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Synergism Between Vancomycin and Gentamicin or Tobramycin for Methicillin-Susceptible and Methicillin-Resistant *Staphylococcus aureus* Strains

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By the time-kill curve method, the combinations of vancomycin-gentamicin and vancomycin-tobramycin were shown to be synergistic against a majority of methicillin-susceptible and -resistant strains of *Staphylococcus aureus*.

Vancomycin is an excellent antibiotic in the treatment of both methicillin-susceptible and -resistant *Staphylococcus aureus* infections (8a). However, there have been occasions on which the response to vancomycin therapy is not satisfactory (2–4). Thus, there appears to be a need to find antibiotics that will enhance the activity of vancomycin. The combination of vancomycin and rifampin has been shown to be partially synergistic, indifferent, or antagonistic in vitro (6, 10, 11). Clinically, the addition of rifampin to vancomycin has not consistently shown beneficial effects (3–5). There have been no studies on the interaction between vancomycin and gentamicin or tobramycin against methicillin-resistant *S. aureus* strains (8, 8a), although gentamicin and tobramycin have been shown to enhance the in vitro activity of vancomycin against methicillin-susceptible *S. aureus* strains by the checkerboard method (9). In this investigation, we studied the interaction between vancomycin and gentamicin or tobramycin against both methicillin-susceptible and -resistant *S. aureus* strains by the time-kill curve method.

Ten methicillin-susceptible and 17 methicillin-resistant *S. aureus* strains were used in this study. Vancomycin and tobramycin were obtained from Eli Lilly & Co., gentamicin was obtained from Schering Corp., methicillin was obtained from Bristol Laboratories, and nafcillin was obtained from Wyeth Laboratories. A standard stock solution of each antibiotic was prepared according to the manufacturer's instructions, stored at −80°C, and thawed immediately before use.

The minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of each antibiotic were determined by the World Health Organization International Collaborative Study broth dilution method (1). Serial twofold dilutions of the antibiotic were made in Mueller-Hinton broth from 25 to 0.0125 μg/ml. The inoculum was 1 ml of 10^5 to 10^6 organisms. The MIC was defined as the lowest concentration of antibiotic that allowed no visible growth after incubation at 37°C for 18 to 24 h. The MBC was defined as the lowest concentration of antibiotic that allowed no growth (or one colony) from a 0.01-ml subculture from each clear tube on agar plates after incubation at 37°C for 18 to 24 h.

The standard time-kill curve method was used to study the interaction between vancomycin and gentamicin or tobramycin. Mueller-Hinton broth was used. The antibiotic concentrations were as follows (in micrograms per milliliter): vancomycin, 10; gentamicin, 1; tobramycin, 1; a combination of vancomycin, 10, with gentamicin, 1; and a combination of vancomycin, 10,

TABLE 1. MICs and MBCs for 10 methicillin-susceptible and 17 methicillin-resistant *S. aureus* strains

Antibiotic	Methicillin-susceptible *S. aureus*				Methicillin-resistant *S. aureus*			
	MIC (μg/ml)		MBC (μg/ml)		MIC (μg/ml)		MBC (μg/ml)	
	Median	Range	Median	Range	Median	Range	Median	Range
Methicillin	3.12	1.56–3.12	25.0	6.25–100	≥25		≥25	
Nafcillin	0.78	0.39–1.56	6.25	1.56–25	≥25		≥25	
Vancomycin	1.56	0.78–3.12	>25	25–>25	1.56	0.78–3.12	25.0	1.56–>25
Gentamicin	3.12	0.78–>25	12.5	6.25–>25	3.12	1.56–>25	25	6.25–>25
Tobramycin	3.12	1.56–>25	12.5	3.12–>25	>25	6.25–>25	>25	6.25–>25

TABLE 2. Synergism between vancomycin and gentamicin or tobramycin for 10 methicillin-susceptible and 17 methicillin-resistant *S. aureus* strains

Antibiotic Regimen	*S. aureus* strain	No. of strains demonstrating synergism at (h)[a]:			
		6	24	48	6, 24, or 48
Vancomycin + gentamicin	Total	10 (9)	11 (8)	13 (9)	17 (13)
	Susceptible	6 (5)	6 (5)	6 (5)	7 (6)
	Resistant	4 (4)	5 (3)	7 (4)	10 (7)
Vancomycin + tobramycin	Total	4 (3)	9 (7)	17 (12)	19 (13)
	Susceptible	2 (2)	5 (5)	9 (7)	9 (7)
	Resistant	2 (1)	4 (2)	8 (5)	10 (6)

[a] The number of strains demonstrating synergy when synergism is defined as at least 2 × $\log_{10}$ reduction in colony counts is shown in parentheses.

with tobramycin, 1. A broth culture with no antibiotic was set up as a control. The inoculum was between 10^5 and 10^6 organisms per ml, made from an 18- to 24-h culture. All tubes were incubated in a Dry Bath (Fisher Scientific Co.) at 37°C. At 0, 6, 24, and 48 h, the viable numbers of organisms were enumerated by serial 10-fold dilutions plated on Mueller-Hinton agar.

When the result of the combination was at least $\log_{10}$ less than that of both drugs alone at a given time, it was defined as synergism. When the result of the combination was at least $\log_{10}$ more than that of either drug alone, it was defined as antagonism.

The MICs and MBCs for 10 methicillin-susceptible and 17 methicillin-resistant *S. aureus* strains are shown in Table 1. Table 2 shows the results of the time-kill curve studies. It also shows results obtained when a more rigid definition of synergism (>2 × $\log_{10}$ reduction in colony counts) was used. Synergism between vancomycin and gentamicin and between vancomycin and tobramycin was demonstrated for the majority of the strains. The synergistic effect was more pronounced at 24 and 48 h (Fig. 1). There appeared to be no difference between the methicillin-susceptible and methicillin-resistant strains. Antagonism between vancomycin and gentamicin was demonstrated against one methicillin-susceptible strain at 24 h only. Vancomycin and tobramycin showed antagonism against three methicillin-resistant strains at 48 h only.

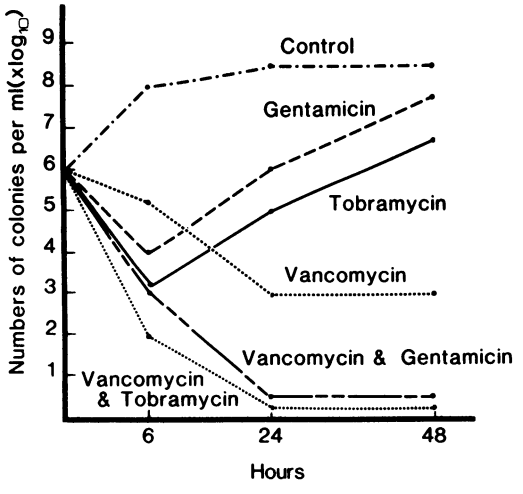

FIG. 1. Time-kill curves of a strain of *S. aureus* showing synergism between vancomycin and gentamicin and between vancomycin and tobramycin.

The results of this study confirmed the findings of enhanced activity of vancomycin-gentamicin and vancomycin-tobramycin against methicillin-susceptible *S. aureus* by the checkerboard method reported previously (9). These findings extended to methicillin-resistant strains as well. Many of the *S. aureus* strains used in this study were tolerant to vancomycin (7, 10). The synergistic effect appeared to be more pronounced with the tolerant strains. There also appeared to be no difference between strains that were susceptible and resistant to gentamicin and tobramycin.

Vancomycin alone appears to be an excellent antibiotic in the therapy of serious *S. aureus* infections in patients who are allergic to penicillin and in whom the infecting *S. aureus* strain is resistant to methicillin (8a). In the unusual circumstance that the response to vancomycin alone is not satisfactory, the addition of gentamicin or tobramycin should be considered. Careful monitoring of serum bactericidal titers before and after the addition of the aminoglycoside should be done. In vitro time-kill curve studies of antibiotic combinations should be performed if it is feasible.

LITERATURE CITED

1. **Ericsson, H. M., and J. C. Sherris.** 1971. Antibiotic sensitivity testing: report of an international collaborative study. Acta Pathol. Microbiol. Scand. Sect. B Suppl. 217:1–90.
2. **Gopal, V., A. L. Bisno, and F. J. Silverblatt.** 1976. Failure

of vancomycin treatment of *Staphylococcus aureus* endocarditis. J. Am. Med. Assoc. **236**:1604–1606.
3. **Faville, R. J., D. E. Zaske, E. L. Kaplan, K. Crossley, L. D. Sabath, and P. G. Quie.** 1978. *Staphylococcus aureus* endocarditis. Combined therapy with vancomycin and rifampin. J. Am. Med. Assoc. **240**:1963–1965.
4. **Massanari, R. M., and S. T. Donta.** 1978. The efficacy of rifampin as adjunctive therapy in selected cases of staphylococcal endocarditis. Chest **73**:371–375.
5. **Tofte, R. W., J. Solliday, J. Rotschafer, and K. B. Crossley.** *Staphylococcus aureus* infection of dialysis shunt: absence of synergy with vancomycin and rifampin. South. Med. J. **74**:612–615.
6. **Tuazon, C. U., M. Y. C. Lin, and J. N. Sheagren.** 1978. In vitro activity of rifampin alone and in combination with nafcillin and vancomycin against pathogenic strains of *Staphylococcus aureus*. Antimicrob. Agents Chemother. **13**:759–761.
7. **Watanakunakorn, C.** 1978. Antibiotic tolerant *Staphylococcus aureus*. J. Antimicrob. Chemother. **4**:561–568.
8. **Watanakunakorn, C.** 1981. The antibacterial action of vancomycin. Rev. Infect. Dis. **3**:S210–S215.
8a. **Watanakunakorn, C.** 1982. Treatment of infections due to methicillin-resistant *Staphylococcus aureus*. Ann. Intern. Med. **97**:376–378.
9. **Watanakunakorn, C., and C. Glotzbecker.** 1974. Enhancement of the effects of anti-staphylococcal antibiotics by aminoglycosides. Antimicrob. Agents Chemother. **6**:802–806.
10. **Watanakunakorn, C., and J. C. Guerriero.** 1981. Interaction between vancomycin and rifampin against *Staphylococcus aureus*. Antimicrob. Agents Chemother. **19**:1089–1091.
11. **Zinner, S. H., H. Lagast, and J. Klastersky.** 1981. Antistaphylococcal activity of rifampin with other antibiotics. J. Infect. Dis. **144**:365–371.