Comparative Activity of Tobramycin, Amikacin, and Gentamicin Alone and with Carbenicillin Against Pseudomonas aeruginosa

RONICA M. KLUGE, HAROLD C. STANDIFORD, BEVERLY TATEM, VIOLA M. YOUNG, WILLIAM H. GREENE, STEPHEN C. SCHIMPFF, FRANK M. CALIA, AND RICHARD B. HORNICK

Department of Medicine, Baltimore VA Hospital, University of Maryland, and the Cancer Research Center, National Cancer Institute, Baltimore, Maryland 21218

Received for publication 9 August 1974

The effect of gentamicin against 130 clinical isolates of Pseudomonas aeruginosa was compared with that of two investigational aminoglycoside antibiotics, tobramycin and amikacin. Minimal inhibitory concentration data indicated that, on a weight basis, tobramycin was two to four times as active as gentamicin against most isolates. However, 14 of 18 organisms highly resistant to gentamicin (≥80 µg/ml) were also highly resistant to tobramycin. Amikacin was the least active aminoglycoside on a weight basis, but none of the isolates were highly resistant to this antibiotic. When therapeutically achievable concentrations were used, adding carbenicillin to gentamicin or to tobramycin enhanced inhibitory activity against those isolates susceptible (≤5 µg/ml) or moderately resistant (10 to 40 µg/ml) to the aminoglycoside. Such synergy was seldom demonstrated for isolates highly resistant to gentamicin or tobramycin. The combination of carbenicillin and amikacin enhanced inhibition against all but two of the isolates. Both tobramycin and amikacin offer in vitro advantages over gentamicin against P. aeruginosa.

Carbenicillin and gentamicin have replaced the polymyxins as the antibiotics of choice for most infections caused by Pseudomonas aeruginosa. Since in vitro synergism has been demonstrated with the combination of carbenicillin and gentamicin (4, 10, 18, 22–24), the two antibiotics frequently are used together. Unfortunately, increasing numbers of strains resistant to both antibiotics have emerged (8, 12, 13, 17, 21, 25).

Two new aminoglycoside antibiotics with activity against pseudomonas have been released for clinical investigation. Tobramycin is similar to gentamicin in structure, pharmacodynamics, and perhaps toxicity (2, 15). Amikacin (BB-K8) resembles kanamycin in these characteristics (3, 19). This study compares these two antibiotics with gentamicin, alone and in combination with carbenicillin, against 130 isolates of P. aeruginosa. Some of the isolates were purposely selected because they were resistant to gentamicin, carbenicillin, or both by routine disk susceptibility testing in our clinical microbiology laboratories.

MATERIALS AND METHODS

One hundred and thirty clinical isolates of P. aeruginosa were obtained from the University Hospital, Baltimore Veterans Administration Hospital, and the Baltimore Cancer Research Center. Cultures were maintained on cystine Trypticase agar (BBL) at 4 C and occasionally in Trypticase soy agar (BBL) at −70 C until studied. Each isolate was plated onto blood agar and passed twice in Trypticase soy broth (BBL) prior to testing to ensure purity and luxurious growth.

Stock antibiotic solutions were prepared freshly each week in concentrations of 1,000 µg/ml for each aminoglycoside antibiotic and 25,000 µg/ml for carbenicillin. The solutions were divided into 2-ml portions and frozen at −20 C. On the day of the study, a portion was thawed and used only once. Susceptibilities were performed in Trypticase soy broth containing 3.4 mg of calcium per 100 ml and 2.9 mg of magnesium per 100 ml.

Minimal inhibitory concentrations (MICs) of each of the antibiotics alone and each of the aminoglycoside antibiotics in combination with carbenicillin were determined by using a microtiter variation of the checkerboard technique. By this method, two-dimensional MICs were obtained in microtiter disposable plates containing 96 wells. Twofold dilutions of each aminoglycoside antibiotic were made in one direction in three separate plates with the automated microtiter dilutor (Cooke Engineering). Twofold dilutions of carbenicillin were added to the plates in the other direction by adding one drop of appropriate concentrations of the antibiotic manually from a disposable pipette dropper delivering 0.05 ml per drop. Where each of the antibiotics was used alone, 0.05 ml of
broth was added to achieve a volume of 0.1 ml. The inoculum of 0.1 ml of a 10^-2 dilution of an overnight culture was added to each well by delivering 2 drops from the 0.05 ml/drop disposable pipette. The plates were sealed with plastic tape and gently agitated, and a small hole was punched in the tape over each well with a 26-gauge needle to allow aerobic conditions. End points were determined (after overnight incubation at 37°C) by noting the wells with the lowest concentrations of antibiotics, alone and in each carbencillin-aminoglycoside combination which contained no visible growth. Thus, each strain was tested against each aminoglycoside antibiotic in twofold dilutions from 10 to 0.156 μg/ml, against carbencillin in concentrations of 400 to 1.6 μg/ml, and against each aminoglycoside-carbencillin combination at every dilution. When inhibition was not seen at these concentrations, the test was repeated with higher concentrations of the appropriate antibiotic.

Preliminary studies revealed that this microtiter variation of the checkerboard technique gave values almost identical to the standard checkerboard technique, with 0.5 ml of each antibiotic and 1.0 ml of a 10^-2 dilution of an overnight culture as the inoculum in a total volume of 2 ml. However, by the microtiter method, as many as 10 strains could be tested simultaneously against three aminoglycoside antibiotics alone and in combination with carbencillin by one technician in a day.

RESULTS

The minimal concentrations of the three aminoglycosides required to inhibit the growth of the 130 isolates are seen in Table 1. Ninety-seven isolates (75%) were inhibited by 5 μg or less of gentamicin per ml, a level which can be achieved in the patient without toxicity. Fifteen isolates were moderately resistant to this antibiotic, requiring concentrations of 10 to 40 μg/ml for inhibition, and the remaining 18 isolates were considered highly resistant with an MIC of 80 μg/ml or greater.

Tobramycin was generally two to four times more active than gentamicin; no strain was more susceptible to gentamicin than to tobramycin. One hundred and sixteen isolates (89%) were inhibited by 5 μg/ml or less, a serum level achieved clinically. All of the strains moderately resistant to gentamicin (10 to 40 μg/ml) were susceptible to tobramycin. However, 14 of the 18 isolants highly resistant to gentamicin (≥ 80 μg/ml), were also highly resistant to tobramycin (Table 2).

On a weight basis, amikacin was the least active of the three aminoglycosides for the majority of strains. However, 114 (88%) of the organisms tested were inhibited by 10 μg/ml or less, a level easily achieved in the serum with this antibiotic. Eight of the 15 isolates moderately resistant to gentamicin and 11 of the 18 isolates highly resistant to gentamicin were susceptible to 10 μg or less of amikacin per ml (Table 2). Only two isolates resistant to amikacin were susceptible to gentamicin. None of the 16 isolates resistant to amikacin had MICs greater than 40 μg/ml.

The MICs of carbencillin for the 130 isolates of P. aeruginosa were comparable to those previously reported. Eighty-five percent were inhibited by 50 or 100 μg/ml. The remaining isolates were considered resistant, requiring 200 to 800 μg of carbencillin per ml for inhibition.

Table 1. Comparative MICs of three aminoglycoside antibiotics for 130 isolates of P. aeruginosa

<table>
<thead>
<tr>
<th>Aminoglycoside antibiotic</th>
<th>≤0.6*</th>
<th>1.2</th>
<th>2.5</th>
<th>5</th>
<th>10</th>
<th>20</th>
<th>40</th>
<th>≥80</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gentamicin</td>
<td>1</td>
<td>2</td>
<td>42</td>
<td>52</td>
<td>12</td>
<td>3</td>
<td>0</td>
<td>18</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>22</td>
<td>68</td>
<td>25</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>14</td>
</tr>
<tr>
<td>Amikacin</td>
<td>0</td>
<td>1</td>
<td>16</td>
<td>56</td>
<td>41</td>
<td>14</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

* MIC in micrograms per milliliter.

Table 2. Comparison of gentamicin susceptibilities to those of tobramycin and amikacin for 130 isolates of P. aeruginosa

<table>
<thead>
<tr>
<th>Gentamicin susceptibility*</th>
<th>No. of isolates</th>
<th>Tobramycin MIC (μg/ml)</th>
<th>Amikacin MIC (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>≤5</td>
<td>10 to 40</td>
</tr>
<tr>
<td>Susceptible (&lt;5)</td>
<td>97</td>
<td>97</td>
<td>0</td>
</tr>
<tr>
<td>Moderately resistant (10 to 40)</td>
<td>15</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>Highly resistant (≥80)</td>
<td>18</td>
<td>4</td>
<td>0</td>
</tr>
</tbody>
</table>

* Parentheses show MIC in micrograms per milliliter.
The effect of adding subinhibitory concentrations of 12.5 µg of carbenicillin per ml to each of the aminoglycoside antibiotics is seen in Fig. 1. Tobramycin inhibited the majority of isolates at the lowest concentrations, whether used alone or with the addition of carbenicillin. A combination of 1.2 µg of tobramycin per ml (approximately one-fourth its serum concentration) with 12.5 µg of carbenicillin per ml inhibited 87% of isolates. Gentamicin in combination with carbenicillin at these same concentrations inhibited only 49%. Amikacin at concentrations of 2.5 µg/ml (approximately one-fourth the achievable serum concentration) in combination with 12.5 µg of carbenicillin per ml inhibited 75% of the isolates.

Although most isolates were inhibited by lower concentrations of tobramycin in combination with carbenicillin, more isolates could be inhibited by the amikacin-carbenicillin combination at concentrations which can be achieved in the serum clinically (Table 3). Concentrations up to 100 µg/ml for carbenicillin and 5 µg/ml for the respective aminoglycoside were considered in this evaluation. By using gentamicin in combination with carbenicillin, 15 organisms were inhibited no better by the antibiotic combination than by the most effective antibiotic alone, and one isolate was not inhibited at all by the combination of these concentrations. For these 16 isolates, no beneficial inhibitory effect was observed at therapeutic concentrations. When the combination of tobramycin and carbenicillin was tested, 10 isolates behaved in the same way. Only 2 of the 130 isolates showed no beneficial inhibitory effect with the amikacin-carbenicillin combination. Both amikacin and tobramycin were also more effective than gentamicin when used in combination with carbenicillin against the 15 carbenicillin-resistant isolates. As the concentration of each of the antibiotics was increased, almost all isolates were inhibited better by the aminoglycoside-carbenicillin combination compared with the most effective of the antibiotics alone, regardless of which aminoglycoside was used in combination. However, concentration much higher than can be achieved in patient’s serum without toxicity were necessary for this to be demonstrated.

The relationship of a beneficial inhibitory effect by each aminoglycoside-carbenicillin combination at therapeutic concentrations to the MIC of the respective aminoglycoside is seen in Table 4. The addition of carbenicillin to either gentamicin or tobramycin increased the inhibitory activity against all of the isolates that were susceptible or moderately resistant to the aminoglycoside in the combination. But 16 of the 18 isolates highly resistant to gentamicin and 10 of the 14 isolates highly resistant to tobramycin were inhibited no better by their respective aminoglycoside-carbenicillin combinations than by the most effective antibiotic alone. None of the 130 isolates tested were highly resistant to amikacin and this antibiotic in combination with carbenicillin inhibited all but two of the isolates.

**DISCUSSION**

The two new aminoglycoside antibiotics, tobramycin and amikacin, have significant in vitro advantage compared to gentamicin against *P. aeruginosa*. Peak serum levels achieved with tobramycin and gentamicin are almost identical (20). However, tobramycin was found to have two to four times the in vitro activity of gentamicin. Thus, strains susceptible to gentamicin were more susceptible to tobramycin, and strains moderately resistant to gentamicin also could be inhibited by concentrations of tobramycin achievable in the serum.

![Fig. 1. Comparative MICs of each aminoglycoside alone and in combination with 12.5 µg of carbenicillin per ml for 130 clinical isolates of *P. aeruginosa*.](http://aac.asm.org/)

<table>
<thead>
<tr>
<th>Aminoglycoside in combination</th>
<th>Total isolates*</th>
<th>Carbenicillin-resistant isolates*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gentamicin .................</td>
<td>16 (12.3)</td>
<td>7 (46.7)</td>
</tr>
<tr>
<td>Tobramycin ...............</td>
<td>10 (7.7)</td>
<td>3 (20.0)</td>
</tr>
<tr>
<td>Amikacin ...............</td>
<td>2 (1.5)</td>
<td>2 (13.0)</td>
</tr>
</tbody>
</table>

*Total number of isolates was 130; total number of carbenicillin-resistant isolates was 15. The parentheses show percentage.
clinically. Amikacin resembles kanamycin in its pharmacology: serum levels peak at 20 μg/ml in the adult following a single intramuscular injection of 7.5 mg/kg (6, 19). In this study, we considered organisms to be susceptible to amikacin if they were inhibited by 10 μg/ml or less. By this criterion, approximately the same percentage of organisms is as susceptible to amikacin as to tobramycin. Protein binding is insignificant for all three antibiotics and therefore should not influence their comparative activity in serum (6, 11).

The question of cross-resistance between gentamicin, tobramycin, and amikacin is controversial and has clinical implications (5, 7, 9). Our data for P. aeruginosa indicate that cross-resistance between gentamicin and both tobramycin and amikacin is present but not complete. Nineteen of the 33 gentamicin-resistant isolates were susceptible to both tobramycin and amikacin. Strains with high-level gentamicin resistance were generally but not invariably highly resistant to tobramycin. These strains were less frequently resistant to amikacin and never at the highly resistant level. On the other hand, all strains resistant to tobramycin and all but two strains resistant to amikacin were highly resistant to gentamicin. Thus, both tobramycin and amikacin may be useful for gentamicin-resistant isolates, but gentamicin will seldom be of use for isolates resistant to either tobramycin or amikacin.

The in vitro synergism of gentamicin and carbenicillin against P. aeruginosa first was reported by Brumfitt et al. in 1967 (4) and soon confirmed by others (10, 18, 22-24). Subsequently, the synergistic effect was shown in rats (1, 16). Klasterkey and colleagues (14) reported suggestive evidence that synergism may also occur in human gram-negative infections, including those due to P. aeruginosa. The present study demonstrated that in vitro synergism between amikacin or tobramycin and carbenicillin also occurs. The addition of carbenicillin to each of the three aminoglycosides lowered the concentrations of both antibiotics in the combination required to inhibit almost all the isolates. At therapeutic concentrations, however, the beneficial effect of the combination occurred mainly against strains that were susceptible or moderately resistant to the aminoglycoside antibiotic. Strains highly resistant to the aminoglycoside seldom were inhibited more effectively by the antibiotic combination as compared with carbenicillin alone. No isolates highly resistant to amikacin were found in the present study, and all but two strains demonstrated an enhanced inhibitory effect with this antibiotic in combination with carbenicillin. Therefore, for strains highly resistant to gentamicin and tobramycin, the amikacin-carbenicillin combination may have a distinct advantage.

MICs of both gentamicin and carbenicillin of susceptible pseudomonas approach those concentrations which can be obtained reliably in the serum clinically. Hence, the use of the two antibiotics in combination appears rational since it reduces concentrations of both antibiotics required for inhibition well below serum levels achieved in therapy. Whether or not the addition of carbenicillin to amikacin or to tobramycin will be necessary for the treatment of the majority of P. aeruginosa infections can only be determined by a controlled clinical trial. However, with or without carbenicillin, these in vitro findings suggest that both tobramycin and amikacin will be valuable additions for the treatment of these infections. Clinical trials are indicated to verify these findings.

ACKNOWLEDGMENT

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