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Antagonism Between Nafcillin or Oxacillin and Rifampin Against *Staphylococcus aureus*

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By the time-kill method, the combinations of nafcillin plus rifampin and oxacillin plus rifampin were antagonistic against 20 strains of *Staphylococcus aureus*. No synergism was demonstrated.

In 1975, Sande and Johnson (5) reported the in vitro antagonism of penicillin and rifampin against a strain of *Staphylococcus aureus*; they also demonstrated that in experimental endocarditis in rabbits, the combination of penicillin and rifampin was less effective than penicillin alone was in eradicating *S. aureus* from the vegetations. In this investigation, we studied the interaction between nafcillin or oxacillin and rifampin against 20 strains of *S. aureus* by the time-kill method.

The *S. aureus* strains were all isolated from blood cultures of patients with septicemia and endocarditis. Nafcillin was obtained from Wyeth Laboratories, oxacillin from Bristol Laboratories, and rifampin 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 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 diluted from an 18-h culture. The MIC was the lowest concentration of an antibiotic that allowed no visible growth after incubation at 37°C for 18 to 24 h. The MBC was the lowest concentration of an 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 nafcillin, oxacillin, and rifampin. Mueller-Hinton broth was used. The antibiotic concentrations were as follows: nafcillin, 10 μg/ml; oxacillin, 10 μg/ml; rifampin, 0.1, 0.5, or 1.0 μg/ml; and combinations of nafcillin and oxacillin with each concentration of rifampin. 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) 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 a combination of drugs was at least $\log_{10}$ less than that from 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 from either drug alone, it was defined as antagonism.

The MIC of nafcillin for the 20 strains of *S. aureus* was between 0.2 and 0.8 μg/ml (median, 0.4 μg/ml), and the MBC was between 0.8 and >25 μg/ml (median, 6.25 μg/ml). The MIC of oxacillin was between 0.4 and 0.8 μg/ml (median, 0.4 μg/ml), and the MBC was between 0.4 and >25 μg/ml (median, 6.25 μg/ml). The MIC of rifampin was between ≤0.0125 and 12.5 μg/ml (median, 0.025 μg/ml), and the MBC was between 0.1 and 12.5 μg/ml (median, 0.8 μg/ml). Several strains exhibited the "skip tubes" phenomenon when tested against rifampin, resulting in the high MIC values.

There was no synergism demonstrated for any antibiotic combination or strain. Strains for which antagonism was shown for different antibiotic combinations at different time intervals are listed in Table 1. The antagonism was mainly manifested as interference of rifampin with the activity of nafcillin or oxacillin at 24 and 48 h (Fig. 1). On the other hand, nafcillin and oxacillin probably prevented the regrowth of *S. aureus* at 24 and 48 h in the presence of rifampin.

The results of this study, which used a large number of strains, confirm the findings of antagonism between a penicillin and rifampin reported by Sande and Johnson (5). We have previously reported the antagonism between vancomycin and rifampin against both methicillin-susceptible

TABLE 1. Antagonism between nafcillin or oxacillin and rifampin against 20 strains of *S. aureus*

Antibiotic regimen and concns (μg/ml)	Antagonism shown (no. of strains)[a] at:			
	6 h	24 h	48 h	6, 24, or 48 h
Nafcillin (10) + rifampin (0.1)	2 (0)	19 (9)	13 (2)	20 (9)
Nafcillin (10) + rifampin (0.5)	1 (0)	18 (5)	13 (2)	20 (7)
Nafcillin (10) + rifampin (1.0)	1 (0)	18 (6)	12 (2)	18 (7)
Oxacillin (10) + rifampin (0.1)	1 (0)	14 (6)	13 (2)	16 (6)
Oxacillin (10) + rifampin (0.5)	2 (0)	17 (7)	15 (5)	17 (8)
Oxacillin (10) + rifampin (1.0)	1 (0)	14 (6)	14 (4)	18 (6)

[a] The number of strains against which antagonism was shown when antagonism was defined as at least a 2 × $\log_{10}$ increase in colony count is given in parentheses.

and methicillin-resistant strains of *S. aureus* (8). Zinner and his colleagues (9) recently reported antagonism between methicillin, oxacillin, or vancomycin and rifampin against *S. aureus* both in vitro and in volunteers given oxacillin and rifampin alone and in combination. However, they also showed that by using very low concentrations of methicillin or oxacillin, there may be enhanced activity with rifampin. In our study, varying the concentrations of rifampin did not make any difference in the results.

Many strains of *S. aureus* showed the skip tubes phenomenon, which has been attributed to rifampin-resistant mutants in the inoculum (4). When rifampin was used alone against *S. aureus*, regrowth of the resistant mutants was usually seen at 24 or 48 h. In the presence of nafcillin or oxacillin, such regrowth did not occur. This was also demonstrated with vancomycin in a previous study (8). In an experimental intraperitoneal infection in mice, Mandell and Moorman (2) showed that methicillin prevents the in vivo development of rifampin resistance when used in combination with rifampin.

Animal and prospective clinical studies are indicated for further elucidation of the in vivo interaction and clinical efficacy of the combination of nafcillin or oxacillin with rifampin in the therapy of serious *S. aureus* infection. Mandell and Vest (3) have shown the excellent entry of rifampin into phagocytic leukocytes. It is conceivable that this unique property may negate the effect of the in vitro antagonism of rifampin with another anti-staphylococcal antibiotic. At present, regardless of anecdotal experience, the use of the combination of nafcillin or oxacillin with rifampin cannot be accepted empirically as superior to the use of nafcillin or oxacillin alone in the therapy of serious *S. aureus* infection. In the unusual circumstances when the clinical response to nafcillin or oxacillin is suboptimal, the addition of an aminoglycoside may be considered. In vitro synergism between nafcillin or oxacillin and an aminoglycoside has been previously demonstrated (6, 7).

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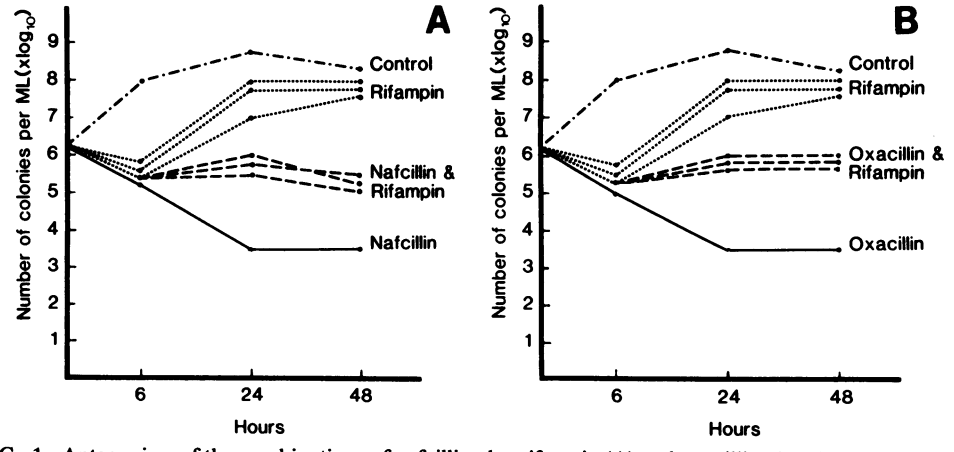

FIG. 1. Antagonism of the combinations of nafcillin plus rifampin (A) and oxacillin plus rifampin (B) against a strain of *S. aureus*. Nafcillin and oxacillin were used at 10 μg/ml. Three concentrations of rifampin were used: 0.1, 0.5, and 1.0 μg/ml.

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