

In Vitro Effects of Carbenicillin Combined with Gentamicin or Polymyxin B Against *Pseudomonas aeruginosa*¹

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Disodium carbenicillin and gentamicin sulfate have both shown promise in the treatment of infections caused by *Pseudomonas aeruginosa*. This study was designed to explore possible synergistic relationships among the new as well as the established antimicrobial agents used to treat such infections. With an agar dilution technique, minimum inhibitory concentrations of 27 strains of *P. aeruginosa* were determined in two-dimensional tests. Graphs of equal biological activity (isobolograms) demonstrated moderate synergistic effects of the carbenicillin-gentamicin combination over therapeutically feasible concentration ranges. In contrast, the combination of carbenicillin and polymyxin B showed only additive or slightly antagonistic effects. Tests of bacterial killing confirmed the presence of carbenicillin-gentamicin synergy in 3 of 6 strains of *P. aeruginosa*, but did not show true antagonism between carbenicillin and polymyxin B. Clinical trials of both drug combinations are advisable to determine whether therapeutic results can be improved, and whether the dosages of gentamicin or polymyxin B can thereby be reduced to lessen their toxic hazards.

Gentamicin sulfate, a potent aminoglycoside antibiotic, has recently been made commercially available after a lengthy period of clinical investigation. Because of its broad spectrum of activity against commonly encountered gram-negative bacilli, including *Pseudomonas aeruginosa*, gentamicin promises to be an exceedingly useful drug. Its nephrotoxic and neurotoxic properties became apparent early in the course of initial clinical investigations, and appear to be of the same order of magnitude as kanamycin sulfate (4, 6, 8, 9, 12).

Carbenicillin, disodium α -carboxybenzylpenicillin, is a semisynthetic derivative of 6-aminopenicillanic acid closely related to ampicillin. In contrast to other penicillins, carbenicillin possesses moderate in vitro activity against certain gram-negative bacilli not susceptible to ampicillin, particularly *P. aeruginosa*, indole-positive proteus species, and *Serratia marcescens* (1, 3, 5, 15). The order of activity against *P. aeruginosa* is small, however, requiring the intravenous administration of 20 to 40 g per day to achieve the necessary serum levels of 100 to 400 μ g/ml.

This study was undertaken to explore possible

synergistic relationships of carbenicillin with both gentamicin sulfate and polymyxin B sulfate, to determine whether the addition of carbenicillin might result in a lessened requirement for either gentamicin or polymyxin, thereby reducing the hazard of toxicity associated with the latter two drugs.

MATERIALS AND METHODS

The antibiotics used were disodium carbenicillin (Pfizer Medical Research Laboratories), gentamicin sulfate (Schering Corp.), polymyxin B sulfate (Burroughs Wellcome and Co., New York, N.Y.), ampicillin trihydrate, and sodium dicloxacillin (Bristol Laboratories). Each was kindly supplied by the manufacturer as the dry powder, and stored at 4 C. Fresh solutions were prepared in pH 7.3 phosphate buffered saline at a concentration of 2,000 μ g/ml and either used the same day or stored at -25 C for no longer than 14 days.

Strains of *P. aeruginosa* studied were isolated from urine, sputum, and blood of infected hospitalized patients in the clinical microbiology laboratories of the University of Colorado Medical Center. Their identity was confirmed by characteristic colonial morphology, positive reaction with oxidase reagent, hemolysis of sheep red blood cells, and oxidative breakdown of glucose by the method of Hugh and Leifson (7).

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Tests for possible antibacterial synergism or antagonism were carried out on 27 strains of *P. aeruginosa* with four drug combinations: carbenicillin plus gentamicin, carbenicillin plus polymyxin B, carbenicillin plus dicloxacillin, and ampicillin plus dicloxacillin. Tests were carried out by a two-dimensional agar dilution method, with twofold increments of each drug in Mueller-Hinton agar (BBL). A 10^{-2} dilution of overnight broth cultures was used in the inoculum replicator of Steers, Foltz, and Graves (17).

The geometric mean minimum inhibitory concentration (MIC) was then calculated for all of the possible combinations of concentrations of each drug pair, and the results presented in the form of isobolograms by the method of Loewe (11) as described by Lacey (10). The concentrations of each drug pair are plotted on an arithmetic scale on the ordinate and abscissa. Each plotted point represents the same biological activity, i.e., the minimum amount of one drug in the presence of various concentrations of the other necessary to inhibit growth. The line joining the points, the isobole, thus represents a continuum of equal antibacterial effect of a pair of drugs. If an isobole connecting the MIC of each drug acting independently follows a straight line, the combined effect is additive; if the isobole is concave (bows in), the combined effect is synergistic; if it is convex (bows out), the combined effect is antagonistic.

To define the drug interrelationships more closely, bacterial killing studies were carried out on 6 strains of *P. aeruginosa* in Mueller-Hinton broth (BBL). Drug concentrations were selected as being approximately the same as the mean MIC of the six strains of *P. aeruginosa* determined in Mueller-Hinton broth. These were 100 $\mu\text{g}/\text{ml}$ for carbenicillin, 1 $\mu\text{g}/\text{ml}$ for gentamicin, 0.1 $\mu\text{g}/\text{ml}$ for polymyxin B, and one-half of those concentrations in the flasks containing drug

TABLE 1. Distribution of responses of 27 strains of *P. aeruginosa* to combinations of carbenicillin with gentamicin sulfate or polymyxin B sulfate, determined by agar-dilution tests

Carbenicillin-polymyxin B	Carbenicillin-gentamicin		
	Synergy	Addition	Antagonism
Synergy	0	0	0
Addition	11	10	0
Antagonism	4	2	0

mixtures. The initial concentration of organisms in the flasks was approximately $5 \times 10^4/\text{ml}$. Flasks were placed in a shaker incubator, and samples were removed periodically. Colony counts were carried out in duplicate.

RESULTS

Figure 1 shows the results of the two-dimensional agar-dilution tests of four antibiotic combinations in the form of isobolograms. The carbenicillin-gentamicin isobole is bowed inward, suggesting a moderate degree of synergism. The carbenicillin-polymyxin B isobole is skewed, and bowed outward slightly, suggesting a slight degree of antagonism, particularly at low concentrations of polymyxin B. The degree of outward bowing, however, rarely exceeds a single twofold dilution. The isoboles of the combinations of ampicillin-dicloxacillin and carbenicillin-dicloxacillin show slight degrees of bowing inward and outward, respectively, suggesting limited synergistic and antagonistic effects.

The responses of individual strains to the 2 drug combinations are summarized in Table 1. Of the 27 strains of *P. aeruginosa*, 6 were considered to show antagonism in the carbenicillin-polymyxin B mixture. Four of these were considered to show synergism in the carbenicillin-gentamicin mixture, and two showed additive effects. The response of a given strain to one drug pair was of no value in predicting its response to the other drug pair.

Figure 2 depicts the bacterial killing curves of six strains of *P. aeruginosa* in carbenicillin, gentamicin, and a half-strength mixture of both drugs. Carbenicillin effected a significant reduction in the numbers of viable organisms after a 2 hr latent period, except for strain 1118, which had an MIC of 200 $\mu\text{g}/\text{ml}$. In no instance did carbenicillin in a concentration of 100 $\mu\text{g}/\text{ml}$ sterilize the culture during the 6 to 8 hr of observation. Gentamicin alone had a more rapid bactericidal effect than the 50% combination of carbenicillin and gentamicin only with one strain,

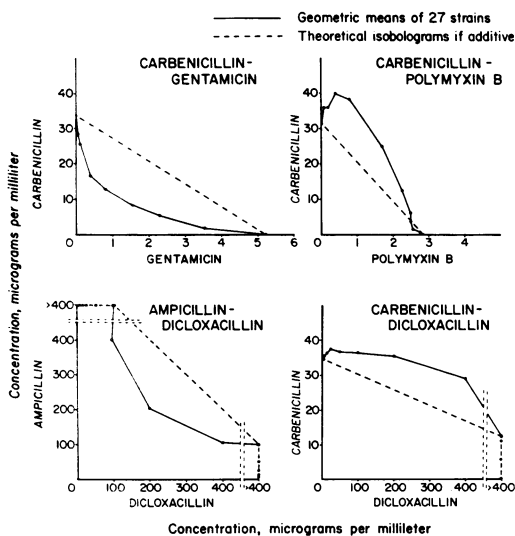


FIG. 1. Isobolograms of activity of four antibiotic combinations against *Pseudomonas aeruginosa*.

number 760. With strains 1118 and 994, gentamicin and its half-strength combination with carbenicillin were approximately equal in rate of bacterial killing. With the remaining three strains, 657, 150, and 2609, the rate of bacterial killing was clearly enhanced by the drug combination.

Figure 3 shows the killing curves of the same six strains of *Pseudomonas aeruginosa* in polymyxin B, carbenicillin, and a half-strength mixture of both drugs. The curves for the control flasks and the flasks containing 100 µg/ml of carbenicillin are similar or identical to those shown in Fig. 2. The differing dynamics of bacterial killing of these two drugs are illustrated. The bactericidal effect of carbenicillin did not become apparent until a 2-hr latent period had elapsed. In contrast, polymyxin B had a very rapid bactericidal action which was essentially complete after 2 hr, and multiplication of surviving organisms ensued thereafter. With two strains, numbers 1118 and 657, the numbers of organisms surviving after 6 to 8 hr of incubation were essentially the same with polymyxin B and with its half-strength combination with carbenicillin. With the other four strains, the numbers of organisms surviving after 6 to 8 hours were significantly less with the 50% carbenicillin-polymyxin B mixture than they were with either drug alone. In no instance was there evidence of drug antagonism.

Table 2 summarizes the responses of the six

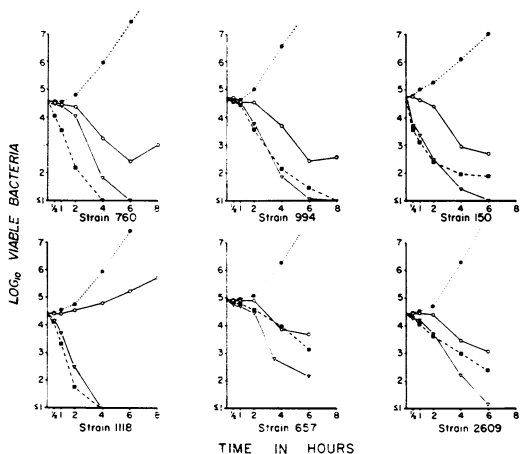


FIG. 2. Growth curves of six strains of *Pseudomonas aeruginosa* in carbenicillin, 100 µg/ml (○); gentamicin, 1.0 µg/ml (■); and a 50% concentration of both combined, 50 µg of carbenicillin plus 0.5 µg of gentamicin per ml (△). Control (●).

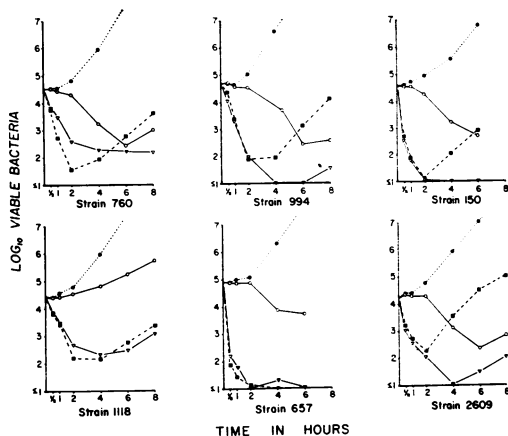


FIG. 3. Growth curves of six strains of *Pseudomonas aeruginosa* in carbenicillin, 100 µg/ml (○); polymyxin B, 0.1 µg/ml (■); and a 50% concentration of both combined, 50 µg of carbenicillin plus 0.05 µg of polymyxin per ml (△). Control (●).

TABLE 2. Comparison of responses of six strains of *P. aeruginosa* to carbenicillin-gentamicin and carbenicillin-polymyxin combinations determined by agar-dilution and bacterial-killing tests^a

Strain	Carbenicillin-gentamicin		Carbenicillin-polymyxin B	
	Agar-dilution	Bacterial-killing	Agar-dilution	Bacterial-killing
2609	+	+	0	+
657	0	+	0	0
150	+	+	0	+
760	+	0	0	+
994	+	0	-	+
1118	0	0	0	0

^a Symbols: +, synergistic; 0, additive; -, antagonistic.

strains to the two drug combinations as determined both by agar-dilution and bacterial-killing tests. Not only was there no correlation in the pattern of response to the two drug pairs but also no correlation in response as measured by the two tests of drug interaction.

DISCUSSION

The isobologram of the carbenicillin-gentamicin combination carried out by two-dimensional agar dilution tests suggested a degree of synergistic activity which was clarified somewhat by the studies of bacterial killing. Three of six strains

showed true antibacterial synergism, which, although not marked, might prove useful clinically. The combined effect of these two drugs in the remaining three strains showed neither synergism nor antagonism, but even an additive effect might also prove useful clinically.

The apparent antagonism between carbenicillin and polymyxin B shown in the isobologram could not be confirmed by tests of bacterial killing, at least with the drug concentrations used in these experiments. As with gentamicin, either an additive or a synergistic effect might have therapeutic application.

The lack of correlation between the two tests of drug interaction used in this study is understandable only when it is considered that different end points are being measured. It is possible that by carrying the tests of bacterial killing out for 12 to 18 hr or by varying the drug concentrations used, or both, different results might have been obtained.

In their initial report on carbenicillin, Brumfitt, Percival, and Leigh (5) described synergy between carbenicillin and gentamicin in all four strains of *P. aeruginosa* that they examined; they also observed synergistic effects in their test strains when carbenicillin was combined with colistin or streptomycin. Standiford and associates (16) also reported a synergistic effect of the carbenicillin-gentamicin combination against *Pseudomonas* strains. Smith and co-workers (14), using a checkerboard microtiter tube dilution technique, found carbenicillin-gentamicin synergy in 5 of 10 strains of *P. aeruginosa* examined, and carbenicillin-polymyxin B synergy in the 5 strains not showing synergy with the former drug pair. This interesting observation could not be confirmed in the present study, in which no predictive relationship was observed among the responses of these two drug pairs.

The apparent synergy between dicloxacillin and ampicillin has been well documented in the past by Sutherland and Batchelor (18) and by Sabath and Abraham (13). The mechanism of this synergistic effect is recognized to be due to competitive inhibition of β -lactamase through binding to a β -lactamase-resistant penicillin (dicloxacillin), allowing the β -lactamase-sensitive analogue (ampicillin) to remain free to exert its antibacterial effect.

No such synergy could be demonstrated with the combination of carbenicillin and dicloxacillin. This is in agreement with the early reports of Brumfitt et al. (5), and of Acred et al. (1), who stated that carbenicillin is not inactivated by β -lactamase produced by *P. aeruginosa*. A combination of carbenicillin and dicloxacillin would therefore not be expected to show synergism.

Whether true antibacterial synergism can be demonstrated is perhaps somewhat less significant than the fact that the activity of carbenicillin combined with gentamicin can be expected to be at least additive in nature. Although the agar-dilution test demonstrated antagonism between carbenicillin and polymyxin B in 6 of 27 strains, the degree of antagonism was not great, and its clinical significance is not known. In vitro screening of this combination would be prudent prior to initiating therapy in a given patient.

Clinical trials of carbenicillin combined with either gentamicin sulfate or polymyxin B sulfate appear to be warranted for several reasons. First, results of therapy of systemic *Pseudomonas* infections with any of the currently available drugs used singly leave ample room for improvement. Second, several recent reports have emphasized the ease with which strains of *P. aeruginosa* may become resistant to carbenicillin, both in vitro and in vivo (2, 15). Finally, the addition of carbenicillin to therapy with either gentamicin or polymyxin B may permit some reduction in dosage of the latter two drugs, thereby reducing the concomitant risk of toxicity.

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