

In Vitro Antibacterial Activity and Susceptibility of Cefsulodin, an Antipseudomonal Cephalosporin, to Beta-Lactamases

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Cefsulodin sodium (SCE-129, CGP-7174/E), active in minimum inhibitory concentrations (MICs) of 0.5 to 64 $\mu\text{g/ml}$, was about 16- to 32-fold more active than carbenicillin against *Pseudomonas aeruginosa*. It was also active against *P. diminuta*, *P. maltophilia*, *P. paucimobilis*, and *P. pseudoalcaligenes* (MICs of 1 to 32 $\mu\text{g/ml}$) but not against other species of *Pseudomonas* or other gram-negative bacteria. Except with highly carbenicillin-resistant isolates, MICs of cefsulodin for *P. aeruginosa* were little affected by an increase in the inoculum. With a small inoculum, minimum bactericidal concentrations (MBCs) were the same as or twice the MIC, but increasing the inoculum had a greater effect on the MBC than on the MIC. Cefsulodin was not hydrolyzed by the β -lactamase induced in *P. aeruginosa* by growth in the presence of benzylpenicillin and was a poor substrate for β -lactamases from *Enterobacter cloacae* and *Proteus morganii*. However, it was hydrolyzed, albeit slowly, by the β -lactamase produced by most of our highly carbenicillin-resistant isolates of *P. aeruginosa* and by TEM-type β -lactamases.

Cefsulodin sodium, previously known as SCE-129 and CGP-7174/E, is a new cephalosporin active against *Pseudomonas aeruginosa* (12). In this paper we report studies of its activity against *P. aeruginosa* and a range of other gram-negative bacteria, and also studies of its susceptibility to hydrolysis by various β -lactamases.

MATERIALS AND METHODS

Bacteria studied. We studied *P. aeruginosa* (131 isolates), *P. acidovorans* (12 isolates), *P. cepacia* (12 isolates), *P. diminuta* (1 isolate), *P. fluorescens* (13 isolates), *P. maltophilia* (10 isolates), *P. paucimobilis* (3 isolates), *P. pseudoalcaligenes* (1 isolate), *P. putida* (27 isolates), *P. putrefaciens* (1 isolate), *P. stutzeri* (1 isolate), *Acinetobacter anitratus* (10 isolates), *A. lwoffii* (1 isolate), *Citrobacter freundii* (5 isolates), *C. koserii* (7 isolates), *Escherichia coli* (35 isolates), *Enterobacter aerogenes* (6 isolates), *E. cloacae* (18 isolates), *Klebsiella aerogenes* (34 isolates), *K. ozaenae* (5 isolates), *Proteus mirabilis* (20 isolates), *P. morganii* (11 isolates), *P. rettgeri* (2 isolates), *P. vulgaris* (4 isolates), *Providencia stuartii* (11 isolates) and *Serratia* spp. (10 isolates). Most were clinical isolates from St. Thomas' Hospital, but we also studied two isolates of *P. aeruginosa* from Birmingham, England that were known to produce TEM-1 β -lactamase.

MICs and MBCs. For determination of minimum inhibitory concentrations (MICs) and minimum bactericidal concentration (MBCs), organisms were grown in nutrient broth (Southern Group Laboratories) at 37°C for 18 h, suitably diluted, and inoculated by a multiple inoculator onto Diagnostic Sensitivity Test Agar (Oxoid CM 261) containing serial doubling dilu-

tions of the antibiotics. The MIC was the lowest concentration of antibiotic that resulted in complete suppression of growth after overnight incubation at 37°C. Unless otherwise stated, the inoculum consisted of about 10^4 colony-forming units (CFU).

MBCs were determined in serial doubling dilutions of antibiotic in Iso-Sensitest Broth (Oxoid CM 473) inoculated with 20 μl of an appropriately diluted suspension of organisms. Tubes showing no growth after overnight incubation at 37°C were subcultured (one 7- μl loopful) onto Oxoid Columbia Agar (CM 331) containing 6% horse blood. The MBC was defined as the lowest concentration of antibiotic preventing visible growth on the subculture plate after overnight incubation at 37°C.

β -Lactamase studies. Suspensions of cells grown at 37°C in nutrient broth, concentrated 10- to 20-fold, were broken with an MSE ultrasonic disintegrator (MSE Scientific Instruments, Crawley, Sussex, England). Isolates of *E. cloacae* and *P. morganii* were grown in the presence of subinhibitory concentrations of cephaloridine. Unless stated otherwise, other organisms were grown without additions to the medium.

Substrate profiles were determined by the ultraviolet spectrophotometric method (6). The rate of decline in absorbance at 260 nm (cephalosporins) or 235 nm (penicillins) in 10-mm-path length cuvettes containing 200 nmol of antibiotic, 200 μmol of potassium phosphate buffer (pH 7), and 30 to 50 μl of enzyme preparation in a total volume of 2 ml was measured in a Pye Unicam SP8-100 ultraviolet spectrophotometer at 37°C. The method was standardized by measurement of the change in absorbance of each compound after complete hydrolysis by an appropriate β -lactamase or by sodium hydroxide (0.8 N).

Protein concentrations of the enzyme preparations

were measured by the biuret method (1).

Isoelectric focusing was performed on a photopolymerized gel (90 by 100 by 2 mm) consisting of 68 g of acrylamide per liter, 6.6 g of methylenebisacrylamide per liter, 5 g of Ampholine per liter (pH range 3.5 to 10; LKB Instruments Ltd., Selsdon, Surrey, England), and 0.7 mg of riboflavin per liter. Ten-microliter spots of enzyme were applied directly to the gel. Current (maximum 10 mA) was passed for 4.5 h with a maximum potential difference of 600 V. After the proteins had been focused, the pH was measured at 5-mm intervals with a surface electrode and the β -lactamases were stained with nitrocefin (supplied by Glaxo Research).

Statistical methods. The correlation coefficients and regression equations were calculated by the method of least squares after conversion of the MICs to \log_2 MIC.

RESULTS

Antibacterial activity of cefsulodin. Cefsulodin, with MICs of 1 to 4 $\mu\text{g/ml}$ for most isolates, was 16- to 32-fold more active than carbenicillin against *P. aeruginosa* (Table 1), but higher concentrations (8 to 64 $\mu\text{g/ml}$) were needed to inhibit the highly carbenicillin-resistant isolates. MICs of cefsulodin and carbenicillin were well correlated (correlation coefficient = 0.85); exclusion of the highly carbenicillin-resistant isolates that produced β -lactamase constitutively increased the correlation coefficient only to 0.87, but this procedure increased the slope of the line of best fit from 0.49 to 0.76 (Fig. 1).

The effect of an increase in inoculum size on MICs of cefsulodin and carbenicillin for *P. aeruginosa* is shown in Table 2. For few isolates was there more than a four-fold increase in the MIC of either agent. However, important exceptions were isolates from St. Thomas' Hospital that produced β -lactamase constitutively. Five such isolates were studied. An increase in the

inoculum from 10^4 to 10^8 CFU was accompanied mostly by an increase in MICs of cefsulodin from 8 to 16 $\mu\text{g/ml}$ to 128 $\mu\text{g/ml}$.

MICs and MBCs of cefsulodin and carbenicillin were determined in a liquid medium for six isolates of *P. aeruginosa*. With one exception, MICs determined in the liquid medium were similar to those obtained on the solid medium, although they were sometimes a little higher. With the same single exception, MBCs of both agents were the same as or twice the MIC if the inoculum was 10^4 CFU. Inoculum effects on broth-determined MICs were similar to those found on solid medium, but there were large

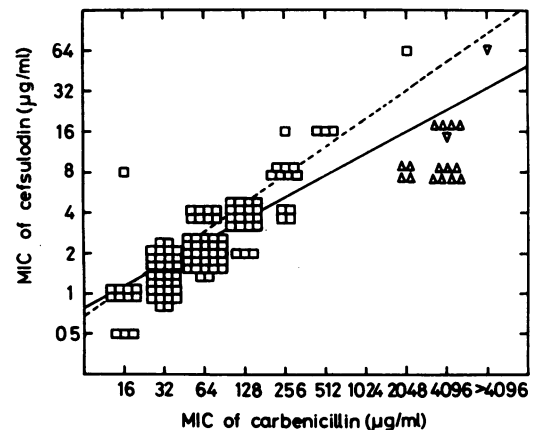


FIG. 1. Comparison of the MICs of cefsulodin and carbenicillin for *P. aeruginosa*. □, Isolates that did not produce β -lactamase constitutively; Δ , β -lactamase-producing isolates from St. Thomas's Hospital; ∇ , β -lactamase-producing isolates from Birmingham; —, calculated line of best fit when MICs for all isolates were included; ---, line of best fit when MICs for isolates that produced β -lactamase were excluded.

TABLE 1. Activity of cefsulodin and carbenicillin against *Pseudomonas* spp., *Acinetobacter* spp., and *Enterobacteriaceae*

Organism	No. of isolates	Concn ($\mu\text{g/ml}$) required to inhibit indicated percentage of isolates							
		Cefsulodin				Carbenicillin			
		50%	75%	90%	100%	50%	75%	90%	100%
<i>P. aeruginosa</i>	131	2	4	8	64	64	128	512	>512
<i>P. acidovorans</i>	12	128	256	512	512	128	256	256	256
<i>P. cepacia</i>	12	32	32	64	128	>512	16	32	128
<i>P. fluorescens</i>	13	256	512	512	<512	32	>512	>512	>152
<i>P. maltophilia</i>	10	8	16	32	32	256	16	16	32
<i>P. putida</i>	27	128	256	256	256	>512	>512	>512	>152
Other species of <i>Pseudomonas</i> ^a	7	16	16	128	128	2	8	64	64
<i>Acinetobacter</i> spp.	11	4	64	64	64	2	32	32	512
<i>Enterobacteriaceae</i>	168	64	128	256	>512	8	512	>512	>512

^a Three isolates of *P. paucimobilis* and one each of *P. diminuta*, *P. pseudoalcaligenes*, *P. putrefaciens*, and *P. stutzeri*.

TABLE 2. Effect of an increase in the inoculum on MICs of cefsulodin and carbenicillin for *P. aeruginosa*

Antibiotic	MIC ($\mu\text{g/ml}$) with an inoculum of 10^4 CFU	No. of isolates with the stated ratio of MICs											
		MIC with an inoculum of 10^8 CFU/ MIC with an inoculum of 10^4 CFU					MIC with an inoculum of 10^8 CFU/MIC with an inoculum of 10^4 CFU						
		1	2	4	8	NT ^a	1	2	4	8	16	32	NT
Cefsulodin	0.5		2	1				1	1		1		
	1	1	18	2				6	13	2			
	2	9	20	3			2	13	16		1		
	4	9	9				2	11	4	1			
	8	2	7				2	2	1		4		
	16	2						1	1	1			
	64	1											
Carbenicillin	16	2	5	3	1			2	8				1
	32	3	21	1				10	12	1	2		
	64	15	11					19	7				
	128	5	7				4	8					
	256	2	3					5					
	2,048					7							7

^a NT, Not tested.

inoculum effects on MBCs, with MBCs of cefsulodin being at least 256 $\mu\text{g/ml}$ and those of carbenicillin being more than 4,096 $\mu\text{g/ml}$ when the inoculum was 10^8 CFU. We observed that, with the intermediate inoculum of 10^6 CFU, the ratio of MBC to MIC tended to be rather higher for cefsulodin than for carbenicillin.

The activities of cefsulodin and carbenicillin on species of *Pseudomonas* other than *P. aeruginosa* are shown in Table 1. The two compounds were more or less equally active against *P. acidovorans* and *P. maltophilia*, but cefsulodin was more active against *P. fluorescens* and *P. putida*, whereas carbenicillin was more active against *P. cepacia*, *P. diminuta*, *P. pseudoalcaligenes*, *P. paucimobilis*, *P. putrefaciens*, and *P. stutzeri*. Cefsulodin was also less active than carbenicillin against *Acinetobacter* spp.

Most isolates of *Enterobacteriaceae* were inhibited by 16 μg of carbenicillin per ml but were less susceptible to cefsulodin (MICs 32 to 128 $\mu\text{g/ml}$ for most isolates, Table 1). However, although not susceptible to either agent, most isolates that could grow in the presence of 64 μg of carbenicillin per ml were less resistant to cefsulodin than to carbenicillin. The same was also true of even more resistant organisms. This category included most isolates of *C. koserii* and *K. aerogenes* as well as a proportion of the isolates from several other species.

β -Lactamase studies. No detectable β -lactamase activity against carbenicillin, cefsulodin, or cephaloridine was found in cultures of five isolates of *P. aeruginosa* that were susceptible (three isolates, MICs 16 to 64 $\mu\text{g/ml}$) or moderately resistant (two isolates, MICs 128 to 256 $\mu\text{g/ml}$) to carbenicillin and that had been incubated

for 5 h in the presence of one-quarter of their MIC of cefsulodin. An isolate for which the MIC of carbenicillin was 2,048 $\mu\text{g/ml}$ and the MIC of cefsulodin was 64 $\mu\text{g/ml}$ also failed to produce β -lactamase.

We also studied 10 isolates of *P. aeruginosa* from St. Thomas' Hospital with MICs of carbenicillin of 2,048 $\mu\text{g/ml}$ and MICs of cefsulodin of 8 to 16 $\mu\text{g/ml}$; nine of these isolates were of the same pyocin type, which indicated probable though unsuspected cross-infection. All the isolates constitutively produced β -lactamases that focused at the same point in an Ampholine pH gradient, with pI of about 5.2. Hydrolysis of cephaloridine occurred at a rate from 0.5 to 1.3 nkat/mg of protein in cultures grown in the absence of antibiotics. Growth in the presence of subinhibitory concentrations of cefsulodin increased β -lactamase activity by 72 and 106% in the two isolates examined. Table 3 shows the substrate profile of the enzyme from one of these isolates (no. 625) together with results for other β -lactamases.

The enzyme from the highly carbenicillin-resistant isolate of *P. aeruginosa* was somewhat similar to TEM-type enzymes but hydrolyzed carbenicillin much more rapidly. It was also less susceptible to inhibition by cloxacillin than was TEM-1 (40% reduction in activity in the presence of 100 μM cloxacillin compared to 90% for TEM-1). Neither cefsulodin nor carbenicillin was a substrate for the Sabbath and Abrahams β -lactamase (9) induced in *P. aeruginosa* NCTC 10662 by growth in the presence of 5,000 μg of benzylpenicillin per ml. Cefsulodin was a poor substrate for the enzymes from *E. cloacae* and *P. morganii*, but some hydrolysis was often de-

TABLE 3. Substrate profiles of various β -lactamases

Enzyme type	Isolate	β -Lactamase activity ^a	Relative hydrolysis rate ^b					
			Cephaloridine	Cefuroxime	Cefsulodin	Benzylpenicillin	Ampicillin	Carbenicillin
TEM-1	<i>E. coli</i> TEM	9.1	100	<2	3.5	635	790	97
TEM-1	<i>P. aeruginosa</i> 69/1822	7.8	100	<2	2.6	564	696	88
TEM-2	<i>E. coli</i> 1725E	43.1	100	<2	4.3	682	907	95
Dalgleish-like	<i>P. aeruginosa</i> 625	1.0	100	<2	13	1,733	1,824	1,670
Cephalosporinase	<i>P. aeruginosa</i> NCTC 10662	2.5	100	<2	<2	152	12	<5
Cephalosporinase	<i>E. cloacae</i> 4.14.76	1.2	100	<2	<2	NT ^c	NT	NT
Cephalosporinase	<i>E. cloacae</i> 6.8.76	32.7	100	<0.2	1.2	NT	NT	NT
Cephalosporinase	<i>P. morgani</i> A131525	16.2	100	<0.3	11	NT	NT	NT
Cephalosporinase	<i>P. morgani</i> A181523	2.7	100	4	<2	NT	NT	NT

^a Nanokatal per milligram of protein with cephaloridine as substrate.

^b Relative to the rate of hydrolysis of cephaloridine.

^c NT, Not tested.

tected and it was mostly more susceptible to these enzymes than was cefuroxime.

DISCUSSION

Our findings that cefsulodin was active against *P. aeruginosa* and *P. maltophilia* and to some extent against *P. paucimobilis*, *P. pseudoalcaligenes* and *P. stutzeri*, but not against other species of *Pseudomonas* or the other gram-negative bacteria that we tested, do not conflict with previous work (11, 12). We agree with Tsuchiya et al. (12) that there was mostly little inoculum effect on the MICs of cefsulodin and carbenicillin and that, with a small inoculum, MBCs of cefsulodin were usually the same as or twice the MIC. However, we found an appreciable inoculum effect on MBCs of both cefsulodin and carbenicillin.

In isoelectric point and substrate profile the enzyme from our highly carbenicillin-resistant isolates of *P. aeruginosa* resembled the β -lactamase produced by the Dalgleish strain of *P. aeruginosa* (5, 9) and also the enzymes from other strains (3, 8). The Dalgleish enzyme is now known as PSE-4 (4) and has been shown to be plasmid determined (1).

Cefsulodin was relatively stable to β -lactamases, being resistant to the inducible Sabath and Abrahams enzyme of *P. aeruginosa* and a poor substrate of the enzymes from *E. cloacae* and *P. morgani*. However, it was hydrolyzed to some extent by TEM-type and PSE-4 β -lactamases, as has been reported before (7, 10). Although the crude extracts of the strains that produced TEM or PSE-4 enzymes were undoubtedly contaminated with chromosomally

determined enzymes, at least for *P. aeruginosa* the hydrolysis of cefsulodin can be wholly attributed to the TEM or PSE-4 enzyme, since the Sabath and Abrahams enzyme does not attack the compound.

It seems probable that the small reduction in susceptibility to cefsulodin shown by the TEM-1- and PSE-4-producing isolates of *P. aeruginosa* was at least partly the result of the slow hydrolysis of the compound. We have come to this conclusion because, compared to those for susceptible isolates, the MICs of carbenicillin for the β -lactamase producers were increased considerably more than the MICs of cefsulodin and because carbenicillin was much more susceptible to the β -lactamases than was cefsulodin. In contrast, carbenicillin-resistant isolates that did not produce TEM-1 or PSE-4 showed increases in resistance to carbenicillin and cefsulodin that were approximately equal.

It seems to us that cefsulodin has a useful, albeit narrow, spectrum of activity and that it might at times be of use in the treatment of infections with *P. aeruginosa*.

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