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Interaction Between Vancomycin and Rifampin Against *Staphylococcus aureus*

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By the time-kill method, vancomycin and rifampin were antagonistic against 43 of 50 strains of *Staphylococcus aureus*, whether susceptible or resistant to methicillin.

Vancomycin is an excellent anti-staphylococcal antibiotic and has been used successfully in the treatment of *Staphylococcus aureus* endocarditis (8). However, there have been cases of staphylococcal endocarditis not responsive to vancomycin therapy that have been cured with the combination of vancomycin and rifampin (2, 3, 5). In a recent study of 20 strains of *S. aureus*, the combination of vancomycin and rifampin was found to be synergistic for 5 strains and antagonistic for 1 strain by the checkerboard method (7).

We studied the interaction between vancomycin and rifampin against both methicillin-susceptible and methicillin-resistant *S. aureus* strains by the time-kill method.

(The results of this study were presented at the 20th Interscience Conference on Antimicrobial Agents and Chemotherapy, 22 to 24 September 1980, New Orleans, La.)

A total of 50 strains of *S. aureus* were studied: 30 methicillin-susceptible strains isolated from blood cultures of patients with endocarditis and 20 methicillin-resistant strains (minimal inhibitory concentration [MIC], $\geq$12.5 μg/ml). Vancomycin hydrochloride was obtained from Eli Lilly & Company, and rifampin was obtained from Dow Chemical Company. 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 MIC and minimal bactericidal concentration of each antibiotic were determined by the World Health Organization-International Collaborative Study broth dilution method with Mueller-Hinton broth (1). Serial twofold dilutions were made from 8 to 0.007 μg/ml. The inoculum was 1 ml of a 10^{-3} dilution of an 18-h culture. The MIC was the lowest concentration of antibiotic that allowed no visible growth after incubation at 37°C for 18 to 24 h. The minimal bactericidal concentration was the lowest concentration of antibiotic that allowed no growth (or $\leq$5 colonies) 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 rifampin. Mueller-Hinton broth was used. The antibiotic concentrations were as follows: vancomycin, 10 μg/ml; rifampin, 1 μg/ml; and the combination of vancomycin and rifampin, 10 and 1 μg/ml, respectively. The inoculum was between 10^5 and 10^6 organisms per ml, diluted from an 18- to 24-h culture. All tubes were incubated in a Dry Bath (Fisher Scientific) 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 from either drug alone at a given time, it was defined as synergism. When the result of the combination was at least $\log_{10}$ more than that from either drug alone, it was defined as antagonism.

Table 1 lists the MICs and minimal bactericidal concentrations of vancomycin and rifampin. All strains of *S. aureus* were susceptible to vancomycin. In the test for the MICs of rifampin, 14 of the 50 strains, regardless of their susceptibility to methicillin, exhibited the "skip-tube" phenomenon. The highest MIC of rifampin for strains without the skip-tube phenomenon was 0.5 μg/ml. Although the MICs of rifampin for the majority of strains were below 1 μg/ml, the minimal bactericidal concentrations were much higher.

Synergism was demonstrated for only 1 strain, and antagonism was demonstrated for 43 strains. Neither synergism nor antagonism was demonstrated for the remainder of the strains. The antagonism was mainly manifested as the interference of rifampin with vancomycin activity at 24 and 48 h. On the other hand, vancomycin

probably prevented the regrowth of *S. aureus* at 24 and 48 h in the presence of rifampin. Figure 1 shows examples of synergism, antagonism, and neither synergism nor antagonism.

In this study, all strains of *S. aureus*, whether methicillin susceptible or methicillin resistant, were susceptible to vancomycin. Most strains were also susceptible to rifampin. However, there were 14 strains which exhibited the skip-tube phenomenon. This phenomenon has been attributed to rifampin-resistant mutants in the inoculum (6).

The apparent antagonism between vancomycin and rifampin against *S. aureus* is an unexpected finding. The previous finding of synergism between vancomycin and rifampin by the checkerboard method (7) may not be true synergism but merely suppression of rifampin resistance by vancomycin. Patients who improved after rifampin was added to the vancomycin regimen (2, 3, 5) probably did well largely because of the excellent entry of rifampin into phagocytic leukocytes (4). When the combination of vancomycin and rifampin is used, vancomycin probably suppresses the resurgence of rifampin-resistant mutants.

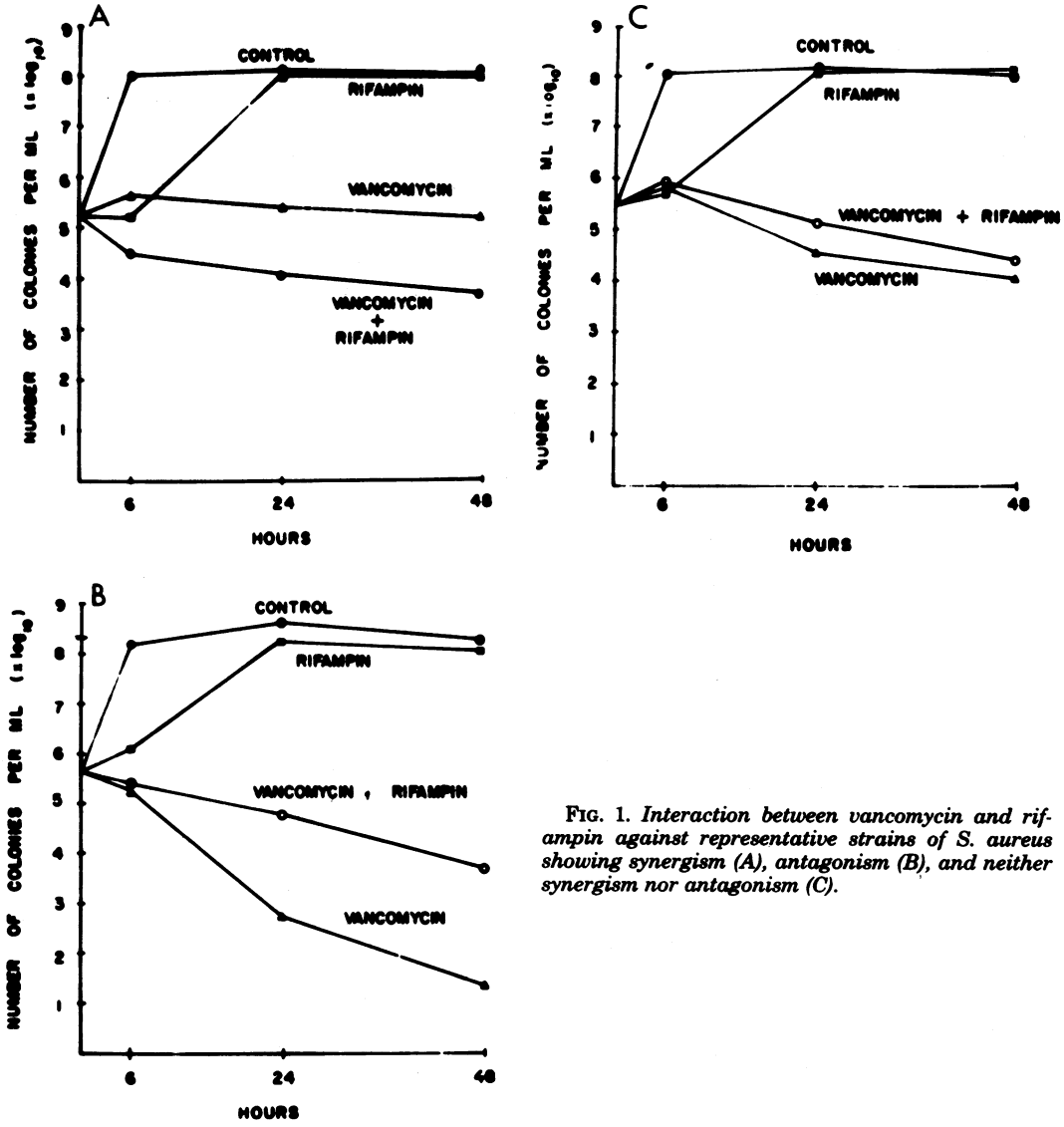

FIG. 1. *Interaction between vancomycin and rifampin against representative strains of S. aureus showing synergism (A), antagonism (B), and neither synergism nor antagonism (C).*

TABLE 1. *In vitro susceptibility of 50 strains of S. aureus*

Drug concn (μg/ml)	No. of strains for which			
	Vancomycin was inhibitory[b]	Vancomycin was bactericidal[c]	Rifampin[a] was inhibitory[b]	Rifampin[a] was bactericidal[c]
≤0.007			5	
0.015			12	
0.03			3	
0.06			6	
0.125			6	
0.25			4	
0.5			3	1
1.0	6		2	2
2.0	40	2		2
4.0	4	5	1	13
8.0		8	3	7
>8.0		35	5	25

[a] For the 14 strains that exhibited the skip-tube phenomenon, the highest MICs were used in the tabulation.

[b] Number of strains for which the given drug concentration was the MIC.

[c] Number of strains for which the given drug concentration was the minimal bactericidal concentration.

Animal and prospective clinical studies are indicated to further elucidate the in vivo interaction and clinical efficacy of the use of the combination of vancomycin and rifampin in the therapy of serious *S. aureus* infection. At the present time, regardless of anecdotal experiences, the use of the combination of vancomycin and rifampin cannot be accepted empirically as superior to the use of vancomycin alone in the therapy of serious *S. aureus* infection.

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