Synergism Between N-Formimidoyl Thienamycin and Gentamicin or Tobramycin Against Enterococci

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By the time-kill curve method, the combination of N-formimidoyl thienamycin and gentamicin showed synergism against 47 of 48 strains of enterococci, whereas the combination of N-formimidoyl thienamycin and tobramycin was synergistic against 46 strains.

N-Formimidoyl thienamycin (MK0787) is a stable derivative of thienamycin, a novel β-lactam not containing a sulfur atom in the secondary ring, derived from Streptomyces catleya. N-Formimidoyl thienamycin has been shown to have a wide antimicrobial spectrum, including gram-positive cocci and gram-negative bacilli (2, 3, 5). In contrast to the third-generation cephalosporins and other new β-lactams, N-formimidoyl thienamycin has been reported to be active against enterococci (2, 3). In this investigation, we studied the in vitro activity of N-formimidoyl thienamycin against enterococci and the effects of combining N-formimidoyl thienamycin with gentamicin or with tobramycin against enterococci by the time-kill method.

Forty-eight strains of enterococci were used in this study. All strains grew in 6.5% NaCl brain heart infusion broth and grew as colonies surrounded by black zones on bile-esculin agar. Identification of the enterococci to species level was performed by the API 20S Streptococcus System (Analytab Products, Plainview, N.Y.). N-Formimidoyl thienamycin was obtained from the Merck Institute for Therapeutic Research, Rahway, N.J.; gentamicin was obtained from Schering Corp., Bloomfield, N.J.; and tobramycin was obtained from Eli Lilly Laboratories, Indianapolis, Ind. A standard stock solution of each antibiotic was prepared according to the instructions of the manufacturer, stored at −80°C, and thawed immediately before use.

The minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of each antibiotic were determined by the World Health Organization-International Collaborative Study broth dilution method (1). Serial twofold dilutions of the antibiotic were made in Mueller-Hinton broth from 64 to 0.06 μg/ml. The inoculum was 1 ml of 10^5 to 10^6 organisms diluted from an 18-h culture. The MIC was defined as the lowest concentration of an antibiotic that allowed no visible growth after incubation at 37°C for 18 to 24 h. The MBC was defined as the lowest concentration of an antibiotic that allowed no growth (or one colony) from a 0.01-ml subculture from each clear tube on agar plates after incubation at 37°C for 18 to 24 h.

The standard time-kill curve method was used to study the interaction between N-formimidoyl thienamycin and gentamicin or tobramycin. Mueller-Hinton broth was used. The antibiotic concentrations (in micrograms per milliliter) were as follows: N-formimidoyl thienamycin, 20; gentamicin, 4; tobramycin, 4; N-formimidoyl thienamycin, 20, combined with gentamicin, 4; and N-formimidoyl thienamycin, 20, combined with tobramycin, 4. A broth culture with no antibiotic was set up as a control. The inoculum contained between 10^5 and 10^6 organisms per ml and was made from an 18- to 24-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 numbers of organisms were enumerated by serial 10-fold dilutions plated on Mueller-Hinton agar.

When the result of the combination was at least log_{10} less than that from both drugs alone at a given time, it was defined as synergy. When the result of the combination was at least log_{10} more than that from either drug alone, it was defined as antagonism.

Forty-seven strains of Streptococcus faecalis and one strain of Streptococcus faecium were used. For the 48 strains of enterococci, the MIC of N-formimidoyl thienamycin was 1 to 2 μg/ml for 44 strains and 8 and 16 μg/ml for 2 strains each. The MIC of N-formimidoyl thienamycin was >64 μg/ml for all strains. The MIC of gentamicin ranged from 4 to 16 μg/ml (median, 16 μg/ml), and the MBC ranged from 8 to 64 μg/ml (median, 32 μg/ml). The MIC of tobramycin ranged from 4 to >64 μg/ml (median, 16 μg/ml),
and the MBC ranged from 16 to >64 μg/ml (median, 32 μg/ml).

The combination of N-formimidoyl thienamycin and gentamicin demonstrated synergism at 6, 24, and 48 h (Fig. 1) against 47 of 48 strains of enterococci, and the combination of N-formimidoyl thienamycin and tobramycin was synergistic against 45 of 48 strains (Table 1). N-Formimidoyl thienamycin–gentamicin and N-formimidoyl thienamycin–tobramycin showed no synergism against a strain of Streptococcus faecalis. N-Formimidoyl thienamycin–tobramycin showed synergism only at 48 h against another strain of Streptococcus faecalis. N-Formimidoyl thienamycin–tobramycin showed no synergism against the only strain of Streptococcus faecium, whereas N-formimidoyl thienamycin–gentamicin did show synergism against this strain.

The results of this study confirm the in vitro activity of N-formimidoyl thienamycin against enterococci (2, 3). As with penicillin and vancomycin, N-formimidoyl thienamycin is not bactericidal against enterococci. Also similar to penicillin and vancomycin (6, 7), synergism of N-formimidoyl thienamycin with gentamicin and N-formimidoyl thienamycin with tobramycin was demonstrated against almost all strains tested. Both combinations failed to show synergism against one strain of Streptococcus faecalis. The combination of N-formimidoyl thienamycin and tobramycin showed no synergism against the only strain of Streptococcus faecium. It has been reported that the combination of penicillin and tobramycin is also not synergistic against Streptococcus faecium (4).

The combination of N-formimidoyl thienamycin and gentamicin or N-formimidoyl thienamycin and tobramycin shows promise as a useful therapeutic regimen in enterococcal endocarditis when penicillin cannot be used. Therapeutic trials in animal models are warranted.

LITERATURE CITED

### TABLE 1. Synergism between N-formimidoyl thienamycin and gentamicin or tobramycin against enterococci

<table>
<thead>
<tr>
<th>Antibiotic combination</th>
<th>No. of strains showing decrease in no. of colonies per ml × log₁₀ at (h):</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>1−2</td>
</tr>
<tr>
<td>N-F thienamycin–gentamicin</td>
<td>41</td>
</tr>
<tr>
<td>N-F thienamycin–tobramycin</td>
<td>2</td>
</tr>
</tbody>
</table>

a As compared with N-formimidoyl thienamycin alone.

FIG. 1. Time-kill curves showing marked synergism of N-formimidoyl thienamycin combined with gentamicin and with tobramycin against a strain of enterococci. The concentrations used (in micrograms per milliliter) were: tobramycin, 4; gentamicin, 4; N-formimidoyl thienamycin (MK0787), 20.