Susceptibility of \textit{Pseudomonas aeruginosa} to Tobramycin or Gentamicin Alone and Combined with Carbenicillin

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Received for publication 25 April 1975

To explore more effective therapy for \textit{Pseudomonas aeruginosa}, 264 recent clinical isolates were tested by agar dilution using gentamicin and tobramycin alone and combined with carbenicillin to seek synergistic effects. Synergism was defined as a fourfold or greater decrease in the minimal inhibitory concentration of each drug in a pair. At a concentration of 3.12 µg/ml, gentamicin inhibited 73% of the strains and tobramycin inhibited 98%. The gentamicin-carbenicillin combination was synergistically active against 57% of the strains, and tobramycin-carbenicillin was active against 46%. The effect did not correlate with either susceptibility or resistance to gentamicin or tobramycin alone. The data suggest that tobramycin or tobramycin plus carbenicillin may provide alternate therapy where susceptibility to gentamicin or synergism between gentamicin and carbenicillin cannot be demonstrated; however, the degree of susceptibility to either aminoglycoside antibiotic alone cannot be used to predict a synergistic effect.

The increasing occurrence and difficulty in managing \textit{Pseudomonas aeruginosa} infections justifies continued efforts to provide new therapeutic regimens (4). Tobramycin, an aminoglycoside antibiotic, has been found to be highly active against \textit{P. aeruginosa} (1, 5, 15, 16). On a weight basis it is frequently two to four times more active than gentamicin against clinical isolates of \textit{P. aeruginosa}. Evidence of synergistic activity by the combination of gentamicin and carbenicillin against \textit{P. aeruginosa} has been well documented in vitro (3, 11, 17). Klastersky et al (9) have presented clinical evidence of in vivo synergism with this combination when in requiring 125 µg or greater of carbinicillin per ml is not well-documented, however, when carbenicillin is combined with tobramycin (10).

More recently Kluge et al. (12, 13) correlated degree of susceptibility of strains of \textit{P. aeruginosa} to gentamicin and other aminoglycoside antibiotics with synergistic response when carbenicillin was added. In general they found the addition of carbenicillin beneficial only for strains susceptible or only moderately resistant to gentamicin or tobramycin. The present study was undertaken to gather additional data on the susceptibility of clinical isolates of \textit{P. aeruginosa} to gentamicin and tobramycin alone and when combined with carbenicillin. The results indicate that the degree of susceptibility to gentamicin or tobramycin alone cannot be used to predict synergistic response when carbenicillin is combined with either of these aminoglycosides.

MATERIALS AND METHODS

Bacterial strains. A total of 264 strains of \textit{P. aeruginosa} was investigated. One hundred and ninety-nine were obtained from the clinical bacteriology laboratory of the Medical University of South Carolina (through the cooperation of E. R. Bannister) where they had been isolated from pus, urine, or blood. Sixty-three were isolated at Emory University Hospital, Atlanta, Ga., and kindly provided by J. A. Shulman. Two tobramycin-resistant strains were supplied by Eli Lilly and Co.

Antibiotic susceptibility testing. All strains were tested for susceptibility to carbenicillin, gentamicin, and tobramycin and to combinations of gentamicin plus carbenicillin and tobramycin plus carbenicillin, using a two-dimensional agar dilution method on Mueller-Hinton agar (Difco Laboratories). An inoculum of approximately 5 x 10^4 organisms was delivered onto the agar surface from an overnight culture in Trypticase soy broth using the inocula replicating

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apparatus of Steers et al. (20). Minimal inhibitory concentration (MIC) was accepted to be the lowest concentration of drug which completely inhibited growth after 18 h of incubation at 37 C. Synergism was defined according to two definitions: a stringent definition, according to which the MIC of each drug in the combination was required to be at least fourfold less than the MIC of either drug alone (14); and a less rigid definition, for which any definite concavity of the isobol obtained by graphing the data was interpreted as synergy (3).

**Bactericidal curves.** Rates of killing of four *Pseudomonas* strains by the antibiotics alone and in combination were studied. An overnight culture in Trypticase soy broth was diluted 1:20 with fresh Mueller-Hinton broth and grown with shaking to an optical density of approximately 0.3 (at a wavelength of 540 nm) on a Coleman Jr. II spectrophotometer. Drugs were then added, and optical density and viable count were measured every 2 h for 6 h.

**RESULTS**

A total of 264 clinical isolates of *P. aeruginosa* was tested for susceptibility to gentamicin and tobramycin both alone and in combination with carbenicillin by two-dimensional agar dilution. The degree of susceptibility of these isolates to either aminoglycoside antibiotic alone was correlated with synergistic response when carbenicillin was added. In addition, the dynamic aspects of the antibiotic effects were studied with four strains to provide further information.

As shown in Table 1, gentamicin and tobramycin were effective in inhibiting the *P. aeruginosa* strains tested, but some differences in activity between the two drugs were observed. At a concentration of 3.12 μg/ml, an achievable serum level (18), 98% of the strains of *P. aeruginosa* were inhibited by tobramycin. Only two strains required greater than 50 μg/ml for inhibition. In comparison, gentamicin at a concentration of 3.12 μg/ml, also a serum level easily achieved clinically (18), inhibited 73% of the strains, whereas 16 required greater than 50 μg/ml. Carbenicillin was moderately active against *P. aeruginosa*. At a concentration of 62.5 μg/ml, 216 (82%) strains were inhibited. Strains requiring 125 μg or greater of carbenicillin per ml for inhibition were considered resistant, as these levels cannot be easily produced clinically (14). No strains were found to be susceptible to gentamicin and resistant to tobramycin.

When either aminoglycoside antibiotic was combined with carbenicillin, synergistic inhibition was observed in a frequency which varied according to the definition of synergism employed. Applying rigid criteria (fourfold or greater decrease in MIC of both drugs in a combination), the gentamicin-carbenicillin combination was synergistically active against 150 strains (57%) and tobramycin-carbenicillin against 121 strains (46%) (Table 1). If any bowing of the isobol was considered as evidence of synergistic activity, the gentamicin-carbenicillin combination synergistically inhibited 220 strains (84%), and the tobramycin-carbenicillin combination was synergistic against 154 strains (58%).

Further examination of the data relative to the degrees of susceptibility to individual antibiotics revealed a lack of correlation with synergistic response. In this evaluation only the rigid criterion of synergism was applied. Of the strains which were susceptible to gentamicin (MIC ≤ 3.12 μg/ml), 86 were not synergistically inhibited by the gentamicin-carbenicillin combination (Table 1). Alternatively, 10 of the strains resistant to gentamicin (MIC > 50 μg/ml) were inhibited synergistically when carbenicillin was added to the combination. There was a similar lack of correlation between susceptibility to tobramycin and response to the tobramycin-carbenicillin combination (Table 1). Less than one-half of the strains susceptible to tobramycin (MIC ≤ 3.12 μg/ml) responded synergistically when carbenicillin was com-

**Table 1.** Correlation between susceptibility of 264 strains of *P. aeruginosa* to tobramycin (Tb) and gentamicin (Gm) alone and synergistic* response when combined with carbenicillin (Cb)

<table>
<thead>
<tr>
<th>Degree of susceptibility</th>
<th>MIC (μg/ml)</th>
<th>Gm</th>
<th>Gm + Cb*</th>
<th>Tb</th>
<th>Tb + Cb*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Susceptible</td>
<td>≤3.12</td>
<td>192</td>
<td>106</td>
<td>258</td>
<td>115</td>
</tr>
<tr>
<td>Moderately resistant</td>
<td>6.25–50</td>
<td>56</td>
<td>35</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Resistant</td>
<td>&gt;50</td>
<td>16</td>
<td>9</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>264</td>
<td>150 (57%)</td>
<td>264</td>
<td>121 (46%)</td>
</tr>
</tbody>
</table>

* MIC for each drug in the combination decreased fourfold or greater than the original individual drug susceptibility.

* Number of strains inhibited synergistically by the combination of antibiotics.
bined with tobramycin. All of the moderately resistant (MIC 6.25 to 50 μg/ml) and resistant strains (MIC > 50 μg/ml) were synergistically inhibited by the combination of tobramycin and carbenicillin.

To determine if tobramycin offered any advantage over the gentamicin-carbenicillin combination in inhibiting strains of *P. aeruginosa*, further analysis of the data was made (Table 2). Strains not inhibited by the combination of gentamicin and carbenicillin were grouped by their degree of susceptibility to gentamicin. Ninety-eight percent of the strains not inhibited synergistically by the gentamicin-carbenicillin combination were inhibited by tobramycin alone. The remaining two strains, which were resistant to gentamicin and carbenicillin in combination and to tobramycin, were inhibited by the combination of tobramycin and carbenicillin.

To define further the effects of the antibiotics alone and in combination, four representative strains of *P. aeruginosa* were studied by killing curves. Two of these strains were susceptible to both gentamicin (MIC 1.56 μg/ml) and tobramycin (MIC 0.78 μg/ml); a third strain was resistant to carbenicillin (MIC 1,000 μg/ml), and a fourth strain was resistant to both gentamicin (MIC 200 μg/ml) and tobramycin (MIC 200 μg/ml). Results obtained with one of the strains which was susceptible to all three drugs are shown in Fig. 1. It is evident that addition of a sublethal concentration of carbenicillin (1 MIC) results in a significant increase in the rate of killing of the organisms by gentamicin. Similar results were obtained when tobramycin was substituted for gentamicin. These results meet the commonly accepted criterion of synergism (6, 14): a decrease of 1 log or more in viable cell count occurring at the end of 4 h with the antibiotic combination as compared with the count obtained with the most active single antibiotic. Synergism was also demonstrated in killing curve studies in the strain resistant to both gentamicin and tobramycin and in the strain resistant to carbenicillin.

**DISCUSSION**

In the present study three antibiotics were evaluated for activity against *P. aeruginosa*. Tobramycin was the most biologically active of the three. This antibiotic has been reported to be more active on a per weight basis against *P. aeruginosa* than gentamicin by other workers (1, 5, 7, 15), whose results compare favorably with ours. This is an especially important finding, as the serum levels of gentamicin and tobramycin are similar after comparable doses (18). Carbenicillin was next in activity against *P. aeruginosa*, with 82% of the 264 strains tested inhibited at a concentration easily achievable in the serum. Smith and Finland (19) reported in 1968 that approximately 90% of 70 strains they tested were susceptible to 62.5 μg or less of carbenicillin per ml. Comparison of these results suggests that the susceptibility of *P. aeruginosa* to carbenicillin has not changed appreciably in the past several years.

Combining aminoglycoside antibiotics with

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**TABLE 2. Tobramycin (Tb) susceptibility of *P. aeruginosa* strains not synergistically* inhibited by gentamicin (Gm) and carbenicillin (Cb)**

<table>
<thead>
<tr>
<th>Degree of susceptibility</th>
<th>Gm MIC (μg/ml)</th>
<th>No. not inhibited</th>
<th>No. susceptible</th>
<th>Gm + Cb</th>
<th>Tb*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Susceptible</td>
<td>≤3.12</td>
<td>86</td>
<td>86</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moderately resistant</td>
<td>6.25-50</td>
<td>21</td>
<td>21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resistant</td>
<td>&gt; 50</td>
<td>7</td>
<td>5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*See Table 1, footnote a, for definition. *MIC of ≤3.12 μg of tobramycin per ml.
carbenicillin may result in synergistic activity against *P. aeruginosa*. Synergistic effects of gentamicin plus carbenicillin have been well documented (3, 8, 11, 17), but the combination of tobramycin and carbenicillin has not been studied as thoroughly (10). Synergistic activity between gentamicin or tobramycin combined with carbenicillin was evaluated by several criteria in the present study. Application of a classical definition of synergism (any bowing of the isobol) to the data resulted in a high rate of synergistic inhibition by both combinations of antibiotics. This criterion leads to more subjective interpretation of data and consequently less ability to compare results between laboratories. A more stringent definition of synergism such as the one employed by Libke et al. (14) lends itself to quantification but a lower incidence of synergistic behavior. Killing curves permit a more precise evaluation of bactericidal activity by antibiotics both alone and in combination.

Recently, Kluge et al. (12, 13) examined the susceptibility of 130 strains of *P. aeruginosa* to gentamicin, tobramycin, and amikacin both alone and combined with carbenicillin. Tobramycin was found to be the most active of the three aminoglycoside antibiotics, inhibiting approximately 90% of the strains at clinically achievable serum levels. Cross-resistance occurred between gentamicin, tobramycin, and amikacin but was not complete. These investigators correlated degree of susceptibility of *P. aeruginosa* strains to the individual antibiotics with the additional inhibition produced when carbenicillin was combined with these agents. Their conclusion was that isolates of *P. aeruginosa* susceptible (MIC ≤ 5 μg/ml) or moderately resistant (MIC 10 to 40 μg/ml) to gentamicin, tobramycin, or amikacin could be additionally inhibited by the presence of carbenicillin, but that no added effect was seen when highly resistant strains (MIC ≥ 80 μg/ml) were tested.

Data from the present study augment information previously available by providing results for a larger number of *P. aeruginosa* strains. In agreement with the work cited (12, 13), it was found that tobramycin was the single most active antibiotic on a per weight basis tested, inhibiting 98% of the strains at levels of ≤3.12 μg/ml. Many strains highly resistant (MIC ≥ 100 μg/ml) to gentamicin were exquisitely susceptible to tobramycin (MIC ≤ 1.56 μg/ml), whereas no strains resistant to tobramycin were susceptible to gentamicin. However, the degree of susceptibility to either gentamicin or tobramycin alone could not be used to predict synergistic response when carbenicillin was added, as reported by Kluge et al. (12, 13). For example, only about one-half of the strains in each of the three categories of gentamicin susceptibility (Table 1) was synergistically inhibited by the gentamicin-carbenicillin combination.

The marked biological activity of tobramycin against *P. aeruginosa* indicates that it may prove valuable in the treatment of infections caused by this species. Any advantage to be gained by using tobramycin and carbenicillin in combination remains to be studied. Appropriate tests should be done for each strain to determine if synergistic activity occurs in vitro, since this cannot be predicted from knowledge of susceptibility to individual antibiotics.

**ACKNOWLEDGMENTS**

The assistance of Wallace A. Clyde in the preparation of this manuscript is gratefully acknowledged.

This work was supported in part by a grant from the Southern Medical Association.

**LITERATURE CITED**


ERRATUM

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Volume 8, no. 3, p. 300, column 1, line 15: Remove entire line and replace with "vitro evidence was shown. Synergistic behavior. . . ."