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Synergy of Imipenem or Penicillin G and Aminoglycosides Against Enterococci Isolated from Patients with Infective Endocarditis

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We tested the synergistic activity of imipenem (formerly imipemide, *N*-formimidoyl thienamycin, or MK 0787) (20 μg/ml) or penicillin (20 μg/ml) in combination with increasing concentrations of either streptomycin (5, 10, and 20 μg/ml) against 13 strains of streptomycin-susceptible enterococci or gentamicin (1, 3, and 5 μg/ml) against 13 strains of streptomycin-susceptible enterococci and 7 strains of streptomycin-resistant enterococci. At 24 h, penicillin together with each increment in streptomycin concentration resulted in a significant increase ($P < 0.001$) in killing of streptomycin-susceptible enterococci compared with imipenem and the corresponding concentration of streptomycin. Similarly, at 24 h, the magnitude of killing of streptomycin-susceptible enterococci by a combination of penicillin plus each increment of gentamicin concentration was significantly greater ($P < 0.001$) than that of the combination of imipenem and the corresponding concentration of gentamicin. Against streptomycin-resistant enterococci, penicillin together with each increment of gentamicin concentration killed significantly more enterococci ($P < 0.02$) than did the combination of imipenem and the corresponding concentration of gentamicin. When combined with an aminoglycoside, the synergistic activity in vitro against enterococci of imipenem was significantly less than that of penicillin.

Imipenem (formerly imipemide, *N*-formimidoyl thienamycin, or MK 0787) is reported to be inhibitory (3, 4, 7, 10) or bactericidal (5) against enterococci. When the macrodilution method for determining the MBC was used with a bactericidal endpoint defined as ≥99.9% kill of the inoculum, Auckenthaler et al. (1) showed that imipenem was not bactericidal in vitro against enterococci. Aukenthaler and associates (1) also showed that imipenem was similar in activity to penicillin G and significantly less effective than a combination of penicillin and gentamicin in the treatment of rabbit experimental enterococcal endocarditis. The purpose of our study was to determine whether imipenem acts synergistically in vitro with streptomycin or gentamicin against enterococci and to compare this activity with penicillin combined with either streptomycin or gentamicin.

MATERIALS AND METHODS

Bacteria. Twenty strains of enterococci isolated from patients with infective endocarditis were tested. All strains were grouped serologically, identified to species, and frozen in defibrinated sheep blood at −70°C. Seventeen strains were *Streptococcus faecalis*, and three were *S. faecium*. Before testing, each strain was thawed and subcultured on tryptic soy agar (BBL Microbiology Systems) containing 5% sheep blood. Thirteen strains (10 *S. faecalis*, 3 *S. faecium*) were susceptible to ≤2,000 μg of streptomycin per ml and the remaining seven strains were highly streptomycin resistant, with a streptomycin MIC of >2,000 μg/ml.

Antibiotics. Stock solutions of penicillin G (Eli Lilly & Co.), streptomycin sulfate (Eli Lilly & Co.), and gentamicin (Schering Corp.) were prepared and stored at −20°C. Solutions of imipenem (Merck Sharp & Dohme Research Laboratories) were prepared daily and adjusted to pH 7.2 with 1.0 mM sodium phosphate. Dilutions used for synergy tests were prepared each day, and tests were performed in Mueller-Hinton broth (Difco Laboratories).

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Determination of MIC and MBC. Inocula were prepared from 6-h broth cultures to yield 10^5 to 10^6 CFU/ml in serial twofold dilutions of antibiotic in broth. After incubation for 18 to 24 h at 37°C, the tubes were inspected for signs of visible growth. The lowest concentration of drug which prevented the appearance of visible turbidity was defined as the MIC. From each clear tube and from the lowest concentration showing turbidity, 0.05 ml was subcultured to and mixed in 10 ml of molten tryptic soy agar. MBC was defined as the lowest concentration of antibiotic which killed ≥99.9% of the original inoculum.

Test of synergy. A 0.25-ml sample from an overnight broth culture of the test strain was inoculated into 4.75 ml of Mueller-Hinton broth which contained antibiotics either singly or in combination. After 0, 4, and 24 h of incubation, 0.2-ml samples were removed and diluted 10-fold in broth. A 0.1-ml sample from each dilution was spread over the surface of a tryptic soy agar plate, pH 7.35. Duplicates of each dilution were made. Colony counts (mean of the duplicates) were performed at 48 h, and the results were expressed as $\log_{10}$ CFU per milliliter. Synergy was defined as an increase of at least 100-fold in killing at 24 h by a combination of imipenem or penicillin G together with an aminoglycoside compared with the killing by imipenem or penicillin G alone (8).

Statistical analysis. Duncan's multiple range test was used

TABLE 1. In vitro susceptibility of 20 strains of enterococci[a]

Antibiotic	MIC (μg/ml)			MBC (μg/ml)
	50%	90%	Range	
Penicillin G	2	2	1–4	>128
Imipenem	1	2	0.5–4	>128
Streptomycin	64	—[b]	—	—
Gentamicin	4	8	4–16	>32

[a] MIC or MBC for cumulative percentage of strains inhibited or killed.
[b] —, Seven strains were highly streptomycin resistant (MIC, >2,000 μg of streptomycin per ml).

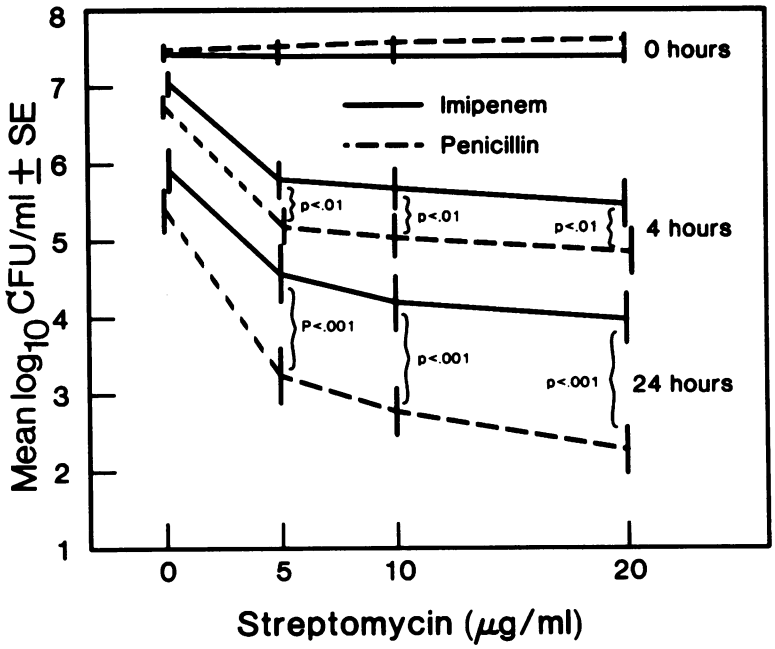

FIG. 1. Imipenem (20 μg/ml) or penicillin (20 μg/ml) and streptomycin synergy against 13 strains of streptomycin-susceptible enterococci. SE, Standard error.

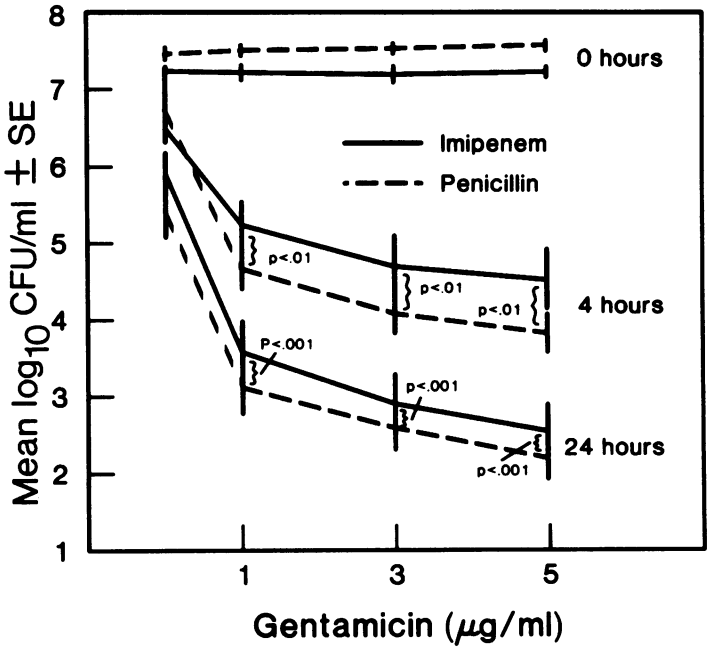

FIG. 2. Imipenem (20 μg/ml) or penicillin (20 μg/ml) and gentamicin synergy against 13 strains of streptomycin-susceptible enterococci. SE, Standard error.

to determine whether increasing concentrations of aminoglycosides combined with either imipenem or penicillin G resulted in a significant decrease in the $\log_{10}$ CFU per milliliter; pair differences were evaluated by Hotelling's T^2 test and Student's t test (2).

RESULTS

The MICs or MBCs of penicillin G, imipenem, streptomycin, and gentamicin for the 20 strains of enterococci are shown in Table 1. The antibiotic MICs for the three strains of *S. faecium* were similar to those for the 17 strains of *S. faecalis*. The synergistic activity of 20 μg of imipenem or 20 μg of penicillin G per ml together with increasing concentrations of streptomycin against 13 strains of streptomycin-susceptible enterococci is shown in Fig. 1. After 24 h of incubation, penicillin in combination with each increment of streptomycin concentration was significantly more active ($P < 0.001$) than was imipenem when combined with the

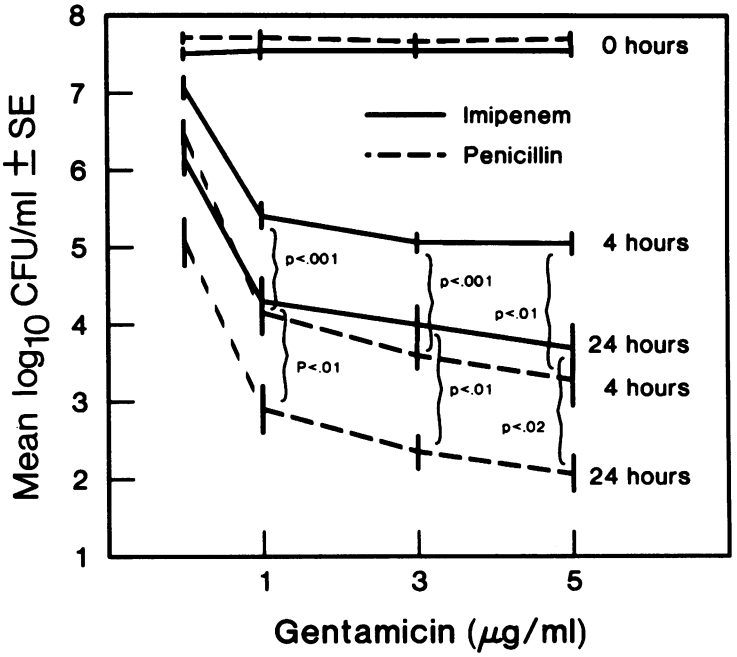

FIG. 3. Imipenem (20 μg/ml) or penicillin (20 μg/ml) and gentamicin synergy against seven strains of streptomycin-resistant enterococci. SE, Standard error.

corresponding concentration of streptomycin. The synergistic effect of penicillin G together with 5 μg of streptomycin per ml was significantly greater ($P < 0.01$) than was the effect of imipenem combined with 20 μg of streptomycin per ml. A combination of imipenem together with 20 μg of streptomycin per ml acted synergistically against only 6 of 13 strains, whereas 12 of the 13 strains were killed synergistically by a combination of penicillin G and 20 μg of streptomycin per ml.

Penicillin G or imipenem together with 5 μg of gentamicin per ml acted synergistically against 12 of 13 strains of streptomycin-susceptible enterococci. However, at 24 h the magnitude of killing of penicillin G together with each concentration of gentamicin was significantly greater ($P < 0.001$) than was the combination of imipenem and the corresponding concentration of gentamicin (Fig. 2).

Similar results were observed with the seven streptomycin-resistant strains of enterococci. Six of seven strains were killed synergistically with 5 μg of gentamicin per ml together with either penicillin G or imipenem. At each concentration of gentamicin, the magnitude of killing of enterococci was significantly greater ($P < 0.02$). when combined with penicillin G than when combined with imipenem (Fig. 3).

DISCUSSION

Watanakunakorn and Tisone (9) reported synergy in vitro between imipenem and gentamicin against 47 of 48 strains of enterococci. These authors defined synergy as a 10-fold increase in killing by the combination of drugs compared with that observed with a single antibiotic, whereas in our study we defined synergy as a 100-fold increase in killing by the combination of antimicrobials. This difference in the definition of synergy may account for the higher percentage of strains of enterococci affected synergistically reported by Watanakunakorn and Tisone versus that observed in our study. Imipenem together with streptomycin acted synergistically in vitro against less than one-half of the strains of streptomycin-susceptible enterococci. More significantly, the magnitude of killing of enterococci by combinations of imipenem and streptomycin or gentamicin was significantly less than when penicillin G was combined with either streptomycin or gentamicin. *S. faecium* is reportedly more resistant in vitro to imipenem than is *S. faecalis* (3). The failure to detect this difference in our study may be a result of the small number of strains of *S. faecium* tested. Eliopoulos and Moellering (3) noted more efficient killing of enterococci by 4 μg than by 10 μg of imipenem per ml. However, when gentamicin was combined with 10 μg of imipenem per ml the synergistic effect was similar to that of gentamicin combined with 4 μg of imipenem per ml. In our study we did not test various concentrations of imipenem because the synergistic effect of the combination of imipenem and gentamicin was independent of the imipenem concentration (3). We do not believe that the concentration of imipenem tested in our study was responsible for the difference between the synergistic activity of the combination of imipenem and gentamicin and that of penicillin G and gentamicin. The magnitude of killing by combinations of imipenem and gentamicin is reportedly similar to that of penicillin-gentamicin combinations (3, 6). The discordant results of these studies compared with those of our study may be related to different concentrations of aminoglycosides tested in the respective studies.

Although there are no published data concerning the use of imipenem together with an aminoglycoside for the treatment of human enterococcal infections, the results of our in vitro study suggest that the use of this combination might be inferior to the use of penicillin G combined with an aminoglycoside. We believe that imipenem combined with an aminoglycoside should be used with caution until the results of additional in vitro and experimental animal studies are available to further clarify the role of this combination for the treatment of serious enterococcal infections.

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