In Vitro Activity of Ceftriaxone Alone and in Combination with Gentamicin, Tobramycin, and Amikacin Against *Pseudomonas aeruginosa*

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Ceftriaxone, a new third-generation cephalosporin with a very long serum half-life, has been shown to have a wide antimicrobial spectrum. Its activity against *Pseudomonas aeruginosa*, however, is rather limited (1, 5, 6, 8). The in vitro activity of ceftriaxone against 50 *P. aeruginosa* strains by the broth dilution method and the effects of combining ceftriaxone with gentamicin, tobramycin, or amikacin against *P. aeruginosa* by the time-kill curve method were studied in this investigation.

Forty-five gentamicin-susceptible (minimal inhibitory concentration [MIC] ≤ 4 μg/ml) and five gentamicin-resistant (MIC, ≥ 8 μg/ml) *P. aeruginosa* strains were used in this study. Ceftriaxone was obtained from Hoffmann-La Roche Inc., Nutley, N.J.; gentamicin was obtained from Schering Corp., Bloomfield, N.J.; tobramycin was obtained from Eli Lilly & Co., Indianapolis, Ind.; and amikacin was obtained from Bristol Laboratories, Syracuse, N.Y. A standard stock solution of each antibiotic was prepared according to the instructions of the manufacturer and either used immediately or stored at −80°C and thawed immediately before use.

The MIC and minimal bactericidal concentration (MBC) of each antibiotic were determined by the broth dilution method of the World Health Organization International Collaborative Study (2). Serial twofold dilutions of the antibiotic from 64 to 0.006 μg/ml were made in Mueller-Hinton broth. The inoculum was 1 ml of 10⁶ CFU diluted from an 18-h culture. The MIC was defined as the lowest antibiotic concentration that resulted in no visible growth after incubation at 37°C for 18 to 24 h. A subculture of each tube containing broth without visible growth was made by streaking 0.01 ml onto the surface of Mueller-Hinton agar. Colonies were counted after incubation at 37°C for 48 h. The MBC was defined as the lowest antibiotic concentration that resulted in 99.9% killing of the original inoculum.

The standard time-kill curve method was used to study the interactions between ceftriaxone and gentamicin, tobramycin, or amikacin. Mueller-Hinton broth was used. The antibiotic concentrations (in micrograms per milliliter) were as follows: ceftriaxone, 32; gentamicin, 4; tobramycin, 4; amikacin, 16; ceftriaxone, 32, combined with gentamicin, 4; ceftriaxone, 32, combined with tobramycin, 4; and ceftriaxone, 32, combined with amikacin, 16. A broth culture with no antibiotic was set up as a control. The inoculum contained 10⁶ CFU/ml and was made from an 18-h culture. All tubes were incubated in a Dry Bath (Fisher Scientific Co., Pittsburgh, Pa.) at 37°C. At 0, 6, 24, and 48 h, the viable CFU were enumerated by serial 10-fold dilutions plated on Mueller-Hinton agar. Colonies were counted after incubation at 37°C for 48 h.

When the result of the combination was at least a 2 × log₁₀ decrease in viable CFU from

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**TABLE 1. In vitro susceptibilities of 50 *P. aeruginosa* strains**

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>MIC/MBC (μg/ml)</th>
<th>50%*</th>
<th>90%b</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gentamicin</td>
<td>2/16</td>
<td>4/32</td>
<td></td>
<td>1−&gt;64/2−&gt;64</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>1/2</td>
<td>2/8</td>
<td>0.125−&gt;64/0.5−&gt;64</td>
<td></td>
</tr>
<tr>
<td>Amikacin</td>
<td>2/8</td>
<td>8/32</td>
<td></td>
<td>1−32/2−32</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>&gt;64/64</td>
<td>&gt;64/64</td>
<td>4−&gt;64/64</td>
<td></td>
</tr>
</tbody>
</table>

*a* MIC/MBC for 50% of strains.

*b* MIC/MBC for 90% of strains.
that of both drugs alone at a given time, it was defined as synergism. When the result of the combination was at least a $2 \times \log_{10}$ increase in viable CFU from that of either drug alone, it was defined as antagonism.

Results of the MIC and MBC determinations are shown in Table 1. The majority of the P. aeruginosa strains were resistant to ceftriaxone, confirming previous reports (1, 5, 6, 8). Using a method that employs an inoculum ($10^6$ CFU/ml) and a subculture volume (0.01 ml) sufficient to allow accurate determination of the MBC (7), we found that an MBC/MIC ratio of $\geq 4$ for ceftriaxone was common among the susceptible strains. The results of time-kill curve studies are shown in Table 2. Both synergism and antagonism were shown, although indifference was shown for the majority of strains. Neu et al. reported that the combination of ceftriaxone and gentamicin demonstrated synergistic inhibitory activity against one of five P. aeruginosa strains tested (6). Fass reported synergistic inhibitory activity of ceftriaxone and tobramycin in combination against 73% of 52 P. aeruginosa strains tested by the checkerboard microtiter method (3). Antagonism was not mentioned by Neu et al. or Fass (3, 6). Antagonism was not found in two other studies in which combinations of aminoglycosides and other new cephalosporins were used (4, 9).

Using the time-kill curve method with a sufficient inoculum size ($10^6$ CFU/ml), we demonstrated both synergism and antagonism between ceftriaxone and an aminoglycoside against P. aeruginosa. Since the effects of such combinations are unpredictable and strain dependent (9), its clinical use against P. aeruginosa infections requires further careful prospective clinical investigation.

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**LITERATURE CITED**