Synergy Between Cephalosporin and Aminoglycoside Antibiotics Against *Providencia* and *Proteus*

PHINEAS J. HYAMS, MICHAEL S. SIMBERKOFF, AND JAMES J. RAHAL, JR.

*Infectious Disease Division, New York Veterans Administration Hospital and Department of Medicine, New York University School of Medicine, New York, New York 10010*

Received for publication 14 January 1974

Clinical isolates of *Providencia* and *Proteus* with relative aminoglycoside resistance were tested for susceptibility to combinations of gentamicin or tobramycin with cephalothin or cefazolin. The minimal bactericidal concentration of aminoglycoside for one-third of the strains was reduced by fourfold or more in the presence of one-fourth of the minimal bactericidal concentration of either cephalosporin. This effect was achieved by clinically attainable concentrations of cephalothin or cefazolin.

Although *Providencia* has been an uncommon cause of clinical infections, recent epidemics have been described in hospitalized patients, particularly in burn units (5, 14, 15). We have noted a gradually increasing incidence of clinical isolates of *Providencia* in our hospital, although serious infections have remained rare. In vitro studies on these strains have indicated relative resistance to all aminoglycoside antibiotics. Most strains have required minimal inhibitory and bactericidal concentrations of 12.5 to 50 μg of gentamicin per ml. A synergistic effect of penicillins or cephalosporins with aminoglycosides has previously been demonstrated against *Enterobacteriaceae* (3, 4, 10) and *Pseudomonas* (6, 12, 17). We have therefore studied nine strains of *Providencia* and seven strains of the related genus, *Proteus*, which were moderately or highly resistant to gentamicin. Our purpose was to evaluate two cephalosporins, cephalothin and cefazolin, in crossover combination with two aminoglycosides, gentamicin and tobramycin, for possible in vitro synergy against these organisms.

**MATERIALS AND METHODS**

*Providencia* and *Proteus* strains were collected from the clinical microbiology laboratory on the basis of gentamicin resistance by routine disk sensitivity testing. Tube dilution assays were done in Mueller-Hinton broth as previously described (9). Grid studies were performed so that double dilutions of cephalothin and cefazolin were combined with double dilutions of gentamicin or tobramycin. This resulted in duplicate minimal inhibitory and bactericidal concentration values for each drug against each strain. Of the 144 such duplications, four differed by two tube dilutions and the remainder differed by one dilution or less. Synergy was defined as a fourfold decline in minimal inhibitory concentrations (MIC) or minimal bactericidal concentration (MBC) of gentamicin or tobramycin after the addition of one-fourth of the MIC or MBC of a cephalosporin antibiotic to each tube.

**RESULTS**

The MIC and MBC values of each drug against the nine strains of *Providencia* and seven strains of *Proteus* tested are shown in Fig. 1 to 4. Eight of nine *Providencia* strains were resistant to clinically attainable concentrations of either cephalothin or cefazolin. One was inhibited by 62 μg/ml but required >500 μg/ml for a bactericidal effect. Three strains were inhibited by 3.1 μg of tobramycin per ml and one by that concentration of gentamicin. However, the MIC and MBC of either drug against most *Providencia* was 12.5 to 50 μg/ml.

All seven *Proteus* strains studied were indole positive and highly resistant to cephalosporins. Unlike *Providencia*, however, a clear difference in sensitivity to gentamicin and tobramycin existed in four of the seven isolates. Two strains of *Proteus morganii* and two of *Proteus vulgaris* were sensitive to tobramycin and relatively resistant to gentamicin. Two strains of *Proteus rettgeri* were resistant to both aminoglycosides, and another *Proteus morganii* isolate was highly resistant to all antibiotics.

Figure 1 indicates the effect of cephalothin and cefazolin on the MICs of tobramycin and gentamicin against *Providencia*. Ninety-one percent of *Providencia* strains were susceptible to a synergistic effect, demonstrated by a fourfold decline in the MIC of tobramycin or gen-
tamicin. This usually required less than 62 \(\mu\)g of cephalosporin per ml, a clinically attainable level of either cefalothin or cefazolin.

Figure 2 shows the effect of each combination of antibiotics on the MBC of Providencia strains. A synergistic effect occurred in 89% of the tests, but in several instances 125 to 250 \(\mu\)g of a cephalosporin per ml was required.

The results of studies carried out with Proteus species are presented in Fig. 3 and 4. Tests for synergy involving tobramycin were not done with four of the seven strains since they were highly sensitive to this antibiotic. Of all tests carried out, a fourfold decline in MIC occurred in 75% and usually required 15 to 62 \(\mu\)g of a cephalosporin per ml (Fig. 3). Figure 4 shows that only 65% of Proteus strains were susceptible to a synergistic effect on the MBC of aminoglycosides and approximately half of these required greater than 62 \(\mu\)g of a cephalosporin per ml to achieve this result.

Table 1 summarizes the percent of cephalosporin-aminoglycoside combinations which produced synergy against Providencia and Proteus. Providencia were somewhat more susceptible than Proteus to the combinations tested. The difference in effect on MBC (but not MIC) was significant with a \(P\) value of <0.05. If only those strains susceptible to synergy with the use of 62 \(\mu\)g/ml or less of either cephalosporin are considered, a significantly greater number of Providencia strains showed fourfold lower MICs. However, the synergistic effect on MBCs occurred in an equal percentage of Providencia and Proteus isolates (36 and 37%, respectively). Thus, a clinically attainable serum concentration of cephalosporin favorably effected the bactericidal activity of tobramycin and gentamicin in approximately one-third of these relatively resistant organisms. The more highly resistant organisms (those with MBCs of 25 50 \(\mu\)g/ml) were usually susceptible to a synergistic
The results of this study indicate that the MIC and MBC of either gentamicin or tobramycin for relatively resistant Providencia or Proteus isolates may be significantly reduced by the addition of cephalothin or cefazolin. Although a bacteriostatic effect is produced by this combination of antibiotics in 60 to 80% of instances, bactericidal activity occurs in only 36 to 37%. Further, when the MBC of gentamicin or tobramycin for a particular strain is 25 μg/ml or greater, a synergistic effect is unlikely to result from clinically attainable concentrations of cephalothin or cefazolin. Nevertheless, since the MIC of gentamicin for a large proportion of Providencia strains tested in our laboratory is 12.5 μg/ml or more, the additional use of a cephalosporin antibiotic may be clinically useful. Cefazolin is slightly more active against Providencia and Proteus than cephalothin, and higher blood levels are attained by usual doses. Our data showed no evident differences in synergistic effect between tobramycin and gentamicin when combined with cephalosporins. Four strains of indole-positive Proteus were...
Fig. 3. Effect of cephalothin (CF) and cefazolin (CZ) on the MIC of gentamicin (G) and tobramycin (T) against Proteus. The left bar of each pair indicates the MIC of tobramycin or gentamicin in the absence of added cephalosporin. The right bar shows the MIC of the aminoglycoside in the presence of one-fourth or less of the MIC of either cephalothin or cefazolin.
FIG. 4. Effect of cephalothin (CF) and cefazolin (CZ) on the MBC of gentamicin (G) and tobramycin (T) against Proteus. The left bar of each pair indicates the MBC of tobramycin or gentamicin in the absence of added cephalosporin. The right bar shows the MBC of the aminoglycoside in the presence of one-fourth or less of the MBC of either cephalothin or cefazolin.
quite sensitive to tobramycin despite moderate to high gentamicin resistance. It is possible that gentamicin resistance in these strains is mediated by an acetylat ing enzyme which does not utilize tobramycin (2).

The phenomenon of cephalosporin-aminoglycoside synergy against *Providencia* and *Proteus* is consistent with previous findings of synergy between inhibitors of cell wall metabolism, e.g., penicillins or cephalosporins, and aminoglycosides. Klustersky et al. have recently shown synergy between cephalothin and gentamicin or tobramycin against gentamicin-resistant *Providencia* (11). Our findings confirm their results and further define the bacteriostatic and bactericidal activity of this combination. In addition, the present data suggest that cefazolin may be more effective than cephalothin in producing synergy with aminoglycosides.

Recent reports have suggested that the combination of cephalothin and gentamicin is potentially more nephrotoxic than either drug alone (1, 7, 8, 13). Since cefazolin has shown somewhat greater nephrotoxic potential than cephalothin in experimental studies (16), renal function should be carefully monitored during the administration of any cephalosporin-aminoglycoside combination.

**Table 1. Percent of tested combinations of cephalosporin plus aminoglycoside which produce synergy against *Providencia* and *Proteus***

<table>
<thead>
<tr>
<th>Genus tested</th>
<th>Tests producing synergy</th>
<th>Tests producing synergy with 62 μg or less of cephalosporin per ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>P</td>
</tr>
<tr>
<td><em>Providencia</em></td>
<td>91 (89)</td>
<td>&gt;0.1 (&lt;0.05)</td>
</tr>
<tr>
<td><em>Proteus</em></td>
<td>75 (63)</td>
<td></td>
</tr>
</tbody>
</table>

*Refers to the percent of tested combinations producing synergy as determined by changes in MIC. Numbers in parenthesis indicate percent of synergy as determined by changes in MBC. P values were determined by the chi-square test.

**Table 2. Percent of tested combinations of cephalosporin plus aminoglycoside which produce synergy against *Providencia* and *Proteus***

<table>
<thead>
<tr>
<th>Antibiotic included in combination</th>
<th>Tests producing synergy</th>
<th>Tests producing synergy with 62 μg/ml or less of cephalosporin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>P</td>
</tr>
<tr>
<td>Cephalothin</td>
<td>75 (73)</td>
<td>&gt;0.05 (&gt;0.1)</td>
</tr>
<tr>
<td>Cefazolin</td>
<td>93 (80)</td>
<td></td>
</tr>
<tr>
<td>Tobramycin</td>
<td>83 (86)</td>
<td>&gt;0.1 (&gt;0.1)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>84 (69)</td>
<td></td>
</tr>
</tbody>
</table>

*Refers to the percent of tested combinations producing synergy as determined by changes in MIC. Numbers in parentheses indicate percent producing synergy as determined by changes in MBC. P values were determined by the chi-square test.

**ACKNOWLEDGMENTS**

This study was supported by a grant from Eli Lilly and Co. We thank Martha Kuepper for valuable technical assistance.

**LITERATURE CITED**

8. Hansten, P. D. 1973. Cephalothin, gentamicin, colistin...