* activity and β-lactamase stability of cefodizime, an aminothiazolyl iminomethoxy cephalosporin. Antimicrob. Agents Chemother. 23: 907～913, 1983
10) NOTO, T.; T. NEHASHI, H. ENDO, M. SAITO, S. MATSUBARA, Y. HARADA, S. SUZUKI, H. OGAWA, K. KOYAMA, Y. KANEKO & S. GOTO: Ceftezole, a new cephalosporin C derivative. I. *In vitro* and *in vivo* antimicrobial activity. J. Antibiotics 29: 1058～1070, 1976