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Synergy of Combinations of Vancomycin, Gentamicin, and Rifampin Against Methicillin-Resistant, Coagulase-Negative Staphylococci

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The activity of combinations of vancomycin (2 or 10 μg/ml), gentamicin (0.3 μg/ml), and rifampin (0.03 μg/ml) against methicillin-resistant, coagulase-negative staphylococcal isolates was determined by the time-kill method. Combinations of rifampin with gentamicin or with vancomycin 2 μg/ml demonstrated enhanced killing against 13 of 17 and 13 of 25 strains, respectively. However, rifampin-resistant strains were selected with the latter combination in the remaining 12 of 25 studies.

Optimal antimicrobial therapy of infections caused by methicillin-resistant, coagulase-negative staphylococci remains uncertain. In vitro and in vivo studies have demonstrated that gentamicin and rifampin are among the most effective antimicrobial agents (8) and that combinations of either drug with vancomycin are often synergistic (2, 4, 5). Results of synergy studies have been less uniform when rifampin has been used in combination with other antibiotics against methicillin-resistant *Staphylococcus aureus* isolates (11, 13). Watanakunakorn and Guerriero recently reported that the combination of vancomycin with rifampin produced antagonism against 43 of 50 *S. aureus* isolates and advised against the empirical use of this regimen (11). We performed time-kill studies using combinations of vancomycin, rifampin, and gentamicin to determine whether antagonism was demonstrable with similar frequency against methicillin-resistant, coagulase-negative staphylococci.

Twenty-five clinical isolates (15 from patients with prosthetic valve endocarditis), characterized as methicillin resistant by Kirby-Bauer disk diffusion tests, were collected (strains were kindly provided by A. W. Karchmer, New England Deaconess Hospital, Boston, Mass., and G. Archer, Medical College of Virginia, Richmond). The isolates were identified to species level by the Staph-Ident System (Analytab Products, Plainview, N.Y.) as follows: *Staphylococcal epidermidis*, 20; *Staphylococcus hominis*, 4; and *Staphylococcal haemolyticus*, 1. Agar dilution minimal inhibitory concentrations (MICs) were performed with a replicator (Craft Machine Inc., Chester, Pa.) by the method of Steers et al. (9, 10). Screening for small-colony gentamicin-resistant mutants in the 24-h gentamicin-containing flasks was performed in 12 studies with Kirby-Bauer gentamicin susceptibility disks (10 μg) as previously described (5).

Time-kill studies were performed in a water bath at 37°C with an overnight suspension of the organism diluted 1/100 into Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.) and preincubated for 1 to 1.5 h to achieve log phase growth. Portions were removed for colony counts at 0, 2, 4, 6, and 24 h. Duplicate, serial 10-fold dilutions were performed in saline and added to heart infusion agar (Difco) at pH 5.5 to minimize carry-over of gentamicin activity (7). The antibiotic concentrations used were as follows: vancomycin, 2 and 10 μg/ml; gentamicin, 0.3 μg/ml; rifampin, 0.03 μg/ml. Synergy and antagonism were defined as differences of ≥ 2 $\log_{10}$ CFU/ml at 6 and 24 h for a combination of drugs as compared with either the single most or the least effective antimicrobial agent, respectively (3).

The agar dilution MICs are shown in Table 1, and a summary of the 25 time-kill studies is shown in Fig. 1. One time-kill study was performed with each strain. Even at relatively low concentrations, rifampin and gentamicin demonstrated rapid and early bactericidal activity. Rifampin-resistant mutants uniformly emerged by 24 h in all studies in which rifampin alone was used. In 2 of the 12 studies in which gentamicin-susceptible isolates were used, gentamicin-resistant variants were detected in the gentamicin-containing flasks at 24 h. The emergence of gentamicin-resistant variants did not appear to account for the slight increase in bacterial density at 24 h shown in the gentamicin curve and the gentamicin-vancomycin (2 μg/ml) curve. Com-

TABLE 1. Agar dilution susceptibility studies of 25 clinical isolates of coagulase-negative, methicillin-resistant staphylococci

Antibiotic	% MIC (μg/ml)[a]	
	50	90
Methicillin[b]	32	>200
Vancomycin	2	4
Gentamicin[c]	0.12	32
Rifampin	0.008	0.02

[a] 50 and 90, MIC inhibiting 50 and 90% of the isolates, respectively.

[b] All isolates were resistant by Kirby-Bauer disk diffusion susceptibility tests.

[c] Eight isolates were resistant by Kirby-Bauer disk diffusion susceptibility tests.

bining gentamicin with a second antimicrobial agent that was active against the eight gentamicin-resistant isolates did not alter the bactericidal rate of the effective drug (data not shown). Combining rifampin with inhibitory concentrations (2 μg/ml) of vancomycin did not prevent the emergence of rifampin resistance in 12 of 25 instances, whereas using a higher concentration (10 μg/ml) of vancomycin did prevent the emergence of rifampin resistance in all studies. Synergy was demonstrated only when rifampin was combined with gentamicin (against gentamicin-susceptible isolates), as shown in the summary curve (Fig. 1). Antagonism was not found with any of the regimens.

The frequency with which synergism was demonstrated in the individual time-kill studies is shown in Table 2. Antagonism was shown in one curve each of rifampin plus vancomycin (2 μg/ml) and rifampin plus gentamicin. Synergy or antagonism was infrequently shown at 2, 4 and 6 h. Defining synergy as a difference of 1 rather than 2 $\log_{10}$ did not significantly alter these results.

In contrast to the results reported in time-kill studies with *S. aureus* (11), antagonism was not demonstrated when the combination of vancomycin plus rifampin was used against methicil-

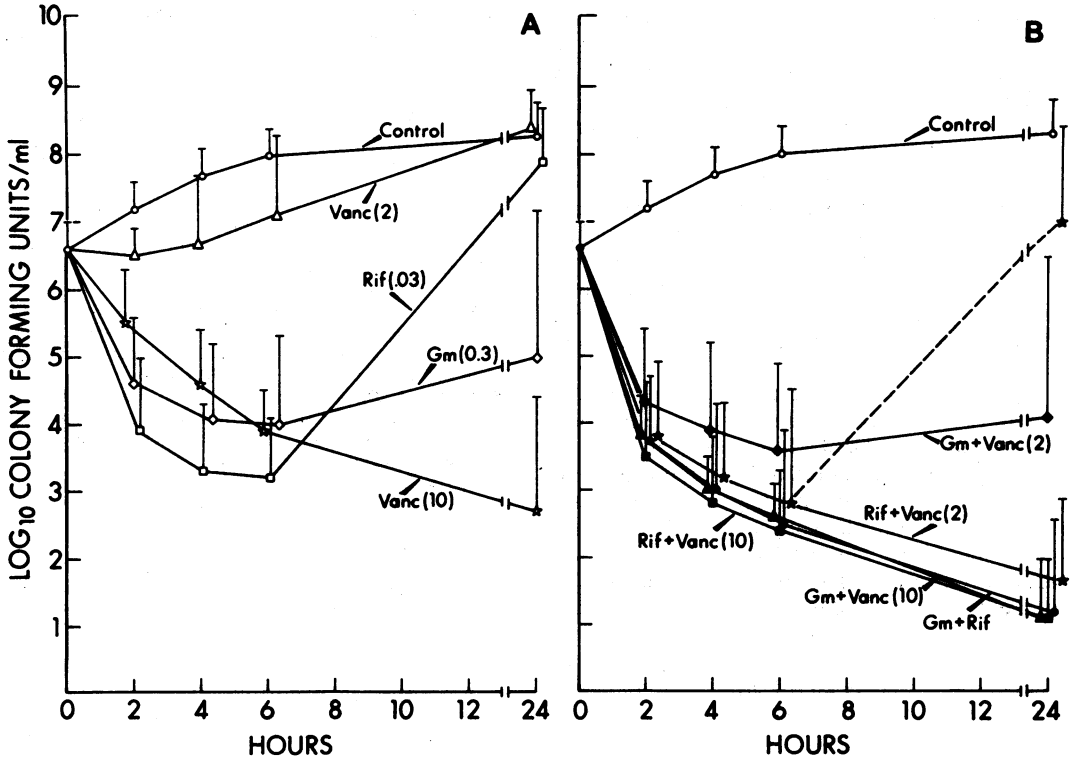

FIG. 1. Cumulative time-kill studies of 25 isolates of coagulase-negative, methicillin-resistant staphylococci. Each point represents the mean plus the standard deviation. The gentamicin curves represent the average of the 17 gentamicin-susceptible strains. Abbreviations: Gm, gentamicin; Vanc, vancomycin; Rif, rifampin. The concentrations of antibiotics used (in micrograms per milliliter) were the same when drugs were employed singly (A) or in combination (B) and are indicated in parentheses. Emergence of rifampin-resistant mutants in 12 of 25 studies in which the combination of rifampin plus vancomycin (2 μg/ml) was used is indicated in (B) by the broken line.

TABLE 2. Synergism among combinations of vancomycin, gentamicin, and rifampin against methicillin-resistant, coagulase-negative staphylococci

Antimicrobial combination and vancomycin concn (μg/ml)	No. of strains tested	No. of strains demonstrating synergism at (h)[a]:	
		6	24
Vancomycin + rifampin			
2	25	0	13
10	25	1	8
Vancomycin + gentamicin			
2	17	1	4
10	17	1	5
Rifampin + gentamicin	17	3	13

[a] Synergism was defined as a difference of ≥2 $\log_{10}$ CFU/ml between the single most effective antimicrobial agent and the combination regimen.

lin-resistant, coagulase-negative staphylococci. Although the use of two different vancomycin concentrations did not alter the frequency with which antagonism was demonstrated, it did alter the number of curves in which synergy was found. The regimen of vancomycin plus gentamicin was effective in this study, although synergy was demonstrated less often than in comparable studies with *S. aureus* (12). Synergy was most frequently demonstrated with the combination of rifampin plus gentamicin when concentrations slightly above the median MIC for these two antibiotics were used. Although this regimen has rarely been used clinically (1), it has been effective in two experimental animal models of infection (4, 5).

Inhibitory concentrations (2 μg/ml) of vancomycin failed to prevent the emergence of rifampin resistance in 12 of 25 studies or to alter the delayed outgrowth noted at 24 h in the gentamicin curve. These findings may be of particular importance in the clinical setting of *S. epidermidis* endocarditis, where relatively low vancomycin concentrations may be achieved in the area of greatest bacterial density, the valvular vegetations. Rifampin-resistant staphylococci have recently been isolated from two patients with endocarditis who relapsed after treatment with vancomycin plus rifampin (B. N. Chamovitz, R. E. Bryant, D. N. Gilbert, A. I. Hartstein, and T. T. Ward, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 22nd, Miami Beach, Fla., abstr. no. 364, 1982). Miller et al. (6) have previously found with *S. aureus* that the emergence of gentamicin-resistant variants in time-kill studies in which low concentrations of gentamicin (0.5 μg/ml) were used was associated with the emergence of similar mutants when gentamicin was used alone in therapeutic doses to treat experimental endocarditis. Similar findings have not been reported with coagulase-negative staphylococci.

Although the combination of vancomycin (2 μg/ml) with rifampin was not antagonistic in this study, it failed to prevent the emergence of rifampin resistance in 48% of the time-kill studies. The clinical importance of this observation is uncertain; however, alternative regimens including either three antibiotics or gentamicin combined with rifampin warrant additional study.

LITERATURE CITED

1. **Archer, G. L., M. J. Tenenbaum, and H. B. Haywood III.** 1978. Rifampin therapy of *Staphylococcus epidermidis*. J. Am. Med. Assoc. **240**:751–753.
2. **Ein, M. D., N. J. Smith, J. F. Arullo, M. S. Heerema, M. W. Bradshaw, and T. W. Williams, Jr.** 1979. Susceptibility and synergy studies of methicillin-resistant *Staphylococcus epidermidis*. Antimicrob. Agents Chemother. **16**:655–659.
3. **Glew, R. H., R. C. Moellering, Jr., and C. Wennersten.** 1975. Comparative synergistic activity of nafcillin, oxacillin and methicillin in combination with gentamicin against enterococci. Antimicrob. Agents Chemother. **7**:828–832.
4. **Lowy, F., 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.
5. **Lowy, F., M. A. Wexler, and N. H. Steigbigel.** 1982. Therapy of methicillin-resistant *Staphylococcus epidermidis* experimental endocarditis. J. Lab. Clin. Med. **100**:94–104.
6. **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.
7. **Sabath, L. D., J. I. Casey, P. A. Ruch, L. L. Stumpf, and M. Finland.** 1971. Rapid microassay of gentamicin, kanamycin, neomycin, streptomycin and vancomycin in serum or plasma. J. Lab. Clin. Med. **78**:457–463.
8. **Sabath, L. D., C. Garner, C. Wilcox, and M. Finland.** 1976. Susceptibility of *Staphylococcus aureus* and *Staphylococcus epidermidis* to 65 antibiotics. Antimicrob. Agents Chemother. **9**:962–969.
9. **Steers, E., E. L. Foltz, B. S. Graves, and J. Riden.** 1959. An inocula replicating apparatus for routine testing of bacterial susceptibility to antibiotics. Antibiotic Chemother. (Basel) **9**:307–311.
10. **Washington, J. A., II, and V. L. Sutter.** 1980. Dilution susceptibility test: agar and macrobroth dilution procedures, p. 453–458. *In* E. H. Lennette, A. Balows, W. Hausler, Jr., and J. P. Truant (ed.), Manual of clinical microbiology. American Society for Microbiology, Washington, D.C.
11. **Watanakunakorn, C., and J. C. Guerriero.** 1981. Interaction between vancomycin and rifampin against *Staphylococcus aureus*. Antimicrob. Agents Chemother. **19**:1089–1091.
12. **Watanakunakorn, C., and J. C. Tisone.** 1982. Synergism between vancomycin and gentamicin or tobramycin for methicillin-susceptible and methicillin-resistant *Staphylococcus aureus* strains. Antimicrob. Agents Chemother. **22**:903–905.
13. **Zinner, S. H., H. Lagast, and J. Klastersky.** 1981. Anti-staphylococcal activity of rifampin with other antibiotics. J. Infect. Dis. **144**:365–371.