Comparative Synergistic Activity of Nafcillin, Oxacillin, and Methicillin in Combination with Gentamicin Against Enterococci

RICHARD H. GLEW, ROBERT C. MOELLERING, JR.,* AND CHRISTINE WENNERSTEN

Department of Medicine, Harvard Medical School, and Infectious Disease Unit, Department of Medicine, Massachusetts General Hospital, Boston, Massachusetts 02114

The effectiveness of three semisynthetic, penicillinase-resistant penicillins alone and in combination with gentamicin was tested against 29 clinical isolates of enterococci. The minimal inhibitory concentrations of nafcillin were considerably lower than those of oxacillin and methicillin but were slightly higher than those of penicillin. At clinically achievable concentrations, the combination of nafcillin plus gentamicin produced enhanced killing against 13 of 14 strains of enterococci and was synergistic (by very rigid criteria) against 10 of 14 strains. In contrast, combinations of oxacillin plus gentamicin were synergistic against only 3 of 14 strains, and methicillin plus gentamicin produced synergistic killing against only 1 of 14 strains.

Although enterococci are relatively resistant to many individual antibiotics (4, 16, 18), combinations of antimicrobial agents have been shown to produce synergism in vitro against these organisms (4–6, 15). Because the combination of penicillin with streptomycin has been demonstrated to act synergistically against many strains of enterococci, this combination has been used extensively to treat infections due to the enterococcus (7, 10). In recent years, however, increasing numbers of enterococci have been found to be resistant to the synergistic effect of penicillin plus streptomycin (11). Fortunately, the combination of penicillin plus gentamicin has been shown to produce enhanced killing against virtually all strains of enterococci in vitro (11, 20, 23). Furthermore, clinical studies have confirmed the in vivo efficacy of this combination against infections due to enterococci (21; J. Carrizosa, and D. Kaye, Clin. Res. 22:705A, 1974). Accordingly, it has been suggested that the combination of penicillin and gentamicin may be the treatment of choice for the initial therapy of serious infections due to enterococci (21, 22).

Patients with presumed septicemia frequently receive initial therapy with gentamicin plus a semisynthetic penicillin, often an anti-staphylococcal, penicillinase-resistant one. Ideally such therapy should protect against all pathogenic gram-negative and gram-positive organisms (including the enterococcus). It has been shown that the various semi-synthetic penicillins vary widely in their in vitro activity against enterococci (18). Furthermore, in our recent clinical experience, the combination of oxacillin plus gentamicin was ineffective in the treatment of enterococcal septicemia in a young girl with extensive burns (see below).

This study was undertaken to determine which, if any, of the penicillinase-resistant penicillins act synergistically with gentamicin against the enterococci, and thus to determine which might be most effectively used for initial antibiotic coverage in situations where enterococcal septicemia is likely.

(This paper was presented in part at the 75th Annual Meeting of the American Society for Microbiology, 27 April–2 May 1975, New York, N.Y.)

CASE REPORT

H. M., a 14-year-old girl, was transferred to the Shriner's Burn Hospital in Boston, Mass., on 20 November 1972 with 66% body surface area burns (second and third degree). Before transfer, she had undergone tracheostomy at an outside hospital because of suspected pulmonary burns.

Upon admission to the Shriner's Burn Institute, the patient was febrile and comatose and required mechanical ventilation. She was begun on penicillin G intravenously (1.8 million U/day) and appropriate fluid replacement. Her burn wounds were treated locally with silver nitrate dressings. On the 5th hospital day, because of deteriorating pulmonary function and spiking fevers, she was begun on gentamicin intravenously. Chest roentgenographs showed no infiltrates at that time. On the 15th hospital day she was begun on total parenteral nutrition.

The patient continued to exhibit hectic, spiking

fevers to 103 to 104 F (39.4 to 40.0 C). On the 19th hospital day she was again begun on gentamicin because of high-grade enterobacter bacteremia (six of six blood cultures positive for enterobacter over 5 days); the same organism had been grown in large numbers from her burn wounds for several days.

On the 28th hospital day the patient developed increasing pulmonary insufficiency, and her chest roentgenographs revealed bilateral fluffy pulmonary infiltrates. Sputum cultures grew *Staphylococcus aureus*; oxacillin (10 g/day intravenously) was begun and gentamicin was continued. A single blood culture that had been drawn 3 days earlier grew out enterococci. Even though all plastic intravenous lines had been removed and despite treatment with oxacillin and gentamicin, four more blood cultures obtained over the next 5 days also grew out enterococci.

In vitro studies demonstrated that the combination of penicillin G and gentamicin was synergistic against the enterococcus isolated from the patient's blood; that is, the combination of penicillin G (10 U/ml) and gentamicin (5 µg/ml) resulted in a 100-fold decrease in colony-forming units as compared with penicillin G alone at 24 h (Fig. 1). The combination of oxacillin plus gentamicin had no bactericidal effect on this organism (Fig. 1).

Because of persistent bacteremia and spiking fevers, oxacillin was discontinued and penicillin G (30 million U/day intravenously) was administered together with gentamicin starting on the 33rd hospital day. During the next several days the patient's temperature fell to normal and blood cultures remained free of enterococci for the remainder of her course.

Unfortunately, on the 40th hospital day, the patient again began to have spiking fevers and her blood cultures grew out serratia and *Candida albicans*. Despite treatment with amphotericin B, the patient died on the 57th hospital day.

Postmortem examination demonstrated disseminated candidiasis, with fungal forms in the kidneys and heart. There was no evidence of endocarditis. Postmortem heart blood grew out only *Enterobacter sp.* and *Clostridium perfringens*.

Comment. It is clear that in this patient enterococcal septicemia was not controlled by the administration of oxacillin and gentamicin and that penicillin plus gentamicin was required for cure. The lack of in vivo effect of oxacillin plus gentamicin against the patient's enterococcal septicemia was distrubing and raised the possibility that the semisynthetic, penicillinase-resistant penicillins in general might not be effective against enterococci, even in combination with gentamicin. However, subsequent in vitro studies with this patient's organism demonstrated effective synergism with nafcillin plus gentamicin, but not with oxacillin or methicillin plus gentamicin (Fig. 1). This led us to undertake a more systematic study of the in vitro effectiveness of the semisynthetic, penicillinase-resistant penicillins against enterococci.

MATERIALS AND METHODS

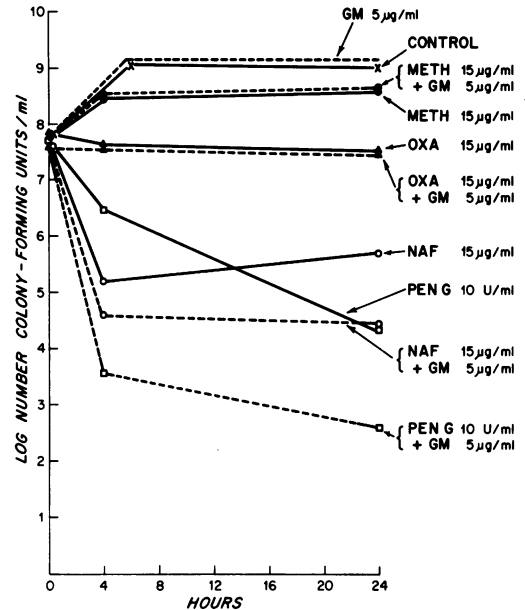The organisms used in this study were isolated from clinical specimens submitted to the Bacteriology

FIG. 1. *Effect of various penicillins alone and in combination with gentamicin against the enterococcus isolated from the blood of patient H. M. Abbreviations: GM, Gentamicin; METH, methicillin; NAF, nafcillin; OXA, oxacillin; PEN G, penicillin G.*

Laboratory of the Massachusetts General Hospital. Each represented a separate infection. Of the 29 strains of enterococci, 28 had been isolated from blood cultures and one had been isolated from a wound culture. These organisms were identified by the usual growth criteria (3, 19), were grouped serologically by using extracts prepared by the method of Rantz and Randall (14) and were further speciated by using a composite of reactions as suggested by Deibel (2).

Antibiotics used in this study were pharmacologic preparations and included sodium nafcillin: (supplied as Unipen by Wyeth Laboratories), sodium oxacillin (supplied as Prostaphlin by Bristol Laboratories), sodium methicillin (supplied as Staphcillin by Bristol Laboratories), potassium penicillin (supplied as Pfizerpen by Pfizer Laboratories), and gentamicin sulfate (supplied as Garamycin by the Schering Corp.). Appropriate dilutions were made in sterile water without preservative.

Minimal inhibitory concentrations (MIC) and minimal bactericidal concentrations (MBC) for all antibiotics were determined by standard tube dilution methods (1) in dextrose-phosphate broth (Pfizer). The inoculum for each organism consisted of 1 ml of a 1:1,000 dilution of an overnight broth culture and contained 10^5 to 10^6 organisms/ml. The final volume in each tube was 2 ml. The MIC and MBC data for penicillin G were derived from earlier work with the same organisms (13); however, the values for penicillin G were converted from units per milliliter to micrograms per milliliter (1 U = 0.6 µg).

Tests of antibiotic synergism were performed in dextrose-phosphate broth (Pfizer), using a starting

concentration of 10^7 organisms/ml diluted from a culture grown overnight in broth. Antibiotics were added as follows: penicillin G, 10 U/ml; nafcillin, oxacillin, and methicillin, 15 µg/ml; and gentamicin, either 2.5 or 5.0 µg/ml (always less than the MIC for gentamicin). The cultures were incubated without agitation at 37 C, and samples (0.5 ml) were removed at 0, 4, and 24 h for determination of colony counts. Details of this method have been described previously (12).

RESULTS

In Fig. 2 are shown the frequency distributions of MIC values for the four penicillins against 29 strains of enterococci. In general, penicillin G was slightly more active than nafcillin, which was, in turn, more active than oxacillin and methicillin. At the clinically attainable concentration of 16 µg/ml, penicillin G inhibited 89% of the strains and nafcillin inhibited 83%, whereas oxacillin and methicillin inhibited only 17 and 0%, respectively. The MBC values were ≥ 250 µg/ml for 86% of the strains with penicillin G, 62% of the strains with nafcillin, 97% of the strains with oxacillin, and 83% of the strains with methicillin.

To evaluate the relative effectiveness in vitro of the three semisynthetic penicillins in combination with gentamicin, we selected 14 strains of enterococci isolated from blood cultures; all had been killed synergistically by the combination of penicillin G (10 U/ml) and gentamicin (5 µg/ml) with an inoculum of 10^7 organisms/ml (13).

The relative effectiveness of the three combinations was evaluated according to several criteria (Table 1). If one uses the rigorous definition of synergism used in our previous studies (a decrease of 100-fold or more in colony-forming units caused by the combination as compared with the most effective of the antibiotics used alone), then the combination of nafcillin plus
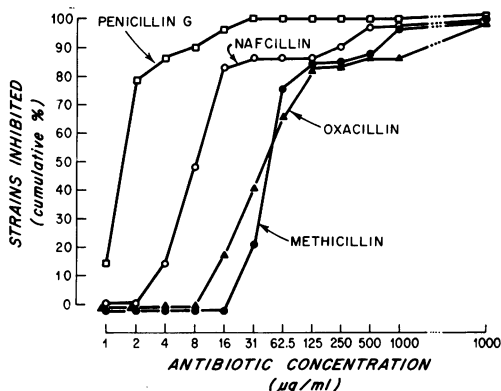
TABLE 1. *Effectiveness of three semisynthetic penicillins in combination with gentamicin against 14 strains of enterococci*

Antibiotics	Increased kill[a]		Absolute kill[b] (≥10³)	Increased kill (≥10²) and absolute kill (≥10³)
	≥10¹	≥10²		
Nafcillin + gentamicin	13	11	13	10
Oxacillin + gentamicin	8	5	3	3
Methicillin + gentamicin	7	5	1	1

[a] Magnitude of increased killing (decrease in viable cell count) after 24 h by the combination as compared with the most effective of the antibiotics used alone. In all cases, the most effective antibiotic alone was semisynthetic penicillin since the aminoglycoside concentration was chosen to be < MIC.

[b] Magnitude of decrease in viable cell count at 24 h as compared with concentration of viable organisms at time zero.
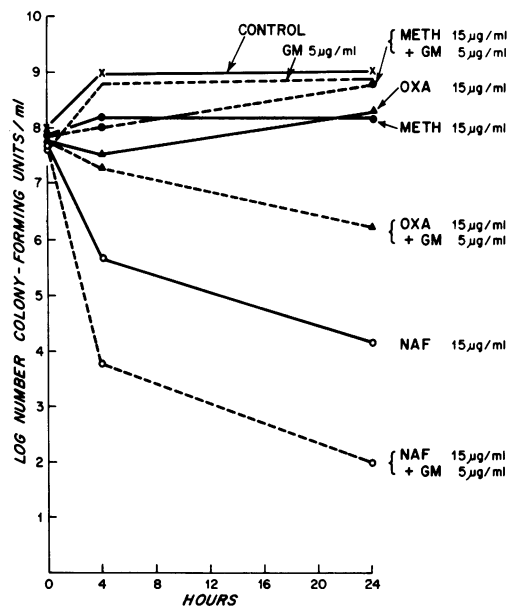

FIG. 3. *Effect of various semisynthetic penicillins alone and in combination with gentamicin against enterococcus strain 447.*

gentamicin was synergistic against 11 of 14 strains, whereas the combinations of oxacillin plus gentamicin and methicillin plus gentamicin were each synergistic against only 5 of 14 strains (Fig. 3). Furthermore, significant enhanced bactericidal effect (at least 10-fold increased killing by the combination) was noted in 13 of 14 strains with nafcillin plus gentami-

FIG. 2. *Frequency distribution of MIC values for various penicillins against 29 strains of enterococci.*

cin, but in just 8 of 14 strains with oxacillin plus gentamicin and 7 of 14 strains with methicillin plus gentamicin.

These data are, in fact, somewhat misleading because in many instances the apparent 100-fold increased killing with oxacillin or methicillin plus gentamicin was related to virtually uninhibited growth in the presence of the oxacillin or methicillin alone, which resulted in increased separation between colony counts from the flasks containing the combination as compared with the flasks containing the oxacillin or methicillin alone (Fig. 3). For example, 1,000-fold killing (relative to initial colony counts) at 24 h was noted in only 3 of 14 strains in the presence of oxacillin plus gentamicin and in 1 of 14 strains with methicillin plus gentamicin; the combination of nafcillin plus gentamicin produced 1,000-fold killing against 13 of 14 strains.

If we apply both criteria for synergy, i.e., 100-fold enhanced killing relative to the semisynthetic penicillin alone and 1,000-fold killing relative to starting colony count, then unequivocal synergism was demonstrated against 10 of 14 strains with nafcillin plus gentamicin, against only 3 of 14 strains with oxacillin plus gentamicin, and against only 1 of 14 strains with methicillin plus gentamicin.

DISCUSSION

Unlike most of the streptococci, the enterococci are resistant to killing by penicillin. Although most strains of enterococci are inhibited by clinically achievable concentrations of penicillin (0.5 to 2.0 U/ml), only a small percentage of strains are killed even by very high concentrations of the antibiotic (4). In vitro studies have demonstrated that combinations of penicillin and an aminoglycoside show synergistic killing against enterococci (4, 6). The combination of penicillin plus gentamicin exhibits enhanced killing in vitro against virtually all strains of enterococci (12, 20, 23), and this combination has been demonstrated to be effective in vivo (21; Carrizosa and Kaye, Clin. Res. 22:705A, 1974).

Certain semisynthetic penicillins have demonstrated in vitro synergism with aminoglycosides against enterococci. In particular, enhanced killing of enterococci has been demonstrated with combinations of an aminoglycoside plus either ampicillin (11, 16, 17, 23) or carbenicillin (8, 9).

Little attention has been devoted to evaluating the activity of the penicillinase-resistant semisynthetic penicillins against enterococci. Toala and co-workers studied the in vitro activity of several antibiotics (alone) against 382 clinical isolates of enterococci at the Boston City Hospital and noted that, extrapolating from MIC levels, the activity of nafcillin ranked below ampicillin and penicillin G, whereas methicillin and cloxacillin were substantially less active than nafcillin (18). At a concentration of 12.5 μg/ml, penicillin G and ampicillin inhibited approximately 100% of the strains of enterococci and nafcillin inhibited about 35%, whereas methicillin and cloxacillin inhibited none. At a concentration of 25 μg/ml, nafcillin inhibited about 75% of the strains, whereas methicillin inhibited about 5% and cloxacillin inhibited essentially none.

Recently we observed a young patient with extensive burns who developed enterococcal septicemia, probably related to intravenous plastic catheters. Her infection was not controlled by discontinuation of her catheters and treatment with high doses of oxacillin and gentamicin parenterally. However, after beginning therapy with penicillin G and gentamicin, blood cultures became negative for enterococci and the patient's temperature returned to normal. In light of this apparent failure of oxacillin plus gentamicin in the in vivo treatment of enterococcal septicemia, we investigated the ability of various penicillinase-resistant, semisynthetic penicillins to act synergistically with gentamicin against enterococci.

In the current study, nafcillin was the most effective of the three semisynthetic, penicillinase-resistant penicillins in combination with gentamicin against enterococci by in vitro testing. The MIC values for nafcillin were lower than for oxacillin or methicillin, and at clinically attainable levels most strains of enterococci tested were inhibited by nafcillin but not by the other two antibiotics. More importantly, at clinically achievable concentrations, the combination of nafcillin plus gentamicin exhibited enhanced killing as compared with nafcillin alone against virtually all strains of enterococci tested, and this combination was synergistic according to very strict criteria against 10 of 14 strains tested. On the other hand, combinations of either oxacillin or methicillin with gentamicin were synergistic against only three strains and one strain, respectively. Thus, of these three penicillinase-resistant, semisynthetic penicillins, nafcillin is the most active in combination with gentamicin against enterococci.

These results demonstrate that the combination of nafcillin plus gentamicin is synergistic for many strains of enterococci and suggest that this combination may be efficacious for initial antibiotic coverage in situations where enterococcal and staphylococcal septicemia are

likely. A clinical trial of nafcillin plus gentamicin in the initial therapy of patients with presumed septicemia is reasonable.

LITERATURE CITED

1. Anderson, T. G. 1970. Testing of susceptibility to antimicrobial agents and assay of antimicrobial agents in body fluids, p. 299–310. In J. E. Black, E. H. Lennette, and J. P. Truant (ed.), Manual of clinical microbiology. American Society for Microbiology, Bethesda, Md.
2. Deibel, R. H. 1964. The group D streptococci. Bacteriol. Rev. 28:330–366.
3. Facklam, R. R. 1973. Comparison of several laboratory media for presumptive identification of enterococci and group D streptococci. Appl. Microbiol. 26:138–145.
4. Fekety, F. R., Jr., and P. Weiss. 1967. Antibiotic synergism: enhanced susceptibility of enterococci to combinations of streptomycin and penicillins or cephalosporins, p. 156–164. Antimicrob. Agents Chemother. 1966.
5. Jawetz, E., and J. B. Gunnison. 1953. Antibiotic synergism and antagonism: assessment of problem. Pharmacol. Rev. 5:175–192.
6. Jawetz, E., J. B. Gunnison, and V. R. Coleman. 1950. Combined action of penicillin with streptomycin or chloromycetin on enterococci in vitro. Science 111:254–256.
7. Jawetz, E., and M. Sonne. 1966. Penicillin-streptomycin treatment of enterococcal endocarditis. N. Engl. J. Med. 274:710–715.
8. Libke, R. D., C. Regamey, J. T. Clarke, and W. M. M. Kirby. 1973. Synergism of carbenicillin and gentamicin against enterococci. Antimicrob. Agents Chemother. 4:564–568.
9. McCracken, G. H., Jr., J. D. Nelson, and M. L. Thomas. 1973. Discrepancy between carbenicillin and ampicillin activities against enterococci and listeria. Antimicrob. Agents Chemother. 3:343–349.
10. Mandell, G. L., D. Kaye, M. E. Levison, and E. W. Hook. 1970. Enterococcal endocarditis: an analysis of 38 patients observed at the New York-Connell Medical Center. Arch. Intern. Med. 125:258–264.
11. Moellering, R. C., Jr., C. Wennersten, T. Medrek, and A. N. Weinberg. 1971. Prevalence of high-level resistance to aminoglycosides in clinical isolates of enterococci, p. 335–340. Antimicrob. Agents Chemother. 1970.
12. Moellering, R. C., Jr., C. Wennersten, and A. N. Weinberg. 1971. Studies on antibiotic synergism against enterococci. 1. Bacteriologic studies. J. Lab. Clin. Med. 77:821–828.
13. Moellering, R. C., Jr., C. Wennersten, and A. N. Weinberg. 1971. Synergy of penicillin and gentamicin against enterococci. J. Infect. Dis. 124:S207–S209.
14. Rantz, L. A., and E. Randall. 1955. Use of autoclaved extracts of hemolytic streptococci for serologic grouping. Stanford Med. Bull. 13:290–291.
15. Robbins, W. C., and R. Tompsett. 1951. Treatment of enterococcal endocarditis and bacteremia. Results of combined therapy with penicillin and streptomycin. Am. J. Med. 10:278–299.
16. Simon, H. J. 1967. Antimicrobial susceptibility of group D hemolytic streptococci (enterococci). Am. J. Med. Sci. 253:44–48.
17. Sonne, M., and E. Jawetz. 1968. Comparison of the action of ampicillin and benzylpenicillin on enterococci in vitro. Appl. Microbiol. 16:645–648.
18. Toala, P., A. McDonald, C. Wilcox, and M. Finland. 1969. Susceptibility of group D streptococcus (enterococcus) to 21 antibiotics in vitro, with special reference to species differences. Am. J. Med. Sci. 258:416–430.
19. von Graevenitz, A., J. J. Redys, and E. Cassidy. 1970. Evaluation of a new differential test for enterococci in the clinical laboratory. J. Med. Microbiol. 3:227–231.
20. Watanakunakorn, C. 1971. Penicillin combined with gentamicin or streptomycin synergism against enterococci. J. Infect. Dis. 124:581–596.
21. Weinstein, A. J., and R. C. Moellering, Jr. 1973. Penicillin and gentamicin therapy for enterococcal infections. J. Am. Med. Assoc. 223:1030–1032.
22. Weinstein, L., and J. Schlesinger. 1973. Treatment of infective endocarditis—1973. Prog. Cardiovasc. Dis. 16:275–302.
23. Wilkowske, C. J., R. R. Facklam, J. A. Washington II, and J. E. Geraci. 1971. Antibiotic synergism: enhanced susecptibility of group D streptococci to certain antibiotic combinations, p. 195–200. Antimicrob. Agents Chemother. 1970.