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Comparative Antipseudomonal Activity of Some Newer β-Lactam Agents

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The antipseudomonal activities of cefotaxime, ceftizoxime, ceftazidime, ceftriaxone, cefoperazone, and moxalactam were tested in conventional minimum inhibitory concentration titrations and according to morphological and turbidimetric criteria. Three groups of pseudomonal strains were tested: carbenicillin hypersusceptible, carbenicillin susceptible, and carbenicillin resistant. In minimum inhibitory concentration titrations, the carbenicillin-hypersusceptible strains did not differ greatly in their susceptibility to other β-lactam agents, although moxalactam appeared to be rather less active than the other drugs. When tested against the remaining strains, ceftazidime was the most active compound, followed by cefoperazone and ceftriaxone. In turbidimetric experiments supported by microscopical observations, all of the agents induced bacterial lysis in the carbenicillin-hypersusceptible strains, but cefoperazone appeared to be less actively bacteriolytic than the rest of the antibiotics. None of the agents was able to prevent growth of the remainder of the *Pseudomonas aeruginosa* strains in the first few hours of drug exposure, during which time the bacteria elongated to form long filaments. However, the various agents differed in the length of time which elapsed before growth was completely halted. Judged in this way, moxalactam was the most active compound in relation to its minimum inhibitory concentration, but in comparative experiments in which the same concentration of each drug (64 μg/ml) was used regardless of the minimum inhibitory concentration, ceftazidime appeared to be the most active antibiotic, followed by ceftriaxone and cefotaxime.

In recent years, a considerable number of β-lactam antibiotics have appeared which include *Pseudomonas aeruginosa* in their antibacterial spectrum (3). The in vitro activity of these compounds against *P. aeruginosa*, as judged by minimum inhibitory concentration (MIC) data, is well documented, but a recent study of eight antipseudomonal β-lactam antibiotics (D. Greenwood and A. Eley, J. Infect. Dis., in press) has led us to believe that MIC data alone may not reveal certain aspects of their comparative antibacterial activity. In that study, continuous turbidimetric monitoring of strains of *P. aeruginosa*, selected on the basis of differential susceptibility to carbenicillin, revealed differences in the response of the bacteria which did not correlate with simple conventional MIC results.

We have now extended the earlier study to examine the response of the selected *P. aeruginosa* strains to six newer antipseudomonal agents (cefotaxime, ceftizoxime, ceftazidime, ceftriaxone, cefoperazone, and moxalactam) which were not included in the previous investigation.

MATERIALS AND METHODS

Antibiotics. Antibiotics were kindly provided by the manufacturers: Roussel Laboratories Ltd. (cefotaxime), Fujisawa Pharmaceutical Co., Ltd. (ceftizoxime), Glaxo Group Research Ltd. (ceftazidime), Hoffmann-La Roche & Co. AG (ceftriaxone), Pfizer Central Research (cefoperazone), and Lilly Research Centre Ltd. (moxalactam). Suitable concentrations were freshly prepared as required in sterile distilled water.

Bacterial strains. Seventeen strains of *P. aeruginosa* were selected for investigation on the basis of their susceptibility to carbenicillin. Thirteen of the strains were clinical isolates, and four were variants of *P. aeruginosa* PAO into which plasmids had been introduced which coded for β-lactamases TEM-1, TEM-2, OXA-3, and PSE-1, respectively. Of the clinical isolates, five exhibited unusual susceptibility to carbenicillin (MIC, 0.25 to 2 μg of carbenicillin per ml), six exhibited normal susceptibility to carbenicillin (MIC, 16 to 64 μg/ml), and two were resistant to carbenicillin (MIC ≥ 256 μg/ml).

Antibiotic titration. MICs of antibiotic were estimated by the agar incorporation method. Serial twofold dilutions of antibiotic were prepared in diagnostic sensitivity test agar (Oxoid Ltd.) containing 5% horse

TABLE 1. Range of MICs obtained with three groups of *P. aeruginosa* strains in agar incorporation titrations with an inoculum of 10^4 bacteria

Carbenicillin susceptibility (no. of strains)	MIC range (μg/ml)						
	Carbenicillin	Cefotaxime	Ceftizoxime	Ceftazidime	Ceftriaxone	Cefoperazone	Moxalactam
Hypersusceptible (5)	0.5–2	≤0.25–4	≤0.25–8	0.25–1	0.25–1	≤0.25–1	4–8
Normal (6)	16–64	4–16	8–32	0.5–1	1–4	2–8	16–32
Resistant (6)	128–>512	4–64	8–256	1–4	4–32	8–32	16–64

blood. The plates were spot inoculated with an automatic multipoint inoculator (Denley-Tech Ltd.), which delivered approximately 10^4 bacteria per spot from overnight broth cultures diluted 1:1,000.

Turbidimetric studies. The growth medium used was "complete" broth (1) supplemented with 1% KNO_3. Ten-milliliter volumes of broth containing appropriate concentrations of antibiotic were inoculated with bacteria from overnight broth cultures to yield an initial concentration of between 10^6 and 10^7 organisms per ml. The cultures were incubated at 37°C in a modified version of the 12-channel continuous opacity monitoring device described by Mackintosh et al. (2). In experiments with carbenicillin-hypersusceptible strains, antibiotic was added during the logarithmic growth phase when bacterial growth had raised the opacity to a level of 30% of maximum, equivalent to a viable count of about 10^8 bacteria per ml.

Microscopy. Antibiotic-induced morphological changes in bacteria were observed in untreated "wet" preparations by interference-contrast microscopy after exposure to antibiotic for 2 h in the turbidimetric system.

RESULTS

MICs. The inhibitory activity of the six compounds for the 17 test strains, as judged by conventional agar dilution titration with an inoculum of ca. 10^4 bacteria, is summarized in Table 1. Ceftazidime was the most active agent against all three groups of strains. Ceftizoxime and moxalactam exhibited the lowest overall activity by MIC criteria.

Morphological response. The morphological response of all cultures was observed after exposure for 2 h to each antibiotic during the course of the turbidimetric experiments. The hypersusceptible strains differed in their response from the other strains in that spheroplast formation was induced by the higher antibiotic concentrations tested. In contrast, no antibiotic was able to elicit the spheroplast response in strains of "normal" β-lactam susceptibility over the range of concentrations tested. Ceftazidime was able

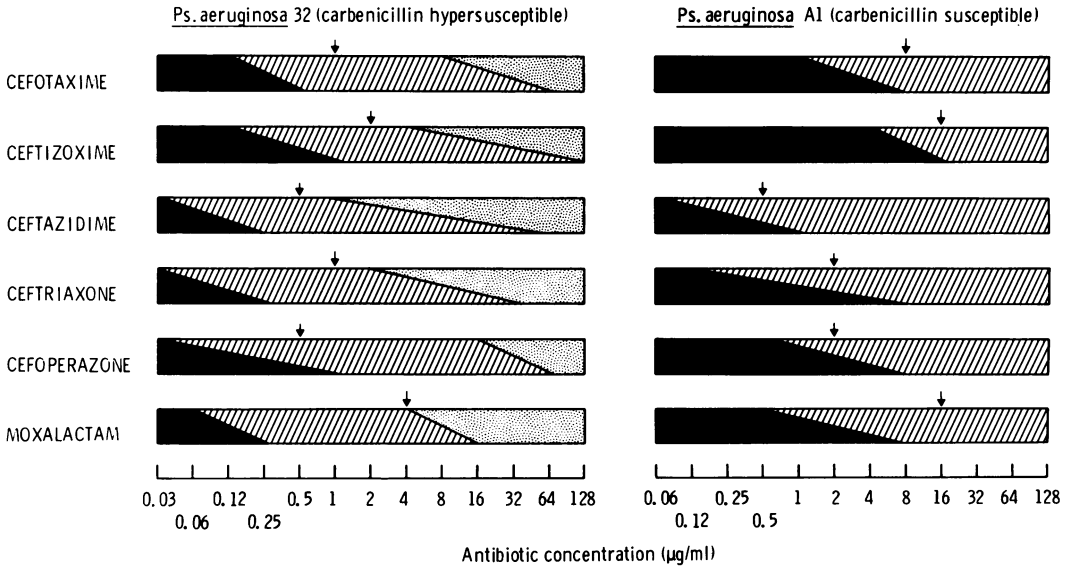

FIG. 1. Morphological response profiles of a carbenicillin-hypersusceptible strain of *P. aeruginosa* and a strain of normal carbenicillin susceptibility. The strains were exposed to each of the six β-lactam antibiotics for 2 h. Black = normal morphology; hatching = filaments; stippling = spheroplasts. Arrow indicates the MICs of the antibiotics for each strain.

to evoke the filamentation response at lower concentrations than the other compounds tested. The range of concentrations over which the six antibiotics induced morphological changes in representative hypersusceptible and normal strains are shown in Fig. 1. Little variation was found between strains of the two susceptibility groups.

No precise correlation was evident between the ability to cause a particular morphological response and the MIC. In the case of moxalactam, the conventionally determined MIC appeared to underestimate markedly the ability of the antibiotic to induce filamentation.

Turbidimetric studies. Since the antibiotics caused spheroplast formation and lysis of the carbenicillin-hypersusceptible strains, it was possible to examine the response of dense populations of these strains turbidimetrically to compare their bacteriolytic activity. Figure 2 shows the time elapsed after the addition of various concentrations of the six antibiotics to exponentially growing cultures of a representative strain of this group before a fall in opacity was observed. Antibiotic was added at a standard point in the growth curve equivalent to a viable count of ca. 10^8 bacteria per ml. Although a spectrum of responses was observed, five of the six agents achieved comparable degrees of bacterial lysis over a similar range of concentrations. The one exception was cefoperazone, which appeared to exhibit rather less bacteriolytic activity.

Because the strains of normal carbenicillin susceptibility responded to exposure to all of the antibiotics by forming filaments, it was not possible to test dense populations of these strains turbidimetrically as the cultures entered the stationary phase of growth before any antibacterial effect was detectable. However, when a bacterial inoculum of between 10^6 and 10^7 bacteria per ml was used, a comparison of antibacterial efficacy could be made. Figure 3 shows the response of an inoculum of ca. 10^6 bacteria of a carbenicillin-susceptible *P. aeruginosa* strain per ml exposed to the six antibiotics at concentrations equivalent to twice the MIC for each

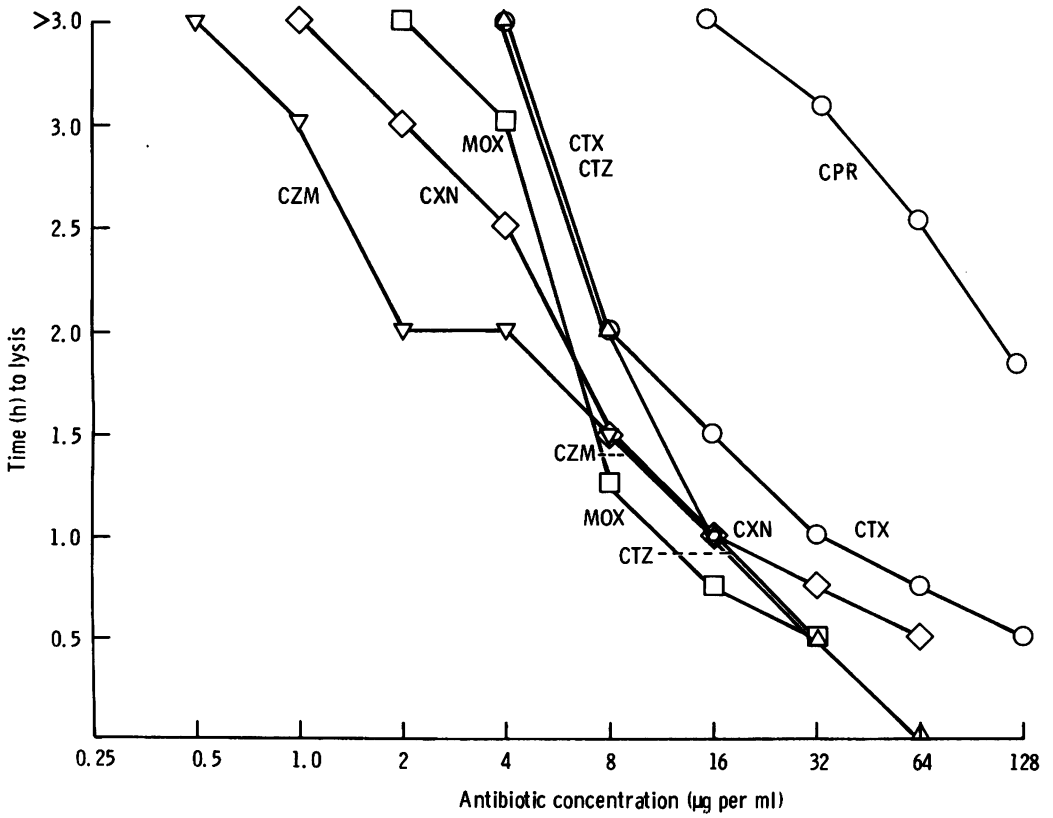

FIG. 2. Lytic response profiles of a carbenicillin-hypersusceptible strain of *P. aeruginosa* (strain 32), showing the time taken to induce a fall in opacity of a dense bacterial culture after exposure to various concentrations of six β-lactam antibiotics. CZM, Ceftazidime; CXN, ceftriaxone; MOX, moxalactam; CTX, cefotaxime; CTZ, ceftizoxime; CPR, cefoperazone.

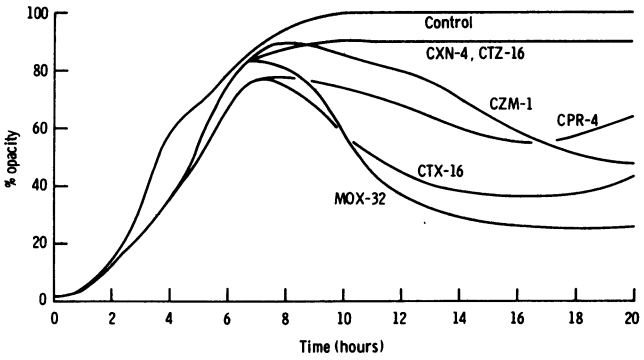

FIG. 3. Continuous opacity records of a carbenicillin-susceptible strain of *P. aeruginosa* exposed (at time zero) to six β-lactam antibiotics at the concentrations (micrograms per milliliter) shown, corresponding to twice the MIC in each case. Abbreviations are as in legend to Fig. 2.

compound. None of the six compounds was able to prevent the opacity from continuing to rise for the first 7 h of drug exposure, although growth slowed slightly. Microscopy confirmed that the bacteria formed filaments during this period. Upon continued incubation, four of the antibiotics (cefotaxime, cefoperazone, ceftazidime, and moxalactam) evoked a subsequent fall in opacity, which was sustained by ceftazidime and moxalactam for the entire 20-h period of observation. Three other *P. aeruginosa* strains were tested at a concentration of twice the MIC of each antibiotic. Moxalactam achieved its antibacterial effect more rapidly than the other compounds in each case.

The amount of residual bacterial growth allowed by the antibiotics was found to be remarkably reproducible, provided that the initial inoculum was carefully standardized. Lowering the amount of inoculum had a marked effect on the response. The effect was particularly striking with cefoperazone (Fig. 4), with which both the initial increase in opacity and subsequent regrowth were totally abolished. With the lower inoculum, the initial opacity increase may have been partially obscured by the poor sensitivity of the turbidimetric system at population densities below ca. 10^6 bacteria per ml.

Turbidimetric experiments were also carried out in which the cultures were exposed to a concentration of 64 μg of each antibiotic per ml, a level that might be achieved in serum by high dosage. The measure of antibacterial efficacy was taken as the amount of residual growth, as judged by a continuing increase in turbidity of the culture, allowed by each drug; to take into account any slowing of bacterial growth, the amount of residual growth and the time elapsed before growth was completely halted were both recorded. Regrowth during the overnight incubation period was also noted. Four strains of normal carbenicillin susceptibility and five carbenicillin-resistant strains were examined in this way (Table 2). Considerable variation was observed in the effectiveness of each antibiotic against different strains, but in general, ceftazidime, ceftriaxone, and cefotaxime achieved their effect more rapidly than the other antibiot-

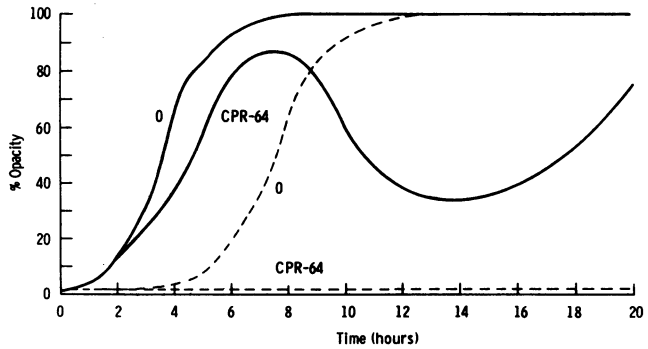

FIG. 4. Continuous opacity records of a carbenicillin-susceptible strain of *P. aeruginosa*. Inocula: 10^6 bacteria per ml (——); 10^4 bacteria per ml (-----). CPR-64, Cefoperazone added at time zero to achieve a concentration of 64 μg/ml.

TABLE 2. Comparative efficacy of six β-lactam agents against nine strains of *P. aeruginosa*, judged by the capacity to halt growth after exposure of 10^6 to 10^7 bacteria per ml to 64 μg of antibiotic per ml

P. aeruginosa strain	Cefotaxime			Ceftizoxime			Ceftazidime		
	%[a]	h[b]	R[c]	%	h	R	%	h	R
A1	44	4.5	+	63	6.5	+	70	5.5	−
1/9	48	3.5	−	82	6.5	+	18	3	−
A2	33	3.5	+	76	7.5	+	30	3.5	−
76/9	24	2.5	+	89	6	+	46	4	−
30/13[d]	33	5	+	80	8	+	8	4	−
PAO (TEM-1)[d]	44	6	−	>90	>8	NA[e]	18	4	−
PAO (TEM-2)[d]	14	5	−	20	8	+	4	4.5	−
PAO (PSE-1)[d]	48	5.5	−	66	7	+	20	4.5	−
PAO (OXA-3)[d]	42	7	+	>90	>8	NA	14	4	−

[a] %, Percent opacity at which growth stopped.
[b] h, Time elapsed (in hours) before growth stopped.
[c] R, Regrowth during overnight incubation.
[d] Carbenicillin-resistant strain.
[e] NA, Not applicable.

ics, and ceftizoxime and cefoperazone were the slowest to act. Only ceftazidime and moxalactam consistently prevented regrowth during the overnight incubation period.

DISCUSSION

The object of this study was not to establish a correlation between MIC results and turbidimetric tests, but to investigate the turbidimetric and morphological response of *P. aeruginosa* to various β-lactam agents in extension of an earlier study (Greenwood and Eley, in press). The results did, however, suggest reasons to suppose that MIC tests may not reveal certain important aspects of the bacterial response. One such aspect is that antipseudomonal agents do not inhibit growth (elongation) of most *P. aeruginosa* strains during the first few hours of the encounter between bacteria and drug, although they do inhibit bacterial division during this period. Present and previous (Greenwood and Eley, in press) results suggest that different β-lactam agents differ in the amount of residual growth that they allow in *P. aeruginosa*. Assessment of the activity of antipseudomonal β-lactam agents by turbidimetric evidence supported by microscopy would lead to a different ranking than that indicated by conventional low-inoculum MIC tests.

All six β-lactam agents tested in this study displayed significant activity against a wide variety of *P. aeruginosa* strains, as judged by MIC titrations with low inocula. Turbidimetric experiments with higher inocula revealed differences in response between different groups of strains and differential activity among the six antibiotics.

When tested against *P. aeruginosa* strains showing unusually high susceptibility to carbenicillin, five of the six antibiotics exhibited bacteriolytic activity similar to that reported elsewhere (Greenwood and Eley, in press) for some other β-lactam agents, including carbenicillin and cefsulodin. Cefoperazone displayed rather poorer bacteriolytic activity against these strains, similar to the activity observed with piperacillin and apalcillin in the earlier study.

The activity of the six agents against strains of normal carbenicillin susceptibility and against carbenicillin-resistant strains (the groups which represent the majority of those encountered in clinical practice) also differed. At a concentration of twice the MIC of each antibiotic, moxalactam appeared to be the most active of the compounds tested by turbidimetric criteria, but this result must be balanced by the observation that in MIC tests moxalactam was among the least active of the six antibiotics. At a likely therapeutic concentration of 64 μg/ml, ceftazidime, ceftriaxone, and cefotaxime were more active than moxalactam. Cefoperazone and ceftizoxime behaved consistently poorly in the turbidimetric system. In the case of cefoperazone, this is particularly surprising in view of the good activity shown by this compound in conventional MIC tests.

We have previously (Greenwood and Eley, in press) drawn attention to the fact that inoculum effects in conventional titrations of agents against *P. aeruginosa* may be difficult to interpret, because the growth of bacteria continues for several hours after the initial exposure to a drug and cultures may achieve visible turbidity during this period which is scored as "growth" after overnight incubation. The present results reemphasize this difficulty. It is clear that high-inoculum MIC titrations in broth read after a 20-h incubation would not distinguish among agents that had had little or no effect on bacterial growth (e.g., ceftizoxime), agents which had

TABLE 2.—*Continued*

Ceftriaxone			Cefoperazone			Moxalactam		
%	h	R	%	h	R	%	h	R
68	6	+	87	7.5	+	50	5.5	−
28	3	+	90	5.5	+	60	4	−
30	3.5	+	64	7.5	+	38	4	−
50	3.5	+	94	6	+	56	4.5	−
8	4	+	80	7	+	42	5.5	−
28	4.5	+	80	7	+	63	6.5	−
4	4.5	+	18	6.5	+	12	6	−
30	5	+	58	8	+	66	6.5	−
23	5	+	44	8	+	44	7	+

achieved an effect but allowed regrowth to occur (e.g., cefoperazone), and agents which had achieved a sustained effect but failed to reduce the final turbidity to below the visible level (e.g., ceftazidime) (Fig. 3).

Inoculum effects undoubtedly play a part in modifying the activity of some antipseudomonal antibiotics as illustrated for cefoperazone (Fig. 4), but conventional MIC tests in broth are not a satisfactory way of evaluating such effects because continued growth of the organisms for several hours after exposure to the drug may bring dense inocula into the visible range. The precise relationship of inoculum effects to the activity of antipseudomonal agents in liquid and on solid media is under investigation.

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