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Comparison of Activity and Beta-Lactamase Stability of Cefotaxime with Those of Six Other Cephalosporins

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A study of the susceptibility to cefotaxime and six other cephalosporins in 213 nonselected strains of nine different bacterial species clearly showed that cefotaxime was the most active against aerobic gram-negative bacilli. The same pattern emerged with 84 cephalothin-resistant strains of five enterobacterial species, but the mean minimal inhibitory concentration values for all cephalosporins were about twofold higher in this group of strains. Cephalothin was the most active antibiotic against *Staphylococcus aureus*. The inoculum effect of 10 cephalothin-resistant strains was relatively small, but it was most marked for cefamandole, as compared with that of three other new cephalosporins, including cefotaxime. The susceptibility of these cephalosporins to beta-lactamases from 12 beta-lactamase-producing enterobacterial strains was determined. Half of these were slightly active against cefotaxime and had similar activity against cefuroxime. Cefoxitin was not degraded at all, and cefamandole was the most susceptible. No correlation between beta-lactamase susceptibility and minimal inhibitory concentration values of different cephalosporins was found. Cefotaxime combined high intrinsic antibiotic activity with marked resistance to beta-lactamase inactivation.

Several new cephalosporins became available and have been investigated in the last few years. Among these are cefamandole, which has high intrinsic antibacterial activity (2, 3), cefuroxime, which appears to be more active than cephalothin and shows resistance to inactivation by beta-lactamases (5, 9), and cefoxitin, which combines a high degree of beta-lactamase resistance with activity against many anaerobes (10, 13). The new cephalosporin, cefotaxime (HR 756), has been studied in vitro (1, 6, 7, 12) and appears to be promising in view of its low minimal inhibitory concentration (MIC) values for cephalothin-resistant *Enterobacteriaceae*.

In this report we present additional data on the high activity of cefotaxime; we also compare this antibiotic with other cephalosporins with regard to their susceptibility to beta-lactamases and its relationship to their MIC values.

MATERIALS AND METHODS

Antibiotics. Cefotaxime was a gift of Roussel Laboratories, Hoevelaken, The Netherlands; cefamandole, cefazolin, and cephalothin were obtained from Eli Lilly Benelux, Brussels, Belgium; cefradin was from Gist-Brocades, Delft, The Netherlands; cefoxitin was from Merck Sharp & Dohme, Haarlem, The Netherlands; and cefuroxime was from Glaxo, Hoofddorp, The Netherlands. Fresh dilutions of the antibiotics were prepared daily before use.

Bacterial strains. For the first series of tests, the strains were 213 randomly selected isolates from the clinical laboratory of the University Hospital, Leiden. From this group of strains and from our laboratory culture collection, 84 cephalothin-resistant strains were selected for the appropriate tests.

Susceptibility testing. The MIC was determined by an agar dilution technique. Twofold dilutions were prepared in Isosensitest agar (Oxoid Ltd., London, England). The inoculum was prepared by diluting 18-h broth cultures to 10^6 organisms per ml as determined with a nephelometer. Inoculation of media was performed with a multipoint inoculator (Denley Instruments Ltd., Bolney, Sussex, England), yielding approximately 0.001 ml/spot. Each spot contained about 10^3 cells. After 18 h of incubation, MIC values were determined as the lowest concentration of antibiotic which prevented bacterial growth. For the determination of the inoculum effect, inocula of 10^6 organisms per spot were also used.

Preparation of cell-free extracts. Cells were pregrown overnight in 5 ml of 3% brain heart infusion medium (Oxoid) at 37°C in a shaker. A 1-ml portion of each culture was used to inoculate 300 ml of brain heart infusion medium, which was subsequently incubated at 37°C. After 2 h of incubation, cephalothin was added at a final concentration of 25 μg/ml to induce beta-lactamase production. After another 3 h of incubation, cells were harvested by centrifugation, washed twice with 0.05 M sodium-potassium phospate buffer (pH 7.0), resuspended in 10 ml of phosphate buffer, and subsequently disrupted in a 100-W MSE ultrasonic disintegrator for 25 s. The sonicated suspension was centrifuged at 4°C for 20 min at 100,000 × *g*. The supernatants (cell-free extracts) were stored at −20°C.

Beta-lactamase assay. Beta-lactamase activity was assayed essentially by the UV spectrophotometric method of O'Callaghan et al. (8). The reaction mixture contained 0.05 to 0.1 μmol of cephalosporin antibiotic and 10 μl of cell-free extract (10 to 25 mg of protein per ml) per ml of 0.1 M sodium-potassium phosphate buffer at pH 7.0. In the blank control cuvette, the antibiotic was omitted. The assays were carried out at room temperature. The optimal wavelengths were determined as the wavelengths with a maximal difference between the spectra of the nonhydrolyzed and those of the corresponding enzymatically hydrolyzed cephalosporin antibiotics. For this hydrolysis of the beta-lactam bond, Whatman beta-lactamase derived from *Bacillus cereus* 569/H9 was used. The optimal wavelengths were 272 nm for cefamandole and 262 nm for cephalothin, cefuroxime, and cefotaxime. Cefoxitin was not hydrolyzed by the Whatman beta-lactamase; for assay, the wavelength of 262 nm was chosen.

RESULTS

The cumulative results of MIC determinations on the nonselected strains are given as the concentrations of the cephalosporins which inhibited 50 and 90% (MIC_{50} and MIC_{90}), respectively, of the strains (Table 1). Except for *Staphylococcus aureus*, all of the organisms tested were most susceptible to cefotaxime, the median of the MIC values being 4 to 128 times lower than those of the next most active cephalosporin investigated. A comparison of the MIC values for cefotaxime, cefamandole, cefuroxime, and cefoxitin against randomly selected strains of five species (*Escherichia coli, Klebsiella, Enterobacter*, indole-positive *Proteus*, and indole-negative *Proteus*) and against cephalothin-resistant strains of the same species yielded only small differences between two groups of strains. MIC_{50} and MIC_{90} values of all four cephalosporins were about twofold higher for the cephalothin-resistant strains, except for MIC_{90} values for cefoxitin, which were fourfold higher in the cephalothin-resistant group. The effect of the inoculum size on the MIC values for the same four cephalosporins was determined by calculating the geometric means of the ratios of the MIC values obtained with two inocula (10^3 and 10^6 colony-forming units) of 10 strains (2 strains each of the same five species). The mean ratios were 5.28 for cefamandole and about 3 for cefotaxime, cefuroxime, and cefoxitin. These differences were not statistically significant ($0.05 < P < 0.10$).

The stability to beta-lactamase of the same four cephalosporins, together with that of cephalothin, was determined. Separate sonic extracts of 12 strains belonging to five genera were prepared for determining beta-lactamase susceptibility. The specific enzyme activities were calculated as micromoles per minute per milligram of protein. Data on these activities, relative to those of cephalothin, are presented in Table 2. In each alternate column, the MIC values of the same strains are given to emphasize the lack of a relationship between beta-lactamase susceptibility and MIC data. The beta-lactamase susceptibility of cefamandole was usually about the same as that of cephalothin. Cefoxitin was completely resistant to beta-lactamases present in the sonic extracts tested, and cefotaxime and

TABLE 1. *Comparative activity of cefotaxime and six other cephalosporins against nine bacterial genera or species*

Antibiotic	%[a]	MIC (μg/ml)								
		E. coli (30)[b]	*Enterobacter* (20)	*Klebsiella* (30)	*Proteus*, indole positive (18)	*Proteus*, indole negative (20)	*P. aeruginosa* (28)	*H. influenzae* (19)	*S. aureus* (20)	*Enterococcus* (28)
Cefotaxime	50	0.06	0.12	0.03	0.03	0.015	16	0.004	2	8
	90	0.12	0.5	0.12	0.06	0.06	32	0.015	4	>128
Cefuroxime	50	4	8	4	64	2	>128	0.5	1	>128
	90	16	16	16	128	16	>128	1	1	>128
Cefamandole	50	2	2	8	4	2	>128	0.25	0.25	32
	90	16	16	32	32	8	>128	0.5	0.5	32
Cefoxitin	50	4	128	4	4	4	>128	1	2	>128
	90	16	>128	16	16	16	>128	2	4	>128
Cefazolin	50	2	32	4	128	8	>128	1	0.25	16
	90	16	>128	>128	>128	32	>128	2	0.5	32
Cefradin	50	16	32	8	128	32	>128	8	4	64
	90	32	>128	64	>128	>128	>128	16	8	64
Cephalothin	50	8	64	16	>128	8	>128	0.5	0.25	32
	90	64	>128	128	>128	>128	>128	2	0.25	32

[a] Percentage of strains inhibited at a given concentration of antibiotic.
[b] Number of strains.

TABLE 2. *Beta-lactamase activity of cell-free extracts of cephalothin-resistant strains against cephalothin, cefotaxime, cefuroxime, cefamandole, and cefoxitin*

Strain		Cefotaxime		Cefuroxime		Cefamandole		Cefoxitin	
		Activity[a]	MIC	Activity	MIC	Activity	MIC	Activity	MIC
Klebsiella	D2982	0.25	0.5	6.7	32	200	32	tr	32
	E6148	11.6	0.06	8.3	4	133	64	0	32
	8105	0	0.12	0	16	58	32	0	>128
Enterobacter	101164	0	1	0	32	183	32	0	>128
	AZL1	0	1	0	8	0	4	0	128
E. coli	L123	0.1	1	0.5	64	64	16	tr	64
	104050	0	2	0	32	0.5	16	0	32
	E3320	0.9	0.06	2.7	64	336	16	0	8
	D8808	0	4	1.7	64	167	>128	0	>128
Proteus vulgaris	2566	0.4	12	0.6	8	23	8	0	2
	B1392	10	0.06	10	64	195	32	tr	64
Proteus mirabilis	M7	0	0.03	0	32	2.4	4	0	16
Whatman beta-lactamase		58		62		187		0	

[a] Calculated as micromoles per minute per milligram of protein, but given as a percentage of activity against cephalothin (100%).

cefuroxime were only slightly susceptible in tests with six sonic extracts and proved to be resistant to six other cell-free extracts.

DISCUSSION

The low MIC values found for cefotaxime (Table 1) agree well with the results published by others (6, 12). The high intrinsic activity even extends to *Pseudomonas aeruginosa*, which shows MIC values of about 16 μg/ml, a concentration which may be attained in vivo. *Haemophilus influenzae*, although susceptible to most cephalosporins, shows marked susceptibility to cefotaxime, also in agreement with earlier reports (1, 6). Only *S. aureus* is more susceptible to cephalothin, with cefazolin and cefamandole being next in order.

The difference between cefamandole and the other three new cephalosporins with regard to the effect of inoculum size, although not statistically significant, seems to reflect the differences in susceptibility to beta-lactamase, as shown in Table 2.

Rates of hydrolysis of cefotaxime by beta-lactamases have only been published by Fu and Neu (4), who also used the spectrophotometric method. Their results show differences from ours since they could not find any beta-lactamase activity against cefotaxime and cefuroxime, whereas we did find some activity against these two antibiotics, i.e., up to 10% of the activity against cephalothin. Other types of strains used for preparing the cell-free extracts or small differences in techniques may explain these differences. Cefoxitin could not be found to be hydrolyzed in either investigation.

The most intriguing phenomenon is lack of a relationship between MIC values and beta-lactamase susceptibility, which has already been recorded for several new cephalosporins (3, 11).

Cefamandole is about equally susceptible to most beta-lactamases as is cephalothin, but the MIC values are much lower than those of cefoxitin, which is not hydrolyzed at all. However, a closer look at the data for cefamandole suggests some correlation, i.e., the higher beta-lactamase activity of strains usually corresponding with higher MIC values and vice versa.

For the purpose of choosing from among these new cephalosporins for clinical use, it has yet to be elucidated what consequences accrue from the lack of the usual relationship between MIC values and beta-lactamase susceptibility. Does this mean that the beta-lactamase susceptibility is unimportant as a parameter for clinical usefulness, or does it imply that we should be careful in using the MIC as such a parameter?

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