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Susceptibility of Enterococci and *Listeria monocytogenes* to *N*-Formimidoyl Thienamycin Alone and in Combination with an Aminoglycoside

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The susceptibilities of 12 strains of enterococci and 10 strains of *Listeria monocytogenes* to *N*-formimidoyl thienamycin were determined by a standard broth dilution method. Minimal inhibitory concentrations for *L. monocytogenes* strains were less than 0.25 μg/ml. As a group, strains of *Streptococcus faecium* were less susceptible than *Streptococcus faecalis* strains, but even for the latter, the minimal inhibitory concentrations were slightly greater than those previously reported. Minimal bactericidal concentrations against all organisms were many-fold higher than the corresponding inhibitory concentrations. In time-kill studies, combinations of *N*-formimidoyl thienamycin with gentamicin were synergistic against (or they completely sterilized cultures of) all of the enterococcal strains and nine of 10 strains of *L. monocytogenes*. The magnitude of killing by the combinations was comparable to that previously observed with penicillin-gentamicin combinations. *N*-Formimidoyl thienamycin-tobramycin combinations were synergistic against those strains of *S. faecalis* and *L. monocytogenes* tested, but not against those of *S. faecium*.

Initial studies have shown that the new β-lactam antibiotic thienamycin and its more stable *N*-formimidoyl derivative are active against a wide variety of bacteria, including enterococci. When tested by microtiter, agar dilution, and disk diffusion techniques, the majority of enterococcal isolates are inhibited by *N*-formimidoyl thienamycin at concentrations of less than 2 μg/ml (5, 8). Published data on the minimal bactericidal concentration of *N*-formimidoyl thienamycin for enterococci are not presently available.

Currently employed antibiotics are only bacteriostatic against enterococci at concentrations readily achievable in the serum, and successful therapy of enterococcal endocarditis usually requires the addition of an aminoglycoside to penicillin, ampicillin, or vancomycin for synergistic killing (14). There are also significant differences between the inhibitory and bactericidal concentrations of penicillin or ampicillin for *Listeria monocytogenes* (11, 19). Here too, enhanced killing by the addition of an aminoglycoside to cell wall-active agents has been demonstrated both in vitro (11, 19) and in animal models (2, 12, 16), but the clinical benefits of such combinations are less clear (13).

In the present study, we examined the susceptibilities to *N*-formimidoyl thienamycin of *Streptococcus faecalis*, *Streptococcus faecium*, and *L. monocytogenes* by a broth dilution method and evaluated combinations of this antibiotic with an aminoglycoside for synergistic activity against these organisms.

MATERIALS AND METHODS

The organisms used were clinical isolates from the Massachusetts General Hospital, except for *L. monocytogenes* strains 6 through 10 which had been obtained from the Centers for Disease Control, Atlanta, Ga. The isolates had been identified as previously reported (3, 11) and stored at −70°C. After thawing, they were maintained on brucella agar plates with 5% horse blood (GIBCO Diagnostics, Madison, Wis.).

N-Formimidoyl thienamycin (MK0787) was provided by the Merck Institute for Therapeutic Research, Rahway, N.J. The concentration of the drug in a prepared solution was confirmed in our laboratory by a differential spectrophotometric assay, as described previously for the parent compound (4). The in vitro activity of the drug was confirmed by susceptibility testing of *Bacillus subtilis* ATCC 6633, the minimal inhibitory concentration (MIC) for which has been reported (5). Solutions were prepared fresh daily in 10 mM phosphate buffer (pH 7.2) at concentrations of 4 mg/ml or less. Solutions of gentamicin sulfate (Schering Corp., Kenilworth, N.J.) and tobramycin sulfate (Eli Lilly & Co., Indianapolis, Ind.) were diluted in sterile demineralized water.

Bacterial susceptibilities were determined by a stan-

dard broth dilution method (17). Appropriate dilutions of an overnight culture of the test organisms in dextrose phosphate broth were added to serial twofold dilutions of antibiotic in broth to yield an inoculum of between 10^5 and 10^6/ml. After 24 h of incubation at 37°C, the lowest antibiotic concentration resulting in the absence of visible turbidity was noted as the MIC, and 1 loopful of culture material from each tube (approximately 0.005 ml) was streaked onto a blood agar plate. The minimal bactericidal concentration (MBC) was defined as the lowest concentration of antibiotic which resulted in a 99.9% reduction in viable cells at 24 h. Because of the small sampling volume, this method may over- or underestimate the MBC in certain individual cases (1). Since MBCs for streptococci may be influenced by the broth medium (6), antibiotic susceptibilities were also determined in Mueller-Hinton and Todd-Hewitt broths (BBL Microbiology Systems, Cockeysville, Md.) for some organisms.

Studies of antimicrobial combinations were carried out in 250-ml flasks to which dextrose phosphate broth, antibiotics, and appropriate dilutions of overnight cultures were added to yield a final volume of 20 ml. The loosely plugged flasks were incubated at 37°C without agitation, and at 0, 4, and 24 h, 0.5-ml samples were withdrawn for colony counts. The aminoglycoside concentrations that we used did not inhibit the growth of the organisms. Synergism was defined as a 100-fold or greater enhancement of killing at 24 h by the antibiotic combination, as compared with that by *N*-formimidoyl thienamycin alone.

RESULTS

Results of *N*-formimidoyl thienamycin susceptibility studies performed in dextrose phosphate broth are shown in Table 1. Studies comparing the activities of the drug against enterococci in various media revealed no significant differences in MICs determined in dextrose phosphate, Mueller-Hinton or Todd-Hewitt broths (all but one MIC were within one dilution). The mean MBC against *S. faecium* was strikingly less in Mueller-Hinton broth (79 μg/ml) than in the other media.

With an inoculum of 10^7 colony-forming units per ml, five of six strains of *S. faecalis* were synergistically killed by the combination of *N*-formimidoyl thienamycin at 10 μg/ml and gentamicin at 5 μg/ml. For the sixth strain, synergism was observed when the inoculum was decreased to 10^5 colony-forming units per ml (Table 2). Combinations of gentamicin with *N*-formimidoyl thienamycin at 4 μg/ml resulted in a 2-$\log_{10}$ enhancement of killing at 4 h in five strains, but at this concentration the β-lactam alone achieved a 10-fold greater kill than at the higher concentration (Table 3), diminishing the magnitude of the difference between killing by the single agent and the combination observed at 24 h. The results of a representative experi-

TABLE 1. *Susceptibility of enterococci and L. monocytogenes to N-formimidoyl thienamycin in dextrose phosphate broth*

Organism (no. of strains tested)	MIC (μg/ml)		MBC (μg/ml)	
	Mean[a]	Range	Mean	Range
S. faecalis (6)	2	1–4	112	16–>250
S. faecium (6)	14	4–32	315	250–>250
L. monocytogenes (10)	0.04	≤0.015–0.125	5	0.25–250

[a] To calculate geometric means, values ≤0.015 or >250 were taken to equal 0.015 or 500, respectively.

TABLE 2. *Effect of N-formimidoyl thienamycin (F-Thien)-gentamicin (GM) combinations on enterococci*

Strain tested	Inoculum (CFU/ml)[a]	Concn (μg/ml)		Magnitude of increased killing ($\log_{10}$)[b]
		F-Thien	GM	
S. faecalis				
1	10^7	10	5	5.0
2	10^7	10	5	1.0
	10^5	10	5	3.0
3	10^7	10	5	3.0
4	10^7	10	5	3.0
5	10^7	10	5	2.5
6	10^7	10	5	3.5
S. faecium				
7	10^5	50	5	2.0
8	10^5	50	5	3.0
9	10^5	50	5	3.5
10	10^5	10	5	3.5
11	10^5	10	5	3.0
12	10^5	50	5	1.5[c]

[a] CFU, Colony-forming units.

[b] Decrease in viable cell count after 24 h by the combination as compared with *N*-formimidoyl thienamycin alone; rounded to the nearest 0.5 $\log_{10}$ unit.

[c] The combination led to sterilization of the culture within 4 h.

TABLE 3. *Comparison of killing by N-formimidoyl thienamycin (F-Thien) alone and in combination with gentamicin (GM)*

Amt of drug (μg/ml)		Mean $\log_{10}$ kill at 24 h	
F-Thien	GM	*S. faecalis*	*L. monocytogenes*
≤1			1.0
≤1	0.5[a]		4.0
10			1.0
10	0.5		4.0
4		4.0	
4	5.0	5.5	
10		3.0	
10	5.0	6.0	

[a] For one strain of *L. monocytogenes*, gentamicin at 0.5 μg/ml was inhibitory, so a concentration of 0.25 μg/ml was used.

ment illustrating this phenomenon are shown in Fig. 1. When tobramycin at 5 μg/ml was employed as a second agent in one experiment, the resulting killing curves were identical to those obtained with gentamicin.

Time-kill studies with *S. faecium* utilized an inoculum of 10^5 colony-forming units per ml to allow comparison with previous penicillin-aminoglycoside synergism studies. *N*-Formimidoyl thienamycin-gentamicin synergism at 24 h was demonstrated for five strains, with a mean cell kill of 5 $\log_{10}$ units. The sixth strain was completely killed by the combination within 4 h, and at that time a 2-$\log_{10}$ difference between the single drug and the combination did exist. In three strains, it was observed that the addition of gentamicin to subinhibitory concentrations of the β-lactam resulted in a sustained reduction in the number of viable cells by 3 $\log_{10}$ units. For three strains of *S. faecium* tested, combinations with tobramycin resulted in no better killing than that seen with *N*-formimidoyl thienamycin alone.

N-Formimidoyl thienamycin-gentamicin synergism was noted in nine strains of *L. monocytogenes* with a low concentration of the former drug, and in eight strains with the drug concentration at 10 μg/ml (Table 4). For both isolates which were not killed synergistically at the higher concentrations, $\log_{10}$ kills by the combinations were as great as those found for strains in which synergism was demonstrated. Lack of synergism, therefore, was attributable to the relative efficiency of killing by *N*-formimidoyl thienamycin alone. In contrast to the situation with *S. faecalis*, mean 24-h cell kills at the two concentrations of the β-lactam were identical (Table 3). *N*-formimidoyl thienamycin-tobramycin synergism was found in the three strains tested.

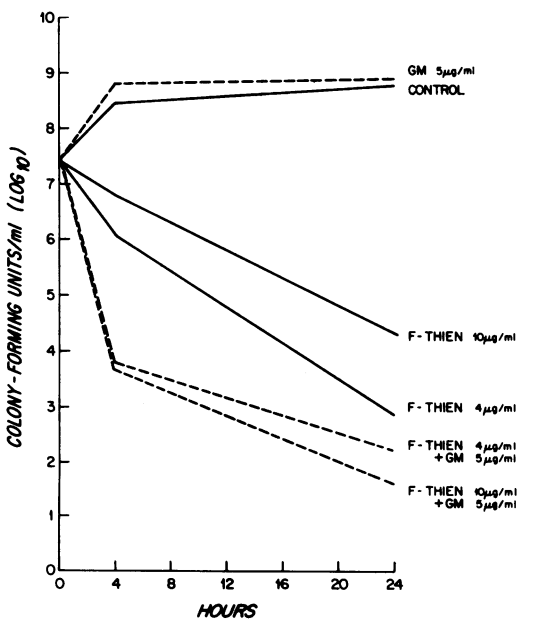

FIG. 1. *Effect of N-formimidoyl thienamycin (F-THIEN) alone and in combination with gentamicin (GM) against enterococcus strain number 4.*

TABLE 4. *Effect of N-formimidoyl thienamycin (F-Thien)-gentamicin (GM) combinations on L. monocytogenes*

Strain	Inoculum (CFU/ml)[a]	Concn (μg/ml)		Magnitude of increased killing ($\log_{10}$)
		F-Thien	GM	
1	10^7	0.25	0.5	4.0
		10	0.5	6.5
2	10^7	0.50	0.5	1.5
		10	0.5	1.5
	10^5	10	0.5	0.5
3	10^7	0.25	0.5	3.5
		10	0.5	3.5
4	10^7	0.50	0.5	5.0
		10	0.5	6.5
5	10^7	0.25	0.5	2.0
		10	0.5	2.0
6	10^7	0.25	0.5	2.0
		10	0.5	2.5
7	10^7	1.0	0.5	2.0
		10	0.5	4.0
8	10^7	0.25	0.5	4.5
		10	0.5	3.0
9	10^7	0.50	0.5	4.0
		10	0.5	3.0
10	10^7	0.50	0.25[b]	2.0
		10	0.25	0.5

[a] CFU, Colony-forming units.
[b] Gentamicin at 0.5 μg/ml was inhibitory.

DISCUSSION

Enterococci. Although only six strains of each species were examined, it was apparent that as a group, *S. faecium* strains were less susceptible to *N*-formimidoyl thienamycin than were *S. faecalis* strains. Such species differences may account in part for the wide range in previously reported MICs for enterococci (8), but are discordant with findings in a preliminary

report describing MICs of ≤1 μg/ml for both *S. faecium* and *S. faecalis* (H. C. Neu and P. Labthavikul, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 20th, New Orleans, La., abstr. no. 260, 1980).

As is the case with penicillin, MBCs of *N*-formimidoyl thienamycin were severalfold higher than its corresponding MICs. This finding is not unexpected since defective killing of enterococci by cell wall-active agents most likely reflects an inherent property of the organisms (7). Despite the fact that these strains of *S. faecalis* are tolerant to both *N*-formimidoyl thienamycin and penicillin, the former drug at concentrations of 4 μg/ml resulted in a 30-fold greater killing at 24 h than did penicillin at 6 μg/ml in time-kill studies previously done by a similar method (10). However, because of less efficient killing at increasing antibiotic concentrations, which has been termed the "paradoxic effect" (1), such comparisons may not be entirely valid.

Interestingly, the magnitude of killing by synergistic combinations of *N*-formimidoyl thienamycin and gentamicin is similar to that seen with penicillin-gentamicin combinations (10). Nevertheless, caution must be exercised in predicting clinical utility from these studies. For example, whereas synergism against enterococci can be demonstrated for nafcillin-gentamicin combinations in broth, the addition of serum, to which the former drug is highly bound, negates this effect, suggesting that such combinations might fail in the treatment of endocarditis (3). Although we have not examined the effect of serum on the activity of *N*-formimidoyl thienamycin-gentamicin combinations in this laboratory, preliminary work suggests that *N*-formimidoyl thienamycin is not highly protein bound (H. Kropp, personal communication).

Recently, the presence of an aminoglycoside 6′-acetyltransferase has been discovered in *S. faecium*, which provides an explanation for the failure of penicillin-tobramycin synergy in this species (18). On the basis of our present studies, we propose that *N*-formimidoyl thienamycin-tobramycin combinations would fail in this situation as well.

L. monocytogenes. In vitro synergism between penicillin or ampicillin and gentamicin against many strains of this organism is well recognized (9, 11, 19). The mean reduction in numbers of viable cells which we have observed with combinations of *N*-formimidoyl thienamycin and gentamicin is equal to that previously seen with penicillin-gentamicin combinations (11). Combinations of the new β-lactam with tobramycin also demonstrated synergistic killing, as might be expected on the basis of reported penicillin-tobramycin synergism (15).

While the potential benefits of synergistic therapy of *Listeria* infections are supported by data from animal models (2, 16) and seem particularly attractive since many patients with such infections have defects in host defense mechanisms, successful therapy of human infections with a single agent has been well documented (13). Whether *N*-formimidoyl thienamycin, alone or in combination with an aminoglycoside, will prove equal or superior to current regimens for the treatment of infections due to *L. monocytogenes* in humans remains to be determined, especially in light of the fact that the extent of penetration of this drug into the cerebrospinal fluid is at present unknown, and because of the wide range in the MBCs of *N*-formimidoyl thienamycin against these organisms.

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