ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Nov. 1986, p. 763–768
0066-4804/86/110763-06$02.00/0

Vol. 30, No. 5

Penicillin-Induced Effects on Streptomycin Uptake and Early Bactericidal Activity Differ in Viridans Group and Enterococcal Streptococci

MICHAEL H. MILLER,* MOHAMED A. EL-SOKKARY, STUART A. FEINSTEIN, AND FRANKLIN D. LOWY

Division of Infectious Diseases, Department of Medicine, Montefiore Medical Center and Albert Einstein College of Medicine, Bronx, New York 10467

Received 21 November 1985/Accepted 2 September 1986

In vitro studies with penicillin and [^{3}H]streptomycin in four strains of streptococci (*S. faecalis*, *S. sanguis*, and *S. mitis*) were performed by simultaneously measuring the rates of bacterial killing and uptake of streptomycin. In *S. faecalis*, penicillin stimulated streptomycin uptake, as has been shown by Moellering and Weinberg (R. C. Moellering, Jr., and A. N. Weinberg, J. Clin. Invest. 50:2580–2584, 1971). Moreover, the antibiotic combination was associated with an enhanced bactericidal rate which temporally correlated with β-lactam-induced aminoglycoside uptake. In contrast, in viridans group streptococci (*S. sanguis* and *S. mitis*) penicillin had no effect on streptomycin uptake and a minimal effect on bactericidal rate when compared with either drug alone. These data suggested that the stimulation of streptomycin uptake in streptococci by penicillin is strain or species specific and that important differences exist between enterococci and viridans group streptococci regarding the mechanisms of β-lactam–aminoglycoside potentiation.

Antibiotic synergy is perhaps best exemplified by the use of a β-lactam combined with an aminoglycoside in the therapy of enterococcal endocarditis. The enhanced antibacterial activity of the combination has been well established both in clinical trials (26) and in an experimental model of endocarditis (14). In studies on the mechanism of synergy in enterococci, Moellering and colleagues (35, 36) demonstrated that penicillin enhanced intracellular entry of otherwise sublethal concentrations of streptomycin, suggesting that the mechanism of synergy in *Streptococcus faecalis* was similar to that shown earlier in *Escherichia coli* by Plotz and Davis (36). However, from a mechanistic standpoint it is not clear how penicillin would enhance uptake of aminoglycosides in most gram-positive bacteria since no barrier to aminoglycoside penetration comparable to that of gram-negative bacilli has been described. Time kill studies (35, 36) also demonstrated that enhancement of streptomycin entry was associated with an increase in the rate of killing during 24 h as determined by the inability of cells to form colonies on agar after overnight incubation. Twenty-four-hour time kill studies have since become a generally accepted in vitro method for examining bactericidal synergism in both gram-positive and gram-negative bacteria.

Numerous 24-h in vitro time kill studies have shown apparent synergy (i.e., a >2 $\log_{10}$ enhanced bactericidal effect) between β-lactams and aminoglycosides in gram-positive organisms (5, 12, 20, 21, 41, 44). In vitro (22, 24, 33) and in vivo (23, 25, 33) studies with *Staphylococcus aureus* and *Staphylococcus epidermidis* in our laboratory, however, indicated that a second mechanism by which cell wall-active antibiotics (β-lactams, vancomycin) enhance the bactericidal effect of aminoglycosides (or rifampin) is by preventing regrowth of relatively resistant subpopulations. Twenty-four-hour time kill studies often do not distinguish between the aforementioned mechanisms of drug potentiation, and extended periods of incubation may be associated with alteration of the growth media (17, 18) or antibiotic degradation (10), further confounding the interpretation of such experiments.

The purpose of the present study was to investigate penicillin effects on streptomycin uptake and killing in several previously characterized strains of viridans group streptococci (3, 15, 16, 23) when compared with *S. faecalis*. The viridans group streptococcus studies included *S. sanguis* and *S. mitis*, not only because these species are a common cause of viridans group streptococcal endocarditis, but also because they demonstrate important differences in penicillin-induced lethal effects (i.e., penicillin tolerance) in vitro (15, 16, 23) and in vivo (3, 13).

MATERIALS AND METHODS

Bacterial strains and antimicrobial susceptibility assays. *S. faecalis* E1 is a previously characterized strain (35, 36), and *S. faecalis* L is a blood culture isolate from a patient with infective endocarditis. *S. sanguis* Wicky is tolerant to the lethal effects of penicillin, and *S. mitis* Sticky is nontolerant (23). *S. faecalis* L was identified as to species by standard biochemical testing (9). MICs were determined for both penicillin and streptomycin in tryptic soy broth (TSB; Difco Laboratories, Detroit, Mich.) by standard techniques (33). Each determination was done in triplicate with a 5×10^5 CFU/ml inoculum with a final volume of 1 ml.

Time kill and streptomycin uptake studies. For time kill and uptake studies, a 1:15 dilution of an overnight culture was added to prewarmed TSB and incubated at 37°C for an additional 60 min to ensure log-phase growth. Cells were then placed into three flasks in a 37°C water bath for determination of cell viability, streptomycin uptake, and optical density (OD) as described previously (27–29, 32). To determine cell viability, samples were removed at specified times, serially diluted in normal saline, added to TSB agar (1%) at 50°C, incubated at 37°C, and counted at 24 to 48 h

* Corresponding author.

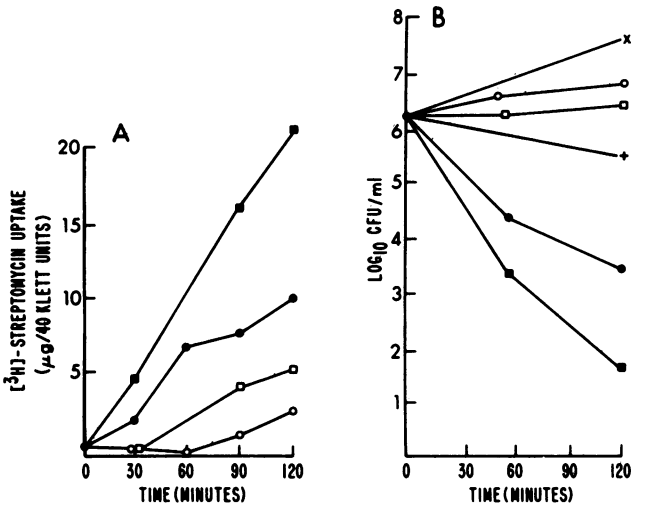

FIG. 1. [^{3}H]streptomycin uptake by *S. faecalis* L at 50 (circles) and 100 μg (squares) of streptomycin per ml in the absence (○, □) or presence (●, ■) of 10 U of penicillin per ml is shown in A. The corresponding bactericidal effects (same symbols) and the effects of penicillin alone (+) and no antibiotic (×) are shown in B.

(32). To prevent the effects of antibiotic carryover, β-lactamase (Difco) was added or cells were immediately diluted 100-fold in iced saline or both. To determine streptomycin uptake, cells were grown as described above. Cell density was monitored turbidimeterically (OD) with a Klett-Summerson photoelectric colorimeter. Samples (0.75 ml) were removed at the times indicated, and samples were washed with 3 ml of 3% NaCl (20°C). Membrane filtration was performed, as previously described (8), with glass microfiber filters (GF/C; Whatman Inc., Clifton, N.J.) presoaked with 100 μg of unlabeled streptomycin per ml. We used [^{3}H]streptomycin at a final specific activity of 20 to 200 μCi/mg after combining unlabeled streptomycin and radiolabeled [^{3}H]streptomycin sesquisulfate (Amersham Corp., Arlington Heights, Ill.; specific activity, 1.2 mCi/mg). Membrane filters were dried at 37°C overnight, and samples were counted in a liquid scintillation counter as described previously (33). Streptomycin uptake was expressed as micrograms of streptomycin per unit of cell mass (40 Klett units) after correction for nonspecific binding with 100 μM carbonyl cyanide-*m*-chlorophenylhydrazone (Calbiochem-Behring, La Jolla, Calif.) (28).

Dose response time kill, aminoglycoside uptake, and OD determinations at aminoglycoside concentrations of 5 to 100 μg/ml for *S. sanguis* and 20 to 100 μg/ml for *S. mitis* were simultaneously performed. The studies with *S. mitis* and *S. sanguis* with streptomycin concentrations of 1 and 2.5 μg/ml, however, were not performed simultaneously with dose-response data at the three higher streptomycin concentrations. All determinations in viridans group streptococcal strains were repeated at least in triplicate. Comparable studies with *S. faecalis* L and E1 were performed as described above except that these studies were done in duplicate. Medium pH was determined with a Corning pH meter (model 109; Corning Glass Works, Corning, N.Y.).

RESULTS

Dose-response, streptomycin uptake, and time kill studies in *S. faecalis*. A representative dose-response, [^{3}H]streptomycin uptake, and time kill study for *S. faecalis* L is shown in Fig. 1. The penicillin and streptomycin MICs for this strain were 3.12 U and 62.5 μg/ml, respectively. In the absence of penicillin, after a delay of 30 to 60 min, uptake proportional to the external streptomycin concentration occurred (Fig. 1A). The addition of penicillin (10 U/ml) was associated with marked stimulation in the rate and amount of streptomycin taken up at streptomycin concentrations of 50 and 100 μg/ml.

Figure 1B shows the corresponding bactericidal effects of the antibiotics. Neither streptomycin concentration was associated with a bactericidal effect, although growth inhibition was demonstrated as it was with penicillin alone. The penicillin-induced stimulation of streptomycin uptake was associated with enhanced bacterial killing (>2 $\log_{10}$) at both streptomycin concentrations. Additional studies with a previously characterized *S. faecalis* (E1) for which the MICs of penicillin and streptomycin were 3.12 U and 125 μg/ml, respectively, were performed. Streptomycin uptake with and without penicillin in E1 was qualitatively similar to that in L. Corresponding time kill studies in E1 showed a greater than 2.5 $\log_{10}$ decrease in CFU/ml at 2 h with 10 U of penicillin per ml and both 100 and 200 μg of streptomycin per ml when compared with either drug alone (data not shown).

Dose-response, streptomycin uptake, and time kill studies in *S. sanguis* and *S. mitis*. Figure 2 shows a representative dose-response, streptomycin uptake, and time kill study for the penicillin-nontolerant *S. mitis* strain with streptomycin at concentrations ranging from 2.5 to 100 μg/ml. The MICs of penicillin and streptomycin were 0.05 U and 32.25 μg/ml. As with enterococci, the rate of aminoglycoside uptake was proportional to external drug concentrations. In marked contrast to enterococcal strains, however, the addition of 10 U of penicillin per ml was not associated with any measurable effect on streptomycin uptake. The effects of lower concentrations of penicillin (1 U/ml) were also examined with and without 2.5 μg of streptomycin per ml. These concentrations were used to more closely approximate the corresponding fraction of the MIC used for the studies with enterococcal strains. As with higher penicillin concentrations (10 U/ml), there was no facilitated aminoglycoside uptake or enhanced killing with the combination (data not shown). Furthermore, neither preincubation with penicillin for 1 h before the addition of streptomycin nor longer incubation with the combination (up to 4 h) was associated with stimulation of streptomycin uptake.

Figure 2B shows the bactericidal effects of these antibiotics used alone or in combination. Whereas the addition of 10 U of penicillin per ml was associated with a slightly en-

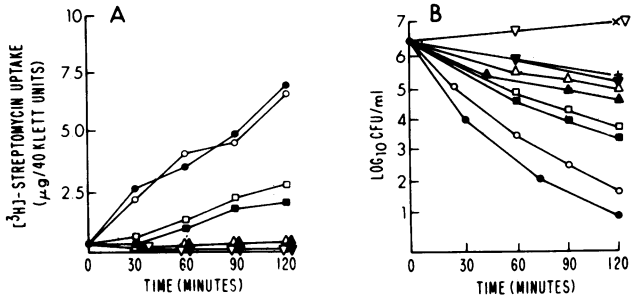

FIG. 2. [^{3}H]streptomycin uptake by *S. mitis* Sticky at 2.5 (inverted triangles), 20 (triangles), 50 (squares), and 100 μg (circles) of [^{3}H]streptomycin per ml in the absence (▽, △, □, ○) or presence (▼, ▲, ■, ●) or 10 U of penicillin per ml are shown in A. The corresponding bactericidal effects and the effects of penicillin alone (+) and no antibiotic (×) are shown in B.

hanced bactericidal effect at the higher streptomycin concentrations (20 to 100 μg/ml), this enhanced killing was no more than would be expected as the sum of the individual lethal effects of each antibiotic alone and was considerably less than that seen with enterococci.

Figure 3 shows a representative dose-response, [^{3}H]streptomycin uptake, and time kill study in tolerant *S. sanguis* Wicky. The MICs of penicillin and streptomycin were 0.05 U and 2 μg/ml. Dose-response data with streptomycin concentrations from 1 to 100 μg/ml are shown; both streptomycin uptake and killing with and without 10 U of penicillin per ml were indistinguishable from those seen with the nontolerant viridans group streptococcus. Penicillin at 1 U/ml also showed no stimulation of streptomycin uptake or enhanced killing. Comparable studies with tolerant *S. sanguis* Durack (3) were identical to those with *S. sanguis* Wicky and *S. mitis* Sticky (data not shown).

Twenty-four-hour time kill studies in viridans group streptococci. To emphasize methodological problems with standard bactericidal assays and to compare the rate of killing in our strains with those using a similar experimental design (5, 12, 20, 21, 41, 44), 24-h time kill studies were done for *S. mitis* Sticky and *S. sanguis* Wicky with a large bacterial inoculum, and samples were obtained at 0, 4, and 24 h. Figure 4 shows a representative study with *S. mitis* Sticky. Streptomycin alone at 20 μg/ml was associated with a decrease in CFU/ml at 4 h followed by regrowth at 24 h. The addition of penicillin to streptomycin caused little enhancement in the bactericidal effect at 4 h. In contrast, at 24 h there was a >2 $\log_{10}$ increased killing effect when compared with that of either drug alone. The kinetics of killing were typical for viridans group streptococci, which characteristically show streptomycin-associated killing or inhibition at 4 h at subinhibitory aminoglycoside concentrations followed by regrowth by 24 h (5, 12, 20, 21, 41, 44) in the absence of penicillin. Similarly, the same concentrations of antibiotics in *S. sanguis* Wicky showed increased killing (3.2 $\log_{10}$) at 24 but not at 4 h (<0.5 $\log_{10}$). There was, however, relatively less killing due to penicillin alone in this tolerant strain (23).

Whereas β-lactam-associated growth suppression of a subpopulation of aminoglycoside-resistant mutants (33) might explain the lack of potentiation at 4 h and its presence at 24 h, consideration of the initial bacterial mass, population dynamics (i.e., a mutation rate of approximately 10^{-7}), and growth rates (doubling time of 1 h) indicated that early regrowth seen at 1 to 4 h in cells exposed to streptomycin

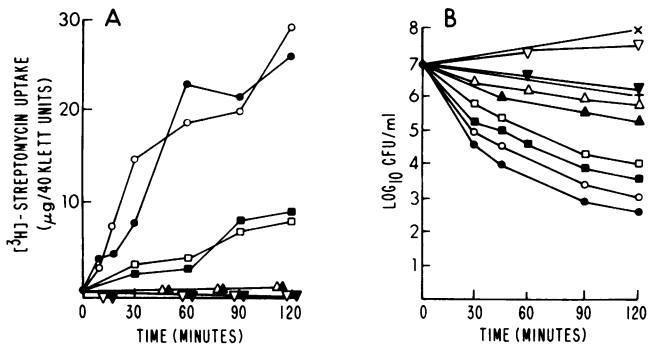

FIG. 3. [^{3}H]streptomycin uptake by *S. sanguis* Wicky at 1 (inverted triangles), 5 (triangles), 50 (squares), and 100 μg (circles) or streptomycin per ml in the absence (▽, △, □, ○) or presence (▼, ▲, ■, ●) or 10 U of penicillin per ml is shown in A. The corresponding bactericidal effects (same symbols) and the effects of penicillin alone (+) and no antibiotic (×) are shown in B.

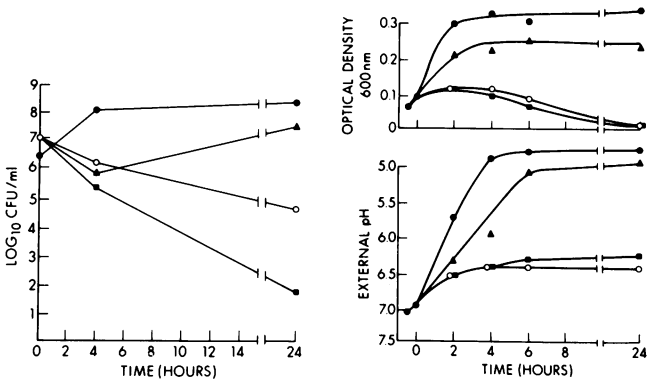

FIG. 4. In vitro bactericidal effects of penicillin at 10 U/ml (○), streptomycin at 20 μg/ml (▲), both penicillin and streptomycin (■), and neither antibiotic (●) on *S. mitis* Sticky are shown in the left panel. The effects (same symbols) on OD at 600 nm with penicillin at 10 U/ml, streptomycin at 20 μg/ml, both penicillin and streptomycin, and neither antibiotic over 24 h are shown in the upper right panel. Corresponding effects on external pH are shown in the lower right panel.

alone was not likely due to mutational resistance. All streptococci are aerotolerant anaerobes which produce acid as a byproduct of metabolism, and growth-associated acidification of media is observed (17). Since acidification of media is associated with both decreased uptake of and increased resistance to aminoglycosides (28), we examined the effect of growth on medium pH in the absence of antibiotics. These studies examined the effects of cell replication in log-phase cells as determined spectrophotometrically (at 600 nm) on external pH in unbuffered TSB. Both *S. sanguis* Wicky and *S. mitis* Sticky lowered pH at a rate which was proportional (r = 0.99 by least-squares analysis) to cell growth as determined by OD ($y = 3.2921x + 7.24$, where x is OD and y is medium pH). In other words, there was a close relationship between cell mass (OD), which increased over several hours, and the rate of medium acidification in the absence of antibiotics. Studies which examined the rate of acidification over 24 h at a 1/10 dilution of overnight cultures in TSB, Todd-Hewitt broth, and brain heart infusion broth (BBL Microbiology Systems, Cockeysville, Md.) were also performed. Acidification was seen in all three media, and there were no apparent differences in the buffering capacities of individual media. Combining data for the different media, mean external pH values for a single representative experiment (n = 3) in *S. mitis* Sticky, *S. sanguis* Wicky, and *S. faecalis* E1, respectively, were 7.50 ± 0.29 at the inception for all strains, 6.16 ± 0.26, 6.30 ± 0.29, and 6.86 ± 0.21 at 3 h, and 6.0 ± 0.35, 5.70 ± 0.26, and 5.6 ± 0.22 at 24 h. Buffering of TSB with 80 mM morpholinepropanesulfonic acid (MOPS) (change in pH, <0.25 pH units per 24 h) did not prevent regrowth in the presence of 20 μg of streptomycin per ml at 24 h in either viridans group streptococcal strain (data not shown). Potentiation at 24 h with antibiotic combinations therefore cannot be attributed to the effect of penicillin on medium pH alone.

Figure 4 (right panel) shows a representative study examining growth as determined spectrophotometrically and external pH with and without antibiotics in log-phase *S. mitis*. In the absence of antibiotics, there was acidification of the media. The addition of 20 μg of streptomycin per ml was associated with decreased growth (OD) when compared with the control. OD, however, increased and pH fell for up to 6 h despite killing (1.2 $\log_{10}$ CFU/ml) of the cells as measured

by the ability to form colonies in agar after overnight incubation. In contrast, penicillin had a greater effect on both the rate of growth (OD) and medium acidification (with or without streptomycin) despite killing, which was comparable to that seen with streptomycin alone at 4 h.

DISCUSSION

It is generally assumed that the mechanism of apparent synergism in viridans group streptococci as demonstrated by in vitro time kill studies (5, 12, 20, 21, 41, 44) or studies in an experimental model of endocarditis (14) is similar to that described for enterococci. Our data suggest that there are important differences in the mechanism(s) of β-lactam–aminoglycoside potentiation between enterococci and viridans group streptococci (*S. mitis* and *S. sanguis*). In *S. faecalis*, as previously shown by Moellering and colleagues, penicillin facilitates the entry of otherwise sublethal streptomycin concentrations. The β-lactam-induced facilitated uptake of streptomycin was associated with a significantly enhanced bactericidal effect when compared with either antibiotic alone. Whereas commonly accepted criteria for in vitro bactericidal synergism are based on 24-h time kill data in enterococci (36), others have also recently shown that synergism may be apparent by 4 h (the earliest time tested) in *S. faecalis* (40). In contrast to *S. faecalis*, penicillin, when used at concentrations above the MIC, demonstrated no effect on streptomycin uptake at 0 to 4 h in *S. mitis* Sticky and *S. sanguis* Wicky and a minimal effect on lethality which was independent of the tolerance phenotype. Comparable studies in an additional tolerant *S. sanguis* strain (*S. sanguis* Durack), which has been used to demonstrate both the potential significance of penicillin tolerance in vivo and the benefit of β-lactam–aminoglycoside potentiation in an experimental endocarditis model (3), also failed to demonstrate β-lactam induction of facilitated aminoglycoside uptake or an enhanced bactericidal effect. It is important to point out, however, that studies in three South African strains of viridans group streptococcus demonstrated enhanced levels of cell-associated radiolabeled streptomycin and tobramycin when cells were exposed to penicillin. By Blount's criteria (1), these strains were relatively resistant or moderately susceptible to penicillin. Whereas there was no enhanced killing with β-lactam–aminoglycoside combinations, these data nevertheless suggest that β-lactam induction of aminoglycoside uptake may be strain specific (Y. Yee, B. Farber, and S. Mates, Program Abstr. Annu. Meet. Am. Soc. Microbiol. 1984, A85, p. 15; S. Mates, personal communication).

Since marked potentiation with the β-lactam–aminoglycoside combination was observed at 24 h in these and other strains of viridans group streptococci (5, 12, 41, 44), penicillin-induced stimulation of streptomycin uptake might have occurred between 4 and 24 h. Whereas this possibility cannot be easily excluded experimentally, it is highly unlikely. Early in vitro synergy (i.e., 2 to 4 h), as indicated by a >2 $\log_{10}$ killing effect, is associated with β-lactam-induced stimulation of aminoglycoside uptake not only in *S. faecalis* but also in other bacterial species including *S. aureus*, *E. coli*, and *Pseudomonas aeruginosa* (J. M. Zenilman, M. H. Miller, and L. J. Mandel, Program Abstr. 25th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 346, 1985; M. H. Miller, unpublished data). Moreover, 30-min preexposure of cells to β-lactams and use of concentrations in excess of the MIC (as was performed for viridans group streptococci) is associated with stimulation of aminoglycoside uptake and killing within minutes. Consistently, chemicals which stimulate aminoglycoside uptake by alteration in the cytoplasmic membrane energy state (27) also show in vitro synergism by 2 h by standard time kill criteria (36). The combination of a β-lactam and an aminoglycoside may be synergistic by mechanisms unrelated to facilitated aminoglycoside uptake. On the other hand, potentiation at 24 h, which is seen with viridans group streptococci (5, 12, 20, 21, 41) when a large inoculum is used to mimic bacterial densities within infected valvular vegetations, may not be synergistic. Streptococci exposed to subinhibitory concentrations of streptomycin characteristically demonstrate killing (5, 12, 20, 21, 41) or inhibition followed by regrowth at 24 h. Whereas the reasons for this early killing or inhibition and regrowth are both unclear and likely multifactorial and each study can be criticized from a methodological standpoint, it is nevertheless important to point out that these in vitro data have been generally accepted as indicating synergistic interaction of these antibiotics. Moreover, extended periods of incubation favor not only regrowth of resistant subpopulations (mutants or phenotypic variants) with varied resistance to aminoglycosides (11, 21, 31, 32) but also antibiotic degradation (10) and alteration in medium pH (17, 18) which might decrease killing due to aminoglycosides (or β-lactams [17]).

Differences exist between enterococci and viridans group streptococci as related to β-lactam–aminoglycoside potentiation. The reason for the increased resistance to aminoglycosides in streptococci in general and enterococci in particular, however, is not understood. Relative aminoglycoside resistance in all streptococci (2), as also suggested by Bryan (4), is likely a result of the decreased electrical potential, $\Delta\psi$, present in streptococci which are aerotolerant anaerobes. Since $\Delta\psi$ is the proximate driving force for aminoglycoside uptake in gram-positive cocci, it in part regulates susceptibility to this class of antibiotics (8, 28, 32). We have shown that anaerobic incubation of *S. aureus* is associated with a diminished level of $\Delta\psi$ (29) comparable to that found in streptococci incubated aerobically (19), as well as decreased uptake and increased resistance to otherwise lethal concentrations of aminoglycosides.

Since enterococci may be more resistant to aminoglycosides than viridans group streptococci (30), other mechanisms must be considered. Unlike *S. mitis* and *S. sanguis*, enterococci and other group D streptococci are resistant to cytoplasmic membrane-active organic detergents (i.e., bile salts), suggesting the possibility of a barrier to some compounds which might include aminoglycosides. Both enterococci and *S. aureus*, unlike other gram-positive cocci, are able to grow on modified MacConkey agar (7), a selective medium for gram-negative bacilli which contains bile salts and sodium chloride. *E. coli acrA* mutants defective in outer-membrane barrier function are hypersensitive to aminoglycosides and bile salts (6) and do not grow on MacConkey agar (M. H. Miller and T. J. Dougherty, Program Abstr. Natl. Meet. AFCR, Washington, D.C., 3 to 6 May 1985). The presence of a barrier to aminoglycoside entry might, in part, explain β-lactam-induced aminoglycoside uptake in enterococci and *S. aureus* (Zenilman et al., 25th ICAAC). The relative resistance of *S. faecalis* to penicillin, on the other hand, is not related to the inability of these compounds to reach lethal target proteins but shows a better correlation with binding affinity to penicillin-binding proteins (42).

The lack of synergy in vitro does not suggest that addition of streptomycin to penicillin would not benefit selected patients or that β-lactam–aminoglycoside potentiation as

seen in standard 24-h time kill studies does not predict enhanced clinical efficacy. In vivo studies (38) and therapeutic trials (39, 43) in patients with viridans group infective endocarditis suggest the benefit of such a regimen in patients receiving abbreviated parenteral therapy. Aside from shortening the duration of therapy, however, the addition of streptomycin to penicillin has not been clearly shown to benefit patients with viridans group endocarditis, and the increased toxicity of this regimen is of concern particularly in elderly individuals. The distinction between additive and synergistic effects as suggested by our studies with viridans group streptococci and enterococci, respectively, has important therapeutic implications, however, and careful examination of the mechanism(s) of potentiation may suggest novel approaches aimed at increasing the efficacy of and decreasing untoward reactions to combination therapy.

ACKNOWLEDGMENTS

During this study, M.A.E.-S. was supported by a fellowship awarded by the Camp David Institute for International Health, Albert Einstein College of Medicine, and S.A.F. was supported by stipend 2-T32-AI07183-06 from the National Institute of Allergy and Infectious Diseases.

LITERATURE CITED

1. **Blount J. G.** 1965. Bacterial endocarditis. Am. J. Med. **38**:909–922.
2. **Bourgault A. M., W. R. Wilson, and J. A. Washington II.** 1979. Antimicrobial susceptibilities of viridans streptococci. J. Infect. Dis. **140**:316–321.
3. **Brennan, R. O., and D. T. Durack.** 1983. Therapeutic significance of penicillin tolerance in experimental streptococcal endocarditis. Antimicrob. Chemother. **23**:273–277.
4. **Bryan, L. E.** 1984. Antimicrobial drug resistance, p. 241–273. Academic Press, Inc., New York.
5. **Carey, R. B., B. D. Brause, and R. B. Roberts.** 1977. Antimicrobial therapy of vitamin B-dependent streptococcal endocarditis. Ann. Intern. Med. **87**:150–154.
6. **Coleman, W. G., Jr., and L. Leive.** 1979. Two mutations which affect the barrier function of the *Escherichia coli* K-12 outer membrane. J. Bacteriol. **139**:899–910.
7. **Difco Laboratories, Inc.** 1984. Dehydrated culture media and reagents for microbiology. Difco manual, 10th ed. Difco Laboratories, Inc., Detroit.
8. **Eisenberg, E. S., L. J. Mandel, H. R. Kaback, and M. H. Miller.** 1984. Quantitative association between electrical potential across the cytoplasmic membrane and early gentamicin uptake and killing in *Staphylococcus aureus*. J. Bacteriol. **157**:863–867.
9. **Faclam, R. R.** 1974. Streptococci, p. 96–108. *In* E. H. Lennette, E. H. Spaulding, and J. P. Truant, (ed.), Manual of clinical microbiology, 2nd ed. American Society for Microbiology, Washington, D.C.
10. **Glew, R. H., and R. A. Pavuk.** 1983. Early synergistic interaction between semisynthetic penicillins and aminoglycosidic aminocyclitols against *Enterobacteriaceae*. Antimicrob. Agents Chemother. **23**:902–906.
11. **Greenwood, D.** 1979. Laboratory methods for the evaluation of synergy, p. 13–33. *In* J. D. Williams (ed.), Antibiotic interactions—synergisms and antagonisms. The Beecham colloquium. Academic Press, Inc., New York.
12. **Herrell W. E., A. Balows, and J. Becker.** 1965. Bactericidal effect of the combination of cephalothin and streptomycin against viridans group streptococci, p. 350–354. Antimicrob. Agents. Chemother. 1964.
13. **Hess, J., J. Dankert, and D. Durack.** 1983. Significance of penicillin tolerance *in vivo*: prevention of experimental Streptococcus sanguis endocarditis. J. Antimicrob. Chemother. **11**:555–564.
14. **Hook, E. W., III, R. B. Roberts, and M. A. Sande.** 1975. Antimicrobial therapy of experimental enterococcal endocarditis. Antimicrob. Agents Chemother. **8**:564–570.
15. **Horne, D., and A. Tomasz.** 1977. Tolerant response of *Streptococcus sanguis* to beta-lactams and other cell wall inhibitors. Antimicrob. Agents Chemother. **11**:888–896.
16. **Horne, D., and A. Tomasz.** 1980. Lethal effect of a heterologous murein hydrolase on penicillin-treated *Streptococcus sanguis*. Antimicrob. Agents Chemother. **17**:235–246.
17. **Horne, D., and A. Tomasz.** 1981. pH-dependent penicillin tolerance of group B streptococci. Antimicrob. Agents Chemother. **20**:128–135.
18. **Houang, E. T., C. Hince, and A. J. Howard.** 1983. The effect of composition of culture media on MIC values of antibiotics, p. 31–48. *In* A. D. Russell and L. B. Quesnel (ed.), Antibiotics. Academic Press, Inc., New York.
19. **Kashket, E. R.** 1981. Proton motive force in growing *Streptococcus lactis* and *Staphylococcus aureus* cells under aerobic and anaerobic conditions. J. Bacteriol. **146**:369–376.
20. **Lam, K., and A. S. Bayer.** 1984. In vitro bactericidal synergy of gentamicin combined with penicillin G, vancomycin, or cefotaxime against group G streptococci. Antimicrob. Agents Chemother. **26**:260–262.
21. **Levine, J. F., B. A. Hanna, A. A. Pollock, M. S. Simberkoff, and J. J. Rahal.** 1983. Case report: penicillin sensitive nutritionally variant streptococcal endocarditis; relapse after penicillin therapy. Am. J. Med. Sci. **286**:31–36.
22. **Lowy, F. D., D. S. Chang, and P. Lash.** 1983. Synergy of combinations of vancomycin, gentamicin, and rifampin against methicillin-resistant, coagulase-negative staplylococci. Antimicrob. Agents Chemother. **23**:932–934.
23. **Lowy, F. D., E. G. Neuhaus, D. S. Chang, and N. H. Steigbigel.** 1983. Penicillin therapy of experimental endocarditis induced by tolerant *Streptococcus sanguis* and nontolerant *Streptococcus mitis*. Antimicrob. Agents Chemother. **23**:67–73.
24. **Lowy, F. D., J. A. Walsh, M. M. Mayers, R. S. Klein, and N. H. Steigbigel.** 1979. Antibiotic activity in vitro against methicillin-resistant *Staphylococcus epidermidis* and therapy of an experimental infection. Antimicrob. Agents Chemother. **16**:314–321.
25. **Lowy, F. D., M. S. Wexler, and N. H. Steigbigel.** 1982. Therapy of methicillin-resistant *Staphylococcus epidermidis* experimental endocarditis. J. Lab. Clin. Med. **100**:94–104.
26. **Mandel, G. L., D. Kaye, M. E. Levison, and E. W. Hook.** 1970. Enterococcal endocarditis: an analysis of 38 patients observed at the New York Hospital Cornell Medical Center. Arch. Intern. Med. **125**:258–264.
27. **Mandel, L. J., E. S. Eisenberg, N. J. Simkin, and M. H. Miller.** 1983. Effect of *N,N'*-dicyclohexylcarbodiimide and nigericin on *Staphylococcus aureus* susceptibility to gentamicin. Antimicrob. Agents Chemother. **24**:440–442.
28. **Mates, S. M., E. Eisenberg, L. J. Mandel, L. Patel, H. R. Kaback, and M. H. Miller.** 1982. Membrane potential and gentamicin uptake in *Staphylococcus aureus*. Proc. Natl. Acad. Sci. USA **79**:6693–6697.
29. **Mates, S. M., L. Patel, H. R. Kaback, and M. H. Miller.** 1983. Membrane potential in anaerobically growing *Staphylococcus aureus* and its relationship to gentamicin uptake. Antimicrob. Agents Chemother. **23**:526–530.
30. **Matsen, J. M., and C. R. Coghlan.** 1972. Antibiotic testing and susceptibility patterns of streptococci. p. 189–204. *In* L. W. Wannamaker and J. M. Matsen, (ed.), Streptococci and streptococcal diseases. Academic Press, Inc., New York.
31. **Mawer, S. L., and D. Greenwood.** 1978. Specific and nonspecific resistance to aminoglycosides in *Escherichia coli*. J. Clin. Pathol. **31**:12–15.
32. **Miller, M. H., S. C. Edberg, L. J. Mandel, C. F. Behar, and N. H. Steigbigel.** 1980. Gentamicin uptake in wild-type and aminoglycoside-resistant small-colony mutants of *Staphylococcus aureus*. Antimicrob. Agents Chemother. **18**:722–729.
33. **Miller, M. H., M. A. Wexler, and N. H. Steigbigel.** 1978. Single and combination antibiotic therapy of *Staphylococcus aureus* experimental endocarditis: emergence of gentamicin-resistant mutants. Antimicrob. Agents Chemother. **14**:336–343.

34. **Moellering, R. C., Jr., B. E. Murray, S. C. Schoenbaum, J. Adler, and C. Wennersten.** 1980. A novel mechanism of resistance to penicillin-gentamicin synergism in *Streptococcus faecalis*. J. Infect. Dis. **141**:81–86.
35. **Moellering, R. C., Jr., and A. N. Weinberg.** 1971. Studies on antibiotic synergism against enterococci. II. Effect of various antibiotics on the uptake of C_{14}-labelled streptomycin by enterococci. J. Clin. Invest. **50**:2580–2584.
36. **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.
37. **Plotz, P. H., and B. D. Davis.** 1962. Synergism between streptomycin and penicillin: a proposed mechanism. Science **135**:1067–1068.
38. **Sande, M. A., and R. G. Irvin.** 1974. Penicillin-aminoglycoside synergy in experimental *Streptococcus viridans* endocarditis. J. Infect. Dis. **129**:572–576.
39. **Tan, J. S., C. A. Terhune, Jr., S. Kaplan, and M. Hamburger.** 1971. Successful two-week treatment schedule for penicillin-susceptible *Streptococcus viridans* endocarditis. Lancet **ii**:1340–1343.
40. **Thauvin, C., G. M. Eliopoulos, C. Wennersten, and R. C. Moellering, Jr.** 1985. Antagonistic effect of penicillin-amikacin combinations against enterococci. Antimicrob. Agents Chemother. **28**:78–83.
41. **Watanakunakorn, C., and C. Glotzbecker.** 1977. Synergism with aminoglycosides of penicillin, ampicillin and vancomycin against non-enterococcal group D streptococci and viridans streptococci. J. Med. Microbiol. **10**:1033–1038.
42. **Williamson, R., S. B. Calderwood, R. C. Moellering, Jr., and A. Tomasz.** 1983. Studies on the mechanism of intrinsic resistance to β-lactam antibiotics in group D streptococci. J. Gen. Microbiol. **129**:813–822.
43. **Wilson, W. R., and J. E. Geraci.** 1984. Treatment of penicillin-sensitive streptococcal endocarditis, p. 101–111. *In* S. A. Merle, D. Kaye, and R. K. Root (ed.), Contemporary issues in infectious diseases. vol. 2. Churchill Livingstone, Ltd., Edinburgh.
44. **Wolf, J. C., and W. D. Johnson, Jr.** 1974. Penicillin-sensitive streptococcus endocarditis: in vitro and clinical observations on penicillin-streptomycin therapy. Ann. Intern. Med. **81**:178–181.