In Vitro Synergistic Activities of Tobramycin and Selected β-Lactams against 75 Gram-Negative Clinical Isolates

ROBERT C. OWENS, JR.,1–3* MARY ANNE BANEVICIUS,4 DAVID P. NICOLAU,4–6 CHARLES H. NIGHTINGALE,4,5 AND RICHARD QUINTILIANI4,5

Department of Clinical Pharmacy Services, Medical Center of Delaware, Newark, Delaware 19718; School of Pharmacy, University of Maryland, Baltimore, Maryland 21201; Philadelphia College of Pharmacy and Science, Philadelphia, Pennsylvania 19104; and Department of Pharmacy Research, Office for Research, and Division of Infectious Diseases, Hartford Hospital, Hartford, Connecticut 06102

Received 14 April 1997/Returned for modification 10 August 1997/Accepted 10 September 1997

To avoid the emergence of resistance during therapy and to improve clinical outcomes, combination antibiotic therapy is often employed in the treatment of serious gram-negative bacterial infections (4, 9a, 11, 19). Moreover, combination therapy broadens the spectrum of bacterial coverage and often achieves synergistic inhibition of the infecting pathogen. Against gram-negative bacteria, a difference exists in the frequency of synergism when a cephalosporin versus a penicillin is combined with an aminoglycoside. To provide insight into the optimal synergistic combination for a variety of nosocomial gram-negative nosocomial isolates, a greater percentage of synergy was consistently observed with combination regimens containing tobramycin and piperacillin-tazobactam or ticarcillin-clavulanate than with the cephalosporin-containing regimens.

The microdilution checkerboard technique was utilized to distinguish synergistic activity between tobramycin and four β-lactams: piperacillin-tazobactam, ticarcillin-clavulanate, ceftazidime, and ceftriaxone. β-Lactam–aminoglycoside combinations were tested against 75 clinical isolates of Pseudomonas aeruginosa, Acinetobacter baumanii, Citrobacter freundii, Serratia marcescens, and Enterobacter cloacae. Despite in vitro susceptibilities, all isolates demonstrated either synergism or indifference; no antagonism was observed. Against pathogenic gram-negative nosocomial isolates, a greater percentage of synergy was consistently observed with combination regimens containing tobramycin and piperacillin-tazobactam or ticarcillin-clavulanate than with the cephalosporin-containing regimens.

The microdilution checkerboard technique was utilized to distinguish synergistic activity between tobramycin and four β-lactams: piperacillin-tazobactam, ticarcillin-clavulanate, ceftazidime, and ceftriaxone. β-Lactam–aminoglycoside combinations were tested against 75 clinical isolates of Pseudomonas aeruginosa, Acinetobacter baumanii, Citrobacter freundii, Serratia marcescens, and Enterobacter cloacae. Despite in vitro susceptibilities, all isolates demonstrated either synergism or indifference; no antagonism was observed. Against pathogenic gram-negative nosocomial isolates, a greater percentage of synergy was consistently observed with combination regimens containing tobramycin and piperacillin-tazobactam or ticarcillin-clavulanate than with the cephalosporin-containing regimens.

The microdilution checkerboard technique was utilized to distinguish synergistic activity between tobramycin and four β-lactams: piperacillin-tazobactam, ticarcillin-clavulanate, ceftazidime, and ceftriaxone. β-Lactam–aminoglycoside combinations were tested against 75 clinical isolates of Pseudomonas aeruginosa, Acinetobacter baumanii, Citrobacter freundii, Serratia marcescens, and Enterobacter cloacae. Despite in vitro susceptibilities, all isolates demonstrated either synergism or indifference; no antagonism was observed. Against pathogenic gram-negative nosocomial isolates, a greater percentage of synergy was consistently observed with combination regimens containing tobramycin and piperacillin-tazobactam or ticarcillin-clavulanate than with the cephalosporin-containing regimens.

To avoid the emergence of resistance during therapy and to improve clinical outcomes, combination antibiotic therapy is often employed in the treatment of serious gram-negative bacterial infections (4, 9a, 11, 19). Moreover, combination therapy broadens the spectrum of bacterial coverage and often achieves synergistic inhibition of the infecting pathogen. Against gram-negative bacteria, a difference exists in the frequency of synergism when a cephalosporin versus a penicillin is combined with an aminoglycoside. To provide insight into the optimal synergistic combination for a variety of nosocomial gram-negative pathogens, we evaluated two penicillin derivative-containing β-lactamase inhibitor combinations (piperacillin-tazobactam and ticarcillin-clavulanate) versus two cephalosporin agents (ceftazidime and ceftriaxone), each combined with tobramycin, by the microdilution checkerboard technique.

(This research was presented, in part, at the 97th General Meeting of the American Society for Microbiology, Miami Beach, Fla., May 4 to 8 1997 [15a].)

Seventy-five clinical isolates, 15 each of Pseudomonas aeruginosa, Acinetobacter baumanii, Citrobacter freundii, Serratia marcescens, and Enterobacter cloacae, were utilized. P. aeruginosa ATCC 27853 was used as the control strain. All strains were stored at −70°C prior to use. Cation-adjusted Mueller-Hinton broth supplemented with Ca2+ (25 mg/liter) and Mg2+ (12.5 mg/liter) (Becton Dickinson Microbiology Systems, Cockeysville, Md.) was utilized in all in vitro testing.

The following antimicrobial agents were obtained for use: piperacillin-tazobactam (Wyeth-Ayerst Laboratories, Carolina, P.R.), ticarcillin-clavulanate (SmithKline Beecham Pharmaceuticals, King of Prussia, Pa.), ceftriaxone (Hoffmann-La Roche Pharmaceuticals, Nutley, N.J.), ceftazidime (Glaxo Pharmaceuticals, Research Triangle, N.C.), and tobramycin (Sigma Chemical Company, St. Louis, Mo.). All stock solutions were prepared in accordance with the guidelines provided by the manufacturers and the National Committee for Clinical Laboratory Standards (21).

All strains were tested by the microdilution checkerboard technique provided by Eliopoulos and Moellering (9). Briefly, bacterial dilutions from the logarithmic-growth phase were prepared and subsequently pipetted into microtiter trays containing various drug regimen concentrations. The final inoculum size in the microtiter trays equaled 10⁵ CFU/ml. As a means of quality control, a sample of inoculating suspension was plated out to assess colony growth both quantitatively and qualitatively. Concentrations of each antibiotic ranged from four to five times below the MIC to two times the MIC for the pathogen being tested. Regimens studied included tobramycin in combination with each of the following four agents: piperacillin-tazobactam and ticarcillin-clavulanate (maintained at fixed inhibitor concentrations of 4 and 2 µg/ml, respectively), ceftazidime, and ceftriaxone. Inoculated microtiter trays were incubated at 37°C for a period of 24 h, after which trays were read for inhibition of bacterial growth. In order to evaluate the outcome of the drug combination, fractional inhibitory concentration (FIC) indices were calculated as FICₐ + FICₕ, where FICₐ and FICₕ represent the minimum concentrations that inhibited organism growth for drugs A and B, respectively (8). Individual checkerboard runs were repeated five times for each isolate and drug combination tested. From the five replicate, a mean FIC index was calculated, applied to a commonly utilized definition of synergies, and classified as either synergistic (≤0.5), indifferent (≤0.6 but ≤4.0), or antagonistic (>4.0). A general agreement of 80% (4/5) between checkerboard replications was defined based on previous checkerboard experience with P. aeruginosa (16).

Statistical differences between the frequency of synergy were compared by the chi-square test for multiple comparisons. A statistical difference was determined by a P value of ≤0.5.

Median MICs and their ranges for the individual agents are presented in Table 1. The results, expressed as the percentage of isolates against which synergy was demonstrated for each combination, are provided in Table 2. Table 2 also shows, for each β-lactam–aminoglycoside combination, the synergistic and each pathogen, the
number of isolates that tested either susceptible or intermediately susceptible to at least one agent. Our results indicated that the penicillin derivatives (piperacillin and ticarcillin) in combination with β-lactamase inhibitors showed a greater degree of synergy against P. aeruginosa and S. marcescens than did the cephalosporin agents, ceftazidime and ceftriaxone, when combined with tobramycin, regardless of susceptibilities to the individual agents. Of the penicillin derivative combinations, piperacillin-tazobactam tested synergistic against a significantly greater percentage of P. aeruginosa and S. marcescens isolates than cephalosporin-containing regimens, whereas ticarcillin-clavulanate did not. As observed in Table 2, both penicillin derivative-containing regimens showed 100% synergy against isolates of A. baumannii, a finding that was not observed with the other isolates tested. All isolates not classified as synergistic were determined to be indifferent, and no isolate showed antagonism to any antibiotic regimen.

Among resistant isolates of P. aeruginosa, several isolates that were resistant to the β-lactam component also had variable susceptibility to tobramycin. Despite resistance to both piperacillin-tazobactam and tobramycin in two isolates, one showed synergy and the other indifference; the same was observed with the other isolates tested. All isolates not classified as synergistic were determined to be indifferent, and no isolate showed antagonism to any antibiotic regimen.

For two isolates showing resistance to both ticarcillin-clavulanate and tobramycin, values for the penicillin are given to the left of the shill and values for the β-lactam–tobramycin combinations against a large number of clinically isolated pathogenic gram-negative bacteria. Our results indicate that the penicillins–β-lactamase inhibitors in combination with tobramycin show a greater degree of synergy compared with the cephalosporins against all isolates tested except those of C. freundii and E. cloacae. For the C. freundii and E. cloacae isolates, the degrees of synergy observed in all aminoglycoside-containing regimens were essentially equal. In addition, piperacillin-tazobactam showed a significantly greater percentage of synergistic activity than the cephalosporin-containing regimens against P. aeruginosa and S. marcescens. Both
tinually changing in quantity and potentially in quality over time, and the ability to capture the dynamics of this complex organism-drug-host relationship is beyond our current technology. We therefore depend on currently available methods for synergy determination to assess the complex nature of these drug relationships; as a result, these data are useful when the limitations (e.g., variability in interpretive criteria and lack of reproducibility) of in vitro testing are respected.

Despite this limitation in the interpretation of synergy data, the observation of synergism is generally considered beneficial in the treatment of infection. The literature provides numerous citations of studies that have evaluated the in vitro synergy of specific drug combinations against a wide range of gram-positive and gram-negative organisms; however, due to the complexity of the pathogen-host-drug triad, limited in vivo data which support the use of synergistic combinations are available. The relationship between in vitro synergy and clinical outcome has been studied for the following infection-specific diagnoses: endocarditis (3, 7, 17, 18, 22, 23), meningitis (20), and bacterial sepsis in both immunocompetent and granulocytopenic patients (1, 2, 5, 11–13). These studies support the use of synergistic combinations.

In the present study, we evaluated in vitro synergism among β-lactam–tobramycin combinations against a large number of clinically isolated pathogenic gram-negative bacteria. Our results indicate that the penicillins–β-lactamase inhibitors in combination with tobramycin show a greater degree of synergy compared with the cephalosporins against all isolates tested except those of C. freundii and E. cloacae. For the C. freundii and E. cloacae isolates, the degrees of synergy observed in all aminoglycoside-containing regimens were essentially equal. In addition, piperacillin-tazobactam showed a significantly greater percentage of synergistic activity than the cephalosporin-containing regimens against P. aeruginosa and S. marcescens. Both

### TABLE 1. Medians and ranges of MICs for individual agents against each set of organisms

<table>
<thead>
<tr>
<th>Organism</th>
<th>P/T</th>
<th>T/C</th>
<th>TAZ</th>
<th>CTX</th>
<th>TOB</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. marcescens</td>
<td>2/4 (1/4–4/4)</td>
<td>8/2 (4/2–32/2)</td>
<td>0.25 (0.125–0.5)</td>
<td>0.25 (0.125–0.5)</td>
<td>1.0 (0.5–16)</td>
</tr>
<tr>
<td>E. cloacae</td>
<td>2/4 (1/4–4/4)</td>
<td>4/2 (2/2–16/2)</td>
<td>0.5 (0.125–4)</td>
<td>0.5 (0.125–1)</td>
<td>0.5 (0.25–1)</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>16/4 (4/4–256/4)</td>
<td>32/2 (8/2–256/2)</td>
<td>4 (2–32)</td>
<td>NT</td>
<td>1.0 (0.25–64)</td>
</tr>
<tr>
<td>C. freundii</td>
<td>2/4 (2/4–64/4)</td>
<td>2/2 (1/2–256/2)</td>
<td>0.5 (0.25–128)</td>
<td>0.5 (0.125–128)</td>
<td>1.0 (0.25–128)</td>
</tr>
<tr>
<td>A. baumannii</td>
<td>8/4 (8/4–256/4)</td>
<td>8/2 (0.5/2–64/2)</td>
<td>8 (2–32)</td>
<td>32 (1–128)</td>
<td>1.0 (0.125–64)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Organism</th>
<th>% Synergy</th>
<th>No. of isolates susceptible</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. marcescens</td>
<td>80</td>
<td>12/12</td>
</tr>
<tr>
<td>E. cloacae</td>
<td>47</td>
<td>7/7</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>93</td>
<td>2/14</td>
</tr>
<tr>
<td>C. freundii</td>
<td>53</td>
<td>2/8</td>
</tr>
<tr>
<td>A. baumannii</td>
<td>100</td>
<td>2/15</td>
</tr>
</tbody>
</table>

**Abbreviations:** P/T, piperacillin-tazobactam; T/C, ticarcillin-clavulanate; TAZ, ceftazidime; CTX, ceftriaxone; TOB, tobramycin; NT, not tested.

**Footnotes:**

- *Fifteen isolates were tested for each species.
- In micrograms per milliliter. Abbreviations: P/T, piperacillin-tazobactam; T/C, ticarcillin-clavulanate; TAZ, ceftazidime; CTX, ceftriaxone; TOB, tobramycin; NT, not tested. For piperacillin-tazobactam and ticarcillin-clavulanate, values for the penicillin are given to the left of the shill and values for the β-lactam–tobramycin combinations against a large number of clinically isolated pathogenic gram-negative bacteria. Our results indicate that the penicillins–β-lactamase inhibitors in combination with tobramycin show a greater degree of synergy compared with the cephalosporins against all isolates tested except those of C. freundii and E. cloacae. For the C. freundii and E. cloacae isolates, the degrees of synergy observed in all aminoglycoside-containing regimens were essentially equal. In addition, piperacillin-tazobactam showed a significantly greater percentage of synergistic activity than the cephalosporin-containing regimens against P. aeruginosa and S. marcescens. Both
tinually changing in quantity and potentially in quality over time, and the ability to capture the dynamics of this complex organism-drug-host relationship is beyond our current technology. We therefore depend on currently available methods for synergy determination to assess the complex nature of these drug relationships; as a result, these data are useful when the limitations (e.g., variability in interpretive criteria and lack of reproducibility) of in vitro testing are respected.

Despite this limitation in the interpretation of synergy data, the observation of synergism is generally considered beneficial in the treatment of infection. The literature provides numerous citations of studies that have evaluated the in vitro synergy of specific drug combinations against a wide range of gram-positive and gram-negative organisms; however, due to the complexity of the pathogen-host-drug triad, limited in vivo data which support the use of synergistic combinations are available. The relationship between in vitro synergy and clinical outcome has been studied for the following infection-specific diagnoses: endocarditis (3, 7, 17, 18, 22, 23), meningitis (20), and bacterial sepsis in both immunocompetent and granulocytopenic patients (1, 2, 5, 11–13). These studies support the use of synergistic combinations.

In the present study, we evaluated in vitro synergism among β-lactam–tobramycin combinations against a large number of clinically isolated pathogenic gram-negative bacteria. Our results indicate that the penicillins–β-lactamase inhibitors in combination with tobramycin show a greater degree of synergy compared with the cephalosporins against all isolates tested except those of C. freundii and E. cloacae. For the C. freundii and E. cloacae isolates, the degrees of synergy observed in all aminoglycoside-containing regimens were essentially equal. In addition, piperacillin-tazobactam showed a significantly greater percentage of synergistic activity than the cephalosporin-containing regimens against P. aeruginosa and S. marcescens. Both

### TABLE 2. Percentage of isolates against which synergy with tobramycin was demonstrated for each of the four β-lactams

<table>
<thead>
<tr>
<th>Organism</th>
<th>P/T</th>
<th>T/C</th>
<th>TAZ</th>
<th>CTX</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. marcescens</td>
<td>80</td>
<td>33(^d)</td>
<td>33(^d)</td>
<td>12/12</td>
</tr>
<tr>
<td>E. cloacae</td>
<td>47</td>
<td>20</td>
<td>53</td>
<td>7/7</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>93</td>
<td>NT</td>
<td>2/14</td>
<td>1/7</td>
</tr>
<tr>
<td>C. freundii</td>
<td>53</td>
<td>40</td>
<td>2/8</td>
<td>0/7</td>
</tr>
<tr>
<td>A. baumannii</td>
<td>100</td>
<td>73</td>
<td>47(^e)</td>
<td>2/15</td>
</tr>
</tbody>
</table>

**Footnotes:**

- *Fifteen isolates were tested for each combination; each checkerboard run was repeated five times.
- Abbreviations: P/T, piperacillin-tazobactam; T/C, ticarcillin-clavulanate; TAZ, ceftazidime; CTX, ceftriaxone; NT, not tested.
- Number of isolates against which synergy was demonstrated that were susceptible or intermediately susceptible to at least one agent/total number of isolates against which synergy was demonstrated.
- Statistically different from piperacillin-tazobactam and ticarcillin-clavulanate (\(P \leq 0.5\)).
penicillin derivative-containing regimens displayed 100% synergy against the isolates of A. baumannii studied. Our results are similar to those of a previous evaluation of cephalosporin-aminoglycoside combinations assessed by the checkerboard microdilution method; that study showed the frequency of synergism to be 30 to 40% for the gram-negative pathogens tested (10).

β-Lactams combined with tobramycin, tested against isolates of P. aeruginosa, resulted in either synergism or indifference, despite the presence of resistance. As a result of these data, the ability to achieve synergy should not be dismissed on the basis of individual susceptibility patterns demonstrated by an isolated pathogen. Since the definition of synergy by the checkerboard method entails at least a fourfold reduction in the MIC of the agent when it is utilized in combination, the magnitude of this increase in susceptibility should be considered in treatment for multiple-drug-resistant isolates, against which optimal treatment strategies should be employed. The initiation of optimal anti-infective treatment regimens which maximize bacterial killing early in the infection process is critical for patients infected with virulent and potentially multidrug-resistant pathogens, such as pseudomonas (6). Although a clear association between in vitro synergy assessments and good clinical outcomes is not always agreed upon, it is for these patients with high associated morbidity and mortality or for infections where resistant organisms are anticipated that the potential for optimal synergistic combinations should be considered. Since an antipseudomonal β-lactam in combination with an aminoglycoside is currently considered the treatment of choice for patients with suspected or proven serious systemic pseudomonal infection, our data suggest that the optimal empirical choice is to use an antipseudomonal penicillin rather than an antipseudomonal cephalosporin (11, 14). In fact, comparison of the antipseudomonal penicillins indicate that piperacillin-tazobactam has an even-greater chance of attaining synergism to be 30 to 40% for the gram-negative pathogens studied. Our results are similar to those of a previous evaluation of cephalosporin-aminoglycoside combinations assessed by the checkerboard microdilution method; that study showed the frequency of synergism to be 30 to 40% for the gram-negative pathogens tested (10).

We acknowledge Wyeth-Ayerst Laboratories for their financial support, and to Yu Ying Xu and Christina Turley for their technical assistance.

REFERENCES


