Comparative Synergistic Activity of Cefoperazone, Cefotaxime, Moxalactam, and Carbenicillin, Combined with Tobramycin, Against *Pseudomonas aeruginosa*

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A broth dilution checkerboard synergy assay was used to assess the activity of cefoperazone, cefotaxime, moxalactam, and carbenicillin, in combination with tobramycin, against 38 strains of *Pseudomonas aeruginosa*. Synergy occurred significantly more often (\(P < 0.001\)) when tobramycin was combined with cefotaxime (63%) than when it was combined with carbenicillin (26%), cefoperazone (21%), or moxalactam (18%). Of the 25 synergistically inhibited strains, the combination cefotaxime-tobramycin was synergistic against 24 and was the only synergistic combination against 10. Of six strains initially resistant to cefotaxime, five were susceptible to this agent (minimum inhibitory concentration, \(\leq 32 \mu g/ml\)) when it was combined with tobramycin. Clinical trials are needed to determine the therapeutic efficacy of cefotaxime-aminoglycoside combinations in the treatment of serious *Pseudomonas* infections.

Antibiotic combinations are widely employed in the treatment of serious *Pseudomonas aeruginosa* infections, particularly in leukopenic or immunosuppressed patients. Empiric regimens traditionally combine an aminoglycoside with an antipseudomonal \(\beta\)-lactam antibiotic such as carbenicillin or ticarcillin to achieve additive or synergistic antibacterial activity (5, 11, 12). Although the clinical relevance of in vitro synergy has not been fully defined (8, 11), a number of studies with experimental animals (1, 2, 13) and with humans (6, 7) indicate that *Pseudomonas* infections respond more favorably to synergistic than to nonsynergistic antibiotic combinations. In addition, by lowering antibiotic minimal inhibitory concentrations (MICs), synergistic combinations permit therapeutically achievable aminoglycoside levels to exceed more effectively the MIC of the infecting *Pseudomonas*.

Cefoperazone (T-1551), cefotaxime (HR-756), and moxalactam (LY127935) are three new semisynthetic \(\beta\)-lactam antibiotics with expanded antibacterial spectra. Thus, they can reasonably be considered candidates for use in combination with an aminoglycoside in the treatment of life-threatening infections due to gram-negative rods, including those caused by *Pseudomonas*. This paper examines the in vitro synergistic activity of these newer agents and of carbenicillin, combined with tobramycin, against 38 strains of *P. aeruginosa*.

MATERIALS AND METHODS

**Microorganisms.** Thirty-seven strains of *P. aeruginosa* were obtained from clinical isolates in the Microbiology Laboratory, Mount Zion Hospital and Medical Center. Organisms were cultured from blood, urine, sputum, and wound specimens submitted from separate patients over a 6-month period. In addition, *P. aeruginosa* ATCC 27853 (American Type Culture Collection, Rockville, Md.) was included as a control. All strains were maintained on tryptic soy agar slants (Bakte Bennett, Berkeley, Calif.) at room temperature until tested. Synergy studies were performed on all isolates during a single 48-h period.

**Antibiotics.** Sterile, standardized antibiotic powders were kindly provided by their respective manufacturers: carbenicillin monohydrate monosodium and cefoperazone by Pfizer Pharmaceuticals, New York, N.Y.; cefotaxime by Hoechst-Roussel Pharmaceuticals, Somerville, N.J.; and moxalactam and tobramycin by Eli Lilly and Co., Indianapolis, Ind.

**Synergy plates.** Antibiotic combinations were prepared to specifications in 80-well microtiter plates by Micro-Media Systems, Inc., San Jose, Calif. Antibiotics were diluted in Mueller-Hinton broth supplemented with calcium (50 \(\mu g/ml\)) and magnesium (25 \(\mu g/ml\)), and dispensed in working volumes of 0.1 ml per well. Twofold serial dilutions of tobramycin were made in vertical columns, and twofold dilutions of the other antibiotics were made in horizontal rows so that each concentration of the aminoglycoside was tested with each concentration of a \(\beta\)-lactam antibiotic (checkerboard pattern). The range of dilutions of the \(\beta\)-lactam agents was 0.25 to 512 \(\mu g/ml\). Tobramycin concentrations ranged from 0.032 to 16 \(\mu g/ml\). On each plate one column of wells contained no \(\beta\)-lactam agent and one row of wells contained no tobramycin; MICs of tobramycin and the various \(\beta\)-lactam antibiotics for the 38 strains of *P. aeruginosa* were read from these columns and rows, respectively. Two wells per plate contained Mueller-Hinton broth only; one served as a growth control, and the other, un inoculated, served as a sterility control. All plates were shipped frozen and received in this laboratory within 72 h of manufacture.
They were kept frozen at −20°C until used within 48 h of receipt. To control for loss of antibiotic potency in the frozen and thawed plates, MICs were determined for P. aeruginosa ATCC 27853 with freshly prepared dilutions of tobramycin and the four β-lactam agents. Results were identical to those obtained with the frozen-thawed plates or differed by no more than a single twofold dilution.

Synergy testing. Four or five colonies of each organism were picked from fresh agar slants, suspended in 0.5 ml of brain heart infusion broth, and incubated for 6 h to achieve a stationary growth phase. The inoculum was then diluted 1:500 by transferring 0.05 ml of the broth culture to a tube containing 25 ml of sterile distilled water supplemented with 0.02% Tween 80. This suspension was poured into a seed trough, and the thawed test plate was inoculated with a transfer lid whose prongs delivered 0.005 ml of bacterial suspension to each well except the sterility control well. The final bacterial concentration was 10⁵ colony-forming units per ml. Immediately after inoculation, 0.001 ml was removed from the growth control well with a calibrated loop and streaked on a blood agar plate to provide a quantitative assessment of inoculum size.

Plates were incubated at 36°C for 18 h and examined for bacterial growth. The MIC was defined as the lowest concentration of antibiotic that inhibited visible growth. Synergy was defined as a fourfold reduction in the MICs of both agents in combination. All strains were tested in duplicate. Results of the two synergy determinations differed in 26 of the 152 replicate runs; in these instances the test was repeated a third time, and the results of that determination were recorded.

RESULTS

The MICs of the five antibiotics tested against 38 strains of P. aeruginosa are shown in Table 1. Cefoperazone was the most active β-lactam agent, followed by moxalactam, cefotaxime, and carbenicillin. The MICs of tobramycin and carbenicillin for P. aeruginosa ATCC 27853 (1 and 32 μg/ml, respectively) agreed with results obtained in a number of reference laboratories (9).

The results of synergy testing are shown in Table 2. Synergy was observed significantly more often when tobramycin was combined with cefotaxime than when it was combined with any of the other β-lactam antibiotics (P < 0.001). Of the 25 synergistically inhibited strains of Pse-

### Table 1. Susceptibilities of 38 strains of P. aeruginosa to five antibiotics

<table>
<thead>
<tr>
<th>Drug</th>
<th>MIC (μg/ml)</th>
<th>Range</th>
<th>For 50% of isolates</th>
<th>For 90% of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tobramycin</td>
<td>0.125–16</td>
<td>2</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Cefoperazone</td>
<td>2–32</td>
<td>4</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Moxalactam</td>
<td>8–64</td>
<td>8</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>8–256</td>
<td>16</td>
<td>64</td>
<td></td>
</tr>
<tr>
<td>Carbenicillin</td>
<td>16–256</td>
<td>64</td>
<td>128</td>
<td></td>
</tr>
</tbody>
</table>

*P < 0.001 for T+CT versus each of the other combinations (McNemar test). Differences among the other antibiotic combinations were not statistically significant.

Synergy with β-lactam antibiotics was synergistic against 24 and was the sole synergistic combination against 10 strains. Tobramycin plus cefoperazone was the sole synergistic combination against one isolate.

The resistance breakpoints for tobramycin and carbenicillin were >4 μg/ml and >128 μg/ml, respectively. Resistance breakpoints for the newer β-lactam agents have not been defined, although resistance to cefotaxime has recently been proposed as >32 μg/ml (4). If a similar level is assumed for cefoperazone and moxalactam, then resistance to a β-lactam agent was observed in 11 instances (cefotaxime, 6; carbenicillin, 3; moxalactam, 2). However, in eight of these instances synergy was achieved, resulting in a decrease in the MIC of the β-lactam antibiotic to within the susceptible range (Table 3). Similarly, the single tobramycin-resistant strain (MIC, 16 μg/ml) was susceptible to 4 μg of that agent per ml when combined with either carbenicillin or cefotaxime.

### DISCUSSION

In this study a stringent definition of synergy (fourfold reduction in MIC of both agents) was applied to the broth dilution checkerboard assay. In a recent comparison of synergy techniques by Norden et al. (10), this proved to be the most rigorous test system evaluated in that it yielded the fewest positive results. Thus, the results obtained in the present study tend to be a conservative estimate of the synergistic potential of the various antibiotic combinations.

The value of in vitro synergy testing against gram-negative organisms can be established only by determining its relevance to the therapeutic outcome of clinical infections (8). To this end, studies with neutropenic (13) and nonneutropenic (1, 2) laboratory animals have demonstrated enhanced survival from lethal P. aeruginosa infections when treated with synergistic antibiotic combinations. Studies with human subjects have suggested improved clinical response rates
among febrile, granulocytopenic cancer patients when treated empirically with gentamicin plus carbenicillin (3, 12). Klastersky and co-workers (6, 7) found significantly better response rates in patients with a variety of gram-negative bacillary infections when treated with synergistic as opposed to nonsynergistic antibiotic combinations, even when the infecting organisms were initially susceptible to both members of the non-synergistic pair (6).

Analysis of the results presented here might suggest that the synergistic increase in activity of cefotaxime when combined with tobramycin does little more than lower the MIC of cefotaxime to that of cefoperazone. More important, however, is the fact that this combination resulted in a fourfold lowering of the tobramycin MIC significantly more often than did any other tobramycin–β-lactam combination (P < 0.001). This enhancement is of particular importance when treating Pseudomonas infections since the toxic-therapeutic ratio of aminoglycosides is considerably lower for this organism than for other gram-negative bacilli.

These data do not establish the superiority of aminoglycoside-cefotaxime combinations in the treatment of serious Pseudomonas infections. They do, however, suggest the need for in vivo studies to compare the therapeutic efficacy of cefotaxime plus an aminoglycoside with currently available antipseudomonal regimens. Such studies must also determine whether simultaneous administration of cefotaxime and an aminoglycoside increases the risk of nephrotoxicity, as has been observed with cephalothin-aminoglycoside combinations (3, 14).

### Table 3. Effect of synergy on the susceptibility of P. aeruginosa strains initially resistant to one or more β-lactam antibiotics

<table>
<thead>
<tr>
<th>Drug</th>
<th>No. of strains:</th>
<th>Initially resistant</th>
<th>Susceptible with synergy*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cefotaxime</td>
<td></td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Carbenicillin</td>
<td></td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Moxalactam</td>
<td></td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

* MIC of β-lactam antibiotic reduced to clinically achievable levels by combination with tobramycin.

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