ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Dec. 1982, p. 1082–1083
0066-4804/82/121082-02$02.00/0

Vol. 22, No. 6

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

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Received 14 June 1982/Accepted 9 September 1982

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 cattleya*. *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 curve 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 synergism. 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 MBC 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),

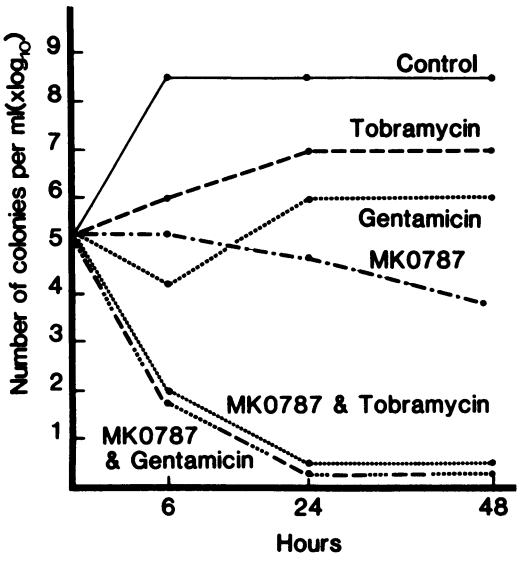

FIG. 1. Time-kill curves showing marked synergism of *N*-formimidoyl thienamycin combined with gentamicin and with tobramycin against a strain of enterococci. The concentrations used (in micrograms per milliliter) were: tobramycin, 4; gentamicin, 4; *N*-formimidoyl thienamycin (MK0787), 20.

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.

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

Antibiotic combination	No. of strains showing decrease in no. of colonies per ml × $\log_{10}$ at (h)[a]:								
	6			24			48		
	1–2	2–4	>4	1–2	2–4	>4	1–2	2–4	>4
N-F thienamycin–gentamicin		41	6	2	30	15	3	28	16
N-F thienamycin–tobramycin	2	26	17		23	22		24	22

[a] As compared with *N*-formimidoyl thienamycin alone.

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.

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