Interaction between Rifampin and Fusidic Acid against Methicillin-Resistant Coagulase-Positive and -Negative Staphylococci

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We studied the interaction between rifampin and fusidic acid against a group of staphylococci. Of the 20 coagulase-positive strains studied, checkerboard studies revealed synergy in 3 and indifference in 17; time-kill studies revealed synergy in 18 of 19 coagulase-positive strains at both 24 and 48 h. Of the 19 coagulase-negative strains, checkerboard studies revealed synergy in 6 and indifference in 13; time-kill studies revealed synergy in 6 of 18 coagulase-negative strains at 24 h and in 17 of 18 coagulase-negative strains at 48 h. The combination of rifampin and fusidic acid warrants further evaluation in the therapy of staphylococcal disease.

Methicillin resistance among Staphylococcus aureus and S. epidermidis has become endemic in many areas of the United States and Europe (4). Vancomycin remains the primary drug used to treat these infections, but its use involves several drawbacks. It must be given intravenously, and it is not tolerated by some patients (2).

Rifampin and fusidic acid are both highly active against staphylococci, but resistance develops rapidly when either agent is used alone (7). For this reason, we sought to study the interaction between rifampin and fusidic acid against a group of coagulase-positive and -negative staphylococci.

A total of 20 strains of methicillin-resistant S. aureus were used in these studies. The strains were collected from five different locations. Of the 20 strains, 2 came from the Clinical Bacteriology Laboratory of the Massachusetts General Hospital, Boston; 3 came from the University of Virginia Hospital, Charlottesville (kindly provided by R. Wenzel); 1 came from the New England Deaconess Hospital, Boston; 13 came from the Veterans Administration Medical Center, Pittsburgh, Pa.; and 1 came from the Presbyterian University Hospital, Pittsburgh, Pa. Methicillin resistance was documented by the presence of visible growth after 10⁵ CFU were inoculated onto a Mueller-Hinton agar plate containing 15 μg of methicillin per ml and incubated at 35°C for 48 h.

Of 19 strains of coagulase-negative staphylococci used, 11 were isolated from patients with prosthetic valve endocarditis. These organisms have been described in detail in a previous report (4). Organisms were stored at -70°C and then were plated onto sheep blood agar before being used. Five strains were isolated from patients with positive blood cultures at the Veterans Administration Medical Center, and three strains were isolated from patients with positive blood cultures at the Presbyterian University Hospital. These strains were also shown to be methicillin resistant in the manner described above.

Antibiotics used included the following: rifampin (Sigma Chemical Company, St. Louis, Mo.), fusidic acid (Leo Pharmaceuticals, Copenhagen, Denmark), and methicillin (Beecham Laboratories, Bristol, Tenn.).

MICs were determined, and checkerboard studies were performed by a microtiter assay in unsupplemented Mueller-Hinton broth. The method of Krogstad and Moellering was used (5). The final volume of each well was 100 μl. The inoculum was prepared by serial dilution of an overnight culture into Mueller-Hinton broth and was checked by serial dilution in saline, with subsequent plating of 25 μl onto Mueller-Hinton agar. Microtiter and agar plates were incubated for 18 h at 37°C. The final inhibitory concentration (FIC) was calculated with a standard formula (5). Synergy was defined as an FIC < 0.5, and antagonism was defined as an FIC > 2.

Antibiotic combinations were tested in Mueller-Hinton broth by a time-kill method previously described (6). Synergism was defined as a decrease of ≥2 log₁₀ CFU/ml produced by the combination compared with either agent alone or both agents in combination. Antagonism was defined as an increase of 2 log₁₀ CFU/ml, the combination being compared with each agent alone. Samples were removed for colony counts at 4, 24, and 48 h, and, after serial dilution in saline, were plated onto Mueller-Hinton agar. They were incubated for 24 h at 35°C and inspected for growth. The combination concentrations were as follows: fusidic acid, 0.5 μg/ml; and rifampin, 5 μg/ml.

All strains of S. aureus were susceptible to rifampin and fusidic acid. The MICs of rifampin for 50 and 90% of the strains tested were 0.004 and 0.006 μg/ml, respectively. The range was 0.007 to 0.017 μg/ml. The MICs of fusidic acid for

| TABLE 1. Results of checkerboard and time-kill studies of antimicrobial agents against staphylococci |
|----------------------------------------|-----------------|-----------------|
| Reaction | No. of coagulase-positive strains killed in the following study | No. of coagulase-negative strains killed in the following study |
| | Checkerboard | Time-kill | Checkerboard | Time-kill |
| | 24 h | 48 h | 24 h | 48 h |
| Synergy | 3 | 18 | 18 | 6 | 6 | 17 |
| Indifference | 17 | 1 | 1* | 13 | 12 | 1 |
| Antagonism | 0 | 0 | 0 | 0 | 0 | 

* Completely killed by both the combination and by fusidic acid alone.
50 and 90% of the strains tested were 0.097 and 0.78 μg/ml, respectively. The range was 0.0007 to 0.78 μg/ml.

Of the 20 strains of S. aureus studied by the checkerboard technique, 3 demonstrated synergy and 17 demonstrated indifference. Antagonism was not noted in any of the isolates. Time-kill studies revealed synergy in 18 of the 19 strains tested at both 24 and 48 h (Table 1). The one strain that did not demonstrate synergy was completely killed by both the combination and the fusidic acid alone. Rifampin and fusidic acid usually produced inhibition at 4 h, but regrowth of resistant organisms was evident at 24 or 48 h in all strains. In no strain did resistance occur when the combination was used. A cumulative (mean) time-kill curve for the studies is shown in Fig. 1.

Of the 19 coagulase-negative strains studied by the checkerboard technique, 6 demonstrated synergy and 13 demonstrated indifference to the combination. Of the 18 strains studied by time-kill curves, 17 demonstrated synergy at 48 h, but only 6 of the 18 demonstrated synergy at 24 h. Antagonism was not seen in any of the strains. Rifampin- and fusidic acid-resistant strains did not emerge in any of the experiments. A mean time-kill curve for the studies is shown in Fig. 1.

Fusidic acid and rifampin are both attractive antistaphylococcal antibiotics. They are relatively nontoxic, can be administered orally, and are highly active against both coagulase-positive and -negative staphylococci.

Our in vitro studies suggest that the combination of rifampin and fusidic acid may be effective in the therapy of S. aureus infections. The time-kill studies demonstrated that these two are synergistically active against the vast majority of isolates. In addition, resistant strains did not emerge upon exposure to these antimicrobial agents, even after 48 h. Foldes and co-workers (3) recently reported that this combination elicited indifferent responses in 37 strains of S. aureus examined by the checkerboard technique. However, their results are difficult to compare with those presented in this report. The results of their checkerboard studies were pooled, and individual FICs were not calculated (3). In addition, time-kill studies were not done. Dixson et al. (1) recently studied this combination by using a modified checkerboard technique. Although no specific results were reported, these investigators stated that most FIC values fell within the additive range.

The combination of fusidic acid and rifampin against coagulase-negative strains produced somewhat different results. The checkerboard and 24-h time-kill curves suggested synergy in 6 of 19 and 6 of 18 strains, respectively. However, by 48 h synergy was seen in 17 of 18 strains examined by the time-kill method. In addition, the magnitude of the effect was much greater against coagulase-positive strains.

The results of these studies suggest that the combination of fusidic acid and rifampin warrants further evaluation in the therapy of both coagulase-negative and -positive staphylococcal infections.

LITERATURE CITED


