Activity of Ceftizoxime Combined with Gentamicin Against 100 Clinical Isolates of Pseudomonas aeruginosa

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The effects of ceftizoxime and gentamicin alone and in combination were determined against 100 Pseudomonas aeruginosa strains. Concentrations of 32 μg of ceftizoxime and 8 μg of gentamicin per ml inhibited 22 and 43% of the strains, respectively. At concentrations of ceftizoxime and gentamicin that are readily achievable in serum, 15 strains were affected synergistically and 17 strains were affected antagonistically. Gentamicin accounted for most of the anti-P. aeruginosa activity of ceftizoxime-gentamicin combinations.

Ceftizoxime is a new, semisynthetic, beta-lactamase-resistant cephalosporin recently approved for use in the United States. In a previous report, we noted synergy between ceftizoxime-gentamicin combinations in 10 of 21 (48%) Pseudomonas aeruginosa strains (7). In this study, we detected synergy in only 19% of the strains when similar techniques were used to determine the in vitro activities of ceftizoxime-gentamicin combinations on 100 recent clinical isolates of P. aeruginosa.

One hundred clinical isolates of P. aeruginosa were collected between June 1982 and June 1983, identified by a standard method (5), and stored at 4°C on brain heart infusion agar slants under sterile mineral oil. Before each experiment, ceftizoxime (Fujiwara SmithKline, Philadelphia, Pa.) and gentamicin (Schering Corp., Kenilworth, N.J.) were dissolved in sterile distilled water and diluted with Mueller-Hinton broth supplemented with 50 μg of calcium and 20 μg of magnesium per ml. The MIC of each antibiotic was determined by a microtiter broth dilution method (3). An automated device (Dynatech Laboratories, Inc., Alexandria, Va.) dispensed 0.1-ml amounts from serial dilutions of ceftizoxime and gentamicin in Mueller-Hinton broth into microtiter wells and inoculated each well with 5 × 10^5 CFU of Pseudomonas strains from 18-h cultures. The MIC was defined as the lowest antibiotic concentration that resulted in no visible growth after incubation at 37°C for 18 h. Reference strains P. aeruginosa ATCC 27853 and ATCC 25619 were used as controls in all experiments and were inhibited by 2 to 4 and 4 to 8 μg of gentamicin per ml, respectively.

The inhibitory activity of ceftizoxime-gentamicin combinations were determined as previously described (7). Serial twofold dilutions of ceftizoxime (range, 0.125 to 128 μg/ml), gentamicin (range, 0.25 to 16 μg/ml), and ceftizoxime-gentamicin combinations were prepared in Mueller-Hinton broth, dispensed into microtiter wells, and inoculated. Microtiter plates were incubated at 37°C for 18 h and examined for visible growth. The effects of ceftizoxime-gentamicin combinations were determined for each organism by calculating the fractional inhibitory concentration (FIC) index (2). Synergy was defined as an FIC index of ≤0.5, indifference as an FIC index of >0.5 but ≤1, and antagonism as an FIC index of >1. The term indeterminate was used to describe antibiotic interactions in which FIC indexes could not be determined because the MICs of one or both drugs were above (37 strains) or below (1 strain) the ranges tested.

The frequencies of synergy in the original and present studies and the frequencies of synergy and antagonism in the present study were analyzed at the 95% confidence level by the estimation-of-proportions method of Dixon and Massey (1). Ceftizoxime concentrations of 8 and 16 μg/ml inhibited 4% of the P. aeruginosa strains, whereas 32 μg of ceftizoxime per ml inhibited 22% of the strains tested. Gentamicin concentrations of 4 and 8 μg/ml inhibited 14 and 43% of the P. aeruginosa strains, respectively. Thus, 8 μg of gentamicin per ml inhibited almost twice as many P. aeruginosa strains as did 32 μg of ceftizoxime per ml. Of the 51 strains inhibited by the combination of 32 μg of ceftizoxime and 8 μg of gentamicin per ml, 43 (84.3%) strains were inhibited by 8 μg of gentamicin per ml and 16 (31.4%) strains were inhibited by both 32 μg of ceftizoxime and 8 μg of gentamicin per ml. Of the 57 strains not inhibited by 8 μg of gentamicin per ml, however, only 6 (10.5%) were inhibited by 32 μg of ceftizoxime per ml.

Table 1 depicts the frequencies of synergy, indifference, and antagonism found with various ceftizoxime-gentamicin combinations in the 62 P. aeruginosa strains for which FIC indexes could be calculated. The remaining 38 P. aeruginosa strains were categorized as indeterminate.

A synergistic interaction between ceftizoxime and gentamicin was detected in 19 of the 100 strains tested. Of these 19 strains, 15 (78.9%) were synergistically affected by 32 μg of ceftizoxime and 8 μg of gentamicin per ml. Four strains required >32 μg of ceftizoxime and >8 μg of gentamicin per ml for demonstration of synergistic interaction.

Other responses to combinations of ceftizoxime and gentamicin were indifference in 20 strains and antagonism in 23 strains. In 10 strains, ≥32 μg of ceftizoxime and ≥4 μg of gentamicin per ml were antagonistic. In the remaining 13 strains, however, ≤3.5 μg of ceftizoxime and ≤16 μg of gentamicin per ml were antagonistic, but >32 μg of ceftizoxime and ≥8 μg of gentamicin per ml were synergistic against 11 strains and indifferent against 2 strains. Each of these 13 latter strains were inhibited by ≥32 μg of ceftizoxime and ≥4 μg of gentamicin per ml.

Our prior observation that 10 of 21 P. aeruginosa strains were synergistically inhibited by ceftizoxime-gentamicin combinations (7) led us to evaluate a larger number of P.
TABLE 1. Interaction between ceftizoxime and gentamicin against 62 P. aeruginosa strains

<table>
<thead>
<tr>
<th>Antibiotic combination concn (µg/ml)</th>
<th>No. of strains with FIC index of:</th>
<th>0.5</th>
<th>0.5 and ≤1</th>
<th>&gt;1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ceftizoxime</td>
<td>Gentamicin</td>
<td>≤0.5</td>
<td>&gt;0.5</td>
<td>&gt;1</td>
</tr>
<tr>
<td>8</td>
<td>4</td>
<td>10</td>
<td>4</td>
<td>8</td>
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<tr>
<td>16</td>
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<td>15</td>
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<tr>
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<td>17</td>
<td>17</td>
</tr>
<tr>
<td>&gt;32</td>
<td>&gt;8</td>
<td>4</td>
<td>3</td>
<td>6</td>
</tr>
</tbody>
</table>

P. aeruginosa strains for inhibition by clinically relevant concentrations of ceftizoxime, gentamicin, and ceftizoxime-gentamicin combinations. In the present study, only 22 strains tested were inhibited by 32 µg of ceftizoxime per ml and 43 strains were inhibited by 8 µg of gentamicin per ml, MICs at which strains are considered moderately susceptible (9). Furthermore, of the 22 strains inhibited by 32 µg of ceftizoxime per ml, 16 (73%) were also inhibited by 8 µg of gentamicin per ml.

Indifferent and indeterminate effects of ceftizoxime-gentamicin combinations occurred in 58 strains. Susceptibility or resistance patterns to either antibiotic did not predict a particular response to the drugs in combination.

A checkerboard broth dilution method which has excellent correlation with other methods of determining antibiotic synergy (10) demonstrated that ceftizoxime in combination with gentamicin was synergistic against 19 P. aeruginosa strains. In 15 of these strains, synergy was achieved at or below the range of susceptibility to ceftizoxime and gentamicin suggested by the National Committee for Clinical Laboratory Standards (9). Although the FIC index may not predict bactericidal activity (4), its use allowed us to compare our present and previous observations. The 15% frequency of synergy to concentrations of ceftizoxime and gentamicin readily achievable in serum was statistically less than that noted in our original study. The MICs required for 50% of the P. aeruginosa strains in our original study were 8 and 64 µg of gentamicin and ceftizoxime per ml, respectively. In the present study, 8 µg of gentamicin per ml inhibited 43% of the strains, but 64 µg of ceftizoxime per ml inhibited only 35% of the strains. Thus, the decreased frequency of synergy in the present study is not explained by increases in the gentamicin MIC but may be partially explained by increases in the ceftizoxime MIC during the year between studies. If increased resistance to ceftizoxime did occur during this period, it did so in the absence of continued ceftizoxime use. Although cross-resistance from use of other expanded-spectrum cephalosporins is possible, these drugs are rarely used in our hospitals. The present results reflect screening of a larger P. aeruginosa population.

Antagonism between ceftizoxime and gentamicin did not occur in the 21 P. aeruginosa strains studied in our original report but was seen in 23 strains in the present study. In the presence of gentamicin, 13 strains demonstrated antagonism at concentrations of ceftizoxime easily achieved in serum, but synergy or indifference occurred when concentrations of ceftizoxime exceeded 32 µg/ml. The reason for the shift from antagonism at low concentrations to synergy or indifference at higher concentrations of ceftizoxime in these 13 strains is unknown. In 10 strains, antagonism between ceftizoxime and gentamicin occurred only when concentrations of ceftizoxime equaled or exceeded the upper limit of the range of susceptibility suggested by the National Committee for Clinical Laboratory Standards (9). However, nine of these latter 10 strains demonstrated antagonism at concentrations of ceftizoxime which can be achieved during therapy (6, 8). In the present study, the frequency of synergy and antagonism were statistically similar.

By presently accepted criteria (9), inhibition of bacterial growth by ≤8 µg of ceftizoxime and ≤4 µg of gentamicin per ml indicate susceptibility. At these concentrations, only 24% of the strains were inhibited by ceftizoxime in combination with gentamicin, whereas at moderately susceptible concentrations, i.e., ≤32 µg of ceftizoxime and ≤8 µg of gentamicin per ml (9), 51% of the strains were inhibited. Although ceftizoxime in combination with gentamicin inhibited more P. aeruginosa strains than did either drug alone, gentamicin accounted for most of the anti-P. aeruginosa activity of ceftizoxime-gentamicin combinations.

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LITERATURE CITED


