ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Feb. 1983, p. 245-247
0066-4804/83/020245-03$02.00/0

Vol. 23, No. 2

Synergistic Effect of *N*-Formimidoyl Thienamycin with Gentamicin and Amikacin Against *Streptococcus faecalis*

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Received 19 July 1982/Accepted 1 December 1982

The in vitro activities of *N*-formimidoyl thienamycin alone and in combination with amikacin and gentamicin were tested against 10 strains of *Streptococcus faecalis*. Synergy was demonstrated in 35% of the combinations tested by the microtiter checkerboard technique; 50% were found to be synergistic with time killing curves.

N-Formimidoyl thienamycin (*N*-f-thienamycin), a stabilized amidine derivative of thienamycin, is a new carbapenem antibiotic that has demonstrated a high order of activity against a wide variety of bacteria (5, 9, 12). Enterococci are uniformly resistant to cephalosporins but have been shown to be highly susceptible to *N*-f-thienamycin, with most strains being inhibited by ≤1 μg/ml. Bactericidal levels of this agent are significantly higher (1, 2).

In this study, we determined the minimal inhibitory concentrations (MICs) and minimal bactericidal concentrations (MBCs) of *N*-f-thienamycin, gentamicin, and amikacin for 10 strains of *Streptococcus faecalis*. In addition, synergy studies were performed with the *N*-f-thienamycin–aminocyclitol combinations.

MATERIALS AND METHODS

Laboratory powders were kindly supplied by the following: *N*-f-thienamycin, Merck & Co., Inc., Rahway, N.J.; amikacin, Bristol Laboratories, Syracuse, N.Y.; and gentamicin, Schering Corp., Kenilworth, N.J. The aminocyclitols were diluted in sterile water as recommended by the manufacturer, and stock solutions were stored at −80°C until used. *N*-f-Thienamycin was diluted at the time of testing. The bacteria used were clinical isolates obtained from patients at the Kings County Hospital and Downstate Medical Centers in Brooklyn, N.Y. Identity was confirmed by standard microbiological methods (3).

MICs were determined by the broth dilution method (13). A 0.01-ml sample from each tube without visible growth was streaked onto a blood agar plate. The MBC was the lowest concentration of drug which produced a 99.9% reduction of the initial inoculum after 24 h of incubation. Synergy was evaluated for the combinations of *N*-f-thienamycin–gentamicin and *N*-f-thienamycin–amikacin by a microtiter checkerboard technique and by timekilling curves. Checkerboard titrations were performed by the serial twofold microdilution method (4), with each well of the microtiter plate containing 0.05 ml of a different concentration of each drug. Volumes of 0.05 ml of log-phase cultures diluted in tryptic soy broth to contain 10^5 to 10^6 CFU/ml were added to the wells. Plates were incubated overnight at 37°C and then read for visible growth.

Each isolate was grown overnight at 37°C in Mueller-Hinton broth (MHB) for the killing curves. Serial 10- and 100-fold dilutions in MHB were prepared to give suspensions of 10^5 CFU/ml as an initial inoculum. The concentration of *N*-f-thienamycin used represented the MIC for each isolate. The concentration of aminocyclitol used represented one-fourth of the MIC for each isolate. Samples (0.5 ml) were taken from each tube at 2, 6, and 24 h of incubation to perform colony counts.

Synergy, by the microtiter checkerboard method, was present when the combination of antibiotics resulted in at least a fourfold reduction in the MIC of both agents. A less than fourfold reduction in the MIC for both antibiotics was considered additive. Indifference was found when neither drug exhibited a decrease in the MIC, and an increase in the MIC for either drug was considered antagonism. Using timekilling curves, we defined synergy as a ≥2-log reduction in the colony counts at 24 h, using the combination of *N*-f-thienamycin and an aminocyclitol as compared with *N*-f-thienamycin alone. A decrease in colony counts between 1 and 2 logs was considered additive. A less than 1-log decrease in colony counts was considered indifferent, and an increase in counts with the combination was antagonistic.

RESULTS

The susceptibilities of 10 isolates of *S. faecalis* to *N*-f-thienamycin, gentamicin, and amikacin are shown in Table 1. The MIC_{90} (the MIC of 90% of isolates) of *N*-f-thienamycin was 2 μg/ml, which is similar to previously reported data (7, 11). The bactericidal levels of this agent, however, were significantly higher. Gentamicin and amikacin were inactive when used alone against these isolates.

Testing for synergy by the microtiter checkerboard technique is based on inhibitory rather

than bactericidal endpoints. The combinations of *N*-f-thienamycin and gentamicin or *N*-f-thienamycin and amikacin were synergistic in 35% of the tests performed. An additive effect was seen in the remaining 65%. No antagonism was found. Synergy was more common in the combination where amikacin was used.

Killing curves showed the combination of agents to be synergistic in 50% of the isolates tested. An additive effect was seen in 30%. Indifference was seen in 20%, and no antagonism was found. There was no difference between the combination groups with amikacin and those with gentamicin. Sixty percent of the synergistic combinations were totally bactericidal at 24 h. Moreover, a more rapid bactericidal effect was observed at all times tested when the *N*-f-thienamycin–aminocyclitol combination was used than when either agent was used alone (Fig. 1).

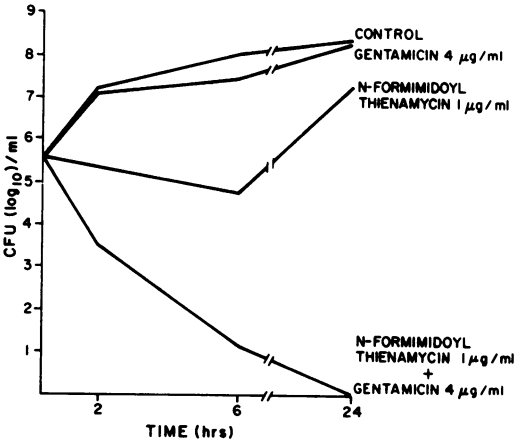

FIG. 1. Effect of *N*-f-thienamycin alone and in combination with aminocyclitol against *S. faecalis* (mean colony counts of synergistic strains).

DISCUSSION

The relative resistance of enterococci to antibiotics is well known (8, 10). Krogstad and Parquette have recently demonstrated that enterococci are tolerant to both β-lactams and non-β-lactams that act on the bacterial cell wall and suggest a defective autolytic enzyme system as a potential mechanism (6). Aukenthaler et al. (1) have recently shown that the MBC/MIC ratio of *N*-f-thienamycin when tested against 21 isolates of enterococci was ≥32 when the MBC was defined as 99.9% killing by the microdilution method. This is in accordance with our data. Moreover, Aukenthaler et al. confirmed their in vitro studies by showing that *N*-f-thienamycin as a single-agent therapy in experimental enterococcal rabbit endocarditis is no better than penicillin G alone and is significantly less effective than penicillin plus gentamicin.

The present studies showed synergy in the *N*-f-thienamycin-aminocyclitol combination against several strains of *S. faecalis*, which is in agreement with the results of Eliopoulos and Moellering (2). The significance of the more rapid and sustained bactericidal action using killing curves by the combination of antibiotics tested could not be assessed in this study. Whether this combination will prove superior to present therapeutic regimens in experimental enterococcal infections warrants further evaluation.

TABLE 1. Susceptibility of 10 isolates of *S. faecalis* to *N*-f-thienamycin, gentamicin, and amikacin

Antibiotic	MIC (μg/ml)		MBC (μg/ml)	
	Mean	Range	Mean	Range
N-f-Thienamycin	1.6	1–4	59.6	4–128
Gentamicin	15.2	8–32	153.6	64–256
Amikacin	179.2	128–256	691.2	256–1,024

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