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Serum Bactericidal Activity of Rifampin in Combination with Other Antimicrobial Agents against *Staphylococcus aureus*

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Because rifampin-resistant strains of *Staphylococcus aureus* emerge during monotherapy with this drug, a search was made for potentially useful companion drugs. Bactericidal titers of spiked serum were determined, and time kill studies were performed for 10 strains of methicillin-susceptible *S. aureus*. We tested rifampin in combination with nafcillin, vancomycin, clindamycin, pefloxacin, ciprofloxacin, trimethoprim, teicoplanin, or erythromycin. The bactericidal activity of nafcillin, vancomycin, and teicoplanin was significantly reduced ($P < 0.05$) when rifampin was added to the drug regimen. In contrast, the addition of rifampin to clindamycin or erythromycin significantly increased bactericidal activity as measured by both bactericidal titers in serum and 6-h killing rates ($P < 0.02$). Bactericidal activity in serum was also increased by the addition of rifampin to trimethoprim, but rifampin-resistant strains emerged with this combination.

Rifampin is highly active against *Staphylococcus aureus* in vitro. Its pharmacologic properties are such that the drug penetrates well into tissues and leukocytes, and it can sterilize abscesses (9, 18). Its clinical use has been limited by the fact that resistant strains emerge when rifampin is used as a single agent (8). The practice has been to use this drug in combination with another antistaphylococcal drug such as nafcillin or vancomycin. Such combinations, when tested in vitro, have shown synergistic, indifferent, or antagonistic activity (1, 15, 18), although there is no indication that in vitro antagonism predicts corresponding in vivo results (17).

For endocarditis and osteomyelitis, the standard of practice is to perform a serum bactericidal test (SBT), usually from a blood sample obtained 30 to 60 min after antibiotic administration, to document the degree of bactericidal activity in the patient's serum. The recommended titer has traditionally been ≥1:8 (12), but a more recent study suggested that titers of ≥1:64 may be preferable (19). We have consistently found that the addition of rifampin to a regimen of either a beta-lactam (nafcillin) or vancomycin often results in loss of bactericidal activity as measured by SBT. To study this further, we simulated SBTs by using pooled human serum containing known concentrations of rifampin in combination with a wide variety of antimicrobial agents. SBTs for rifampin alone or in combination with nafcillin, vancomycin, clindamycin, ciprofloxacin, pefloxacin, teicoplanin, trimethoprim, or erythromycin were measured against 10 strains of methicillin-susceptible *S. aureus*. We also compared the bactericidal activity of the combination versus that of each drug alone by the time kill method in broth.

MATERIALS AND METHODS

Bacterial strains. Ten methicillin-susceptible strains of *S. aureus*, all blood culture isolates from San Francisco General Hospital, were studied. To avoid repeated subculture, each strain was kept on beads and stored at −70°C.

Antimicrobial agents. The antimicrobial agents used were: nafcillin sodium (Wyeth Laboratories, Philadelphia, Pa.); vancomycin hydrochloride (Eli Lilly & Co., Indianapolis, Ind.); rifampin (Dow Chemical Co., Midland, Mich.); clindamycin (The Upjohn Co., Kalamazoo, Mich.); ciprofloxacin (Miles Laboratories, Inc., Elkhart, Ind.); pefloxacin mesylate (Rhone Polounc, Paris, France), teicoplanin (Gruppo Lepetit, Milan, Italy); trimethoprim (Burroughs Wellcome Co., Research Triangle Park, N.C.); and erythromycin (Lilly). Stock solutions were prepared per manufacturer instructions and were either stored at −70°C until the day of use or prepared fresh on that day.

Susceptibilities. MICs and MBCs were determined by macrotube dilution in Mueller-Hinton broth with an inoculum of 3×10^5 log-phase organisms per ml (2). The MIC was defined as the lowest concentration that allowed no visible growth after 18 to 24 h of incubation at 35°C. The MBC was the lowest concentration at which there was a ≥99.9% reduction in organisms as determined by quantitative subculture of 0.01 ml from each tube onto blood agar plates.

SBTs. The SBT was simulated by spiking samples of pooled human serum with each of the drugs at concentrations approximating those found in human serum 1 h after drug administration. Samples of the undiluted serum contained 6 μg of rifampin per ml alone or in combination with 30 μg of vancomycin, 40 μg of nafcillin, 10 μg of clindamycin, or 5 μg of ciprofloxacin, pefloxacin, teicoplanin, trimethoprim, or erythromycin per ml. The samples were diluted in Mueller-Hinton broth, and an inoculum of 3×10^5 log-phase organisms per ml was added to each tube (13). After incubating the tubes at 35°C for 18 to 24 h, we determined the bactericidal titer by streaking 0.01 ml from each tube showing no visible growth onto blood agar plates and incubating the plates overnight. The SBT was the dilution at which there was a ≥99.9% reduction in the original inoculum.

Time kill curves. Killing curves were determined with Mueller-Hinton broth by previously described methods (7). A volume of 10 ml containing the previously mentioned concentration(s) of the antibiotic(s) was inoculated with log-phase *S. aureus* to give a final concentration of 5×10^6 CFU/ml. A control flask containing no antibiotic was also inoculated each time. The flasks were incubated on a shaker at 35°C. Samples were quantitatively cultured at 0, 6, and 24 h. To test for the presence of rifampin-resistant ogranisms

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TABLE 1. Geometric means of the MICs and MBCs of various drugs against 10 *S. aureus* isolates

Drug	MIC (μg/ml)	MBC (μg/ml)
Nafcillin	0.25	1.2
Vancomycin	1.5	5.4
Teicoplanin	0.5	1.5
Clindamycin	0.29	>64.0
Erythromycin	1.15	>64.0
Trimethoprim	5.6	>64.0
Ciprofloxacin	0.7	1.6
Pefloxacin	1.0	2.5
Rifampin	0.017	0.29

after 24 h, we streaked a 0.05-ml sample onto blood agar plates containing 10 μg of rifampin per ml. Synergy was defined as ≥2 log increased killing at 24 h by the combination compared with the single most effective drug. Antagonistic activity was defined as a ≥2 log reduction in killing by the combination compared with either drug alone. If the drugs were additive or indifferent to each other, the killing effects of the combination were within 2 log of either drug alone. The rate of bacterial killing for the first 6 h was defined as [change in bacterial titer($\log_{10}$ CFU/ml)]/6.

Student's paired *t* test was used to analyze differences in SBTs and killing rates between each drug alone or in combination with rifampin.

RESULTS

Susceptibilities. The MICs and MBCs of the various drugs against the 10 strains of *S. aureus* are shown in Table 1. The isolates were all susceptible to nafcillin. They were all very susceptible to the bactericidal effects of the two quinolones examined as well as to those of vancomycin and teicoplanin. Clindamycin, trimethoprim, and erythromycin were bacteriostatic against these strains.

SBTs. There was a marked reduction in the SBT when rifampin was added to nafcillin (mean reduction from 1:32 to <1:2; $P < 0.05$) (Table 2). The addition of rifampin to vancomycin or teicoplanin also reduced the SBT, but not significantly ($P > 0.05$). The combination of rifampin and a quinolone (ciprofloxacin or pefloxacin) had an additive effect; there was a twofold increase in SBTs compared with either drug alone ($P > 0.05$). Clindamycin, trimethoprim, and erythromycin by themselves were bacteriostatic, but the addition of rifampin significantly increased the mean SBTs to 1:4, 1:8, and 1:8, respectively ($P < 0.02$).

TABLE 2. Bacterial titers in serum for the drug regimens tested

Drug(s) (concn [μg/ml])	Bactericidal titer in serum		
	Median	Mean[a]	Range
Nafcillin (40)	1:32[b]	1:28	1:8–1:64
Nafcillin-rifampin	≤1:2	≤1:2	≤1:2–1:4
Vancomycin (30)	1:4	1:3	≤1:2–1:8
Vancomycin-rifampin	1:2	1:3	≤1:2–1:8
Teicoplanin (5)	1:8	1:6	≤1:2–1:16
Teicoplanin-rifampin	1:4	1:4	≤1:2–1:16
Clindamycin (10)	≤1:2[b]	≤1:2	≤1:2–1:4
Clindamycin-rifampin	1:4	1:5	≤1:2–1:32
Erythromycin (5)	≤1:2[b]	≤1:2	≤1:2–1:4
Erythromycin-rifampin	1:8	1:10	≤1:2–1:32
Trimethoprim (5)	≤1:2[b]	≤1:2	≤1:2
Trimethoprim-rifampin	1:8	1:11	1:2–1:64
Ciprofloxacin (5)	1:4	1:6	1:4–1:8
Ciprofloxacin-rifampin	1:8	1:9	1:4–1:64
Pefloxacin (5)	1:4	1:3	≤1:2–1:8
Pefloxacin-rifampin	1:8	1:7	≤1:2–1:32
Rifampin (6)	1:8	1:8	1:4–1:16

[a] Geometric mean for 10 isolates.
[b] $P < 0.02$ compared with SBT when the drug was combined with rifampin (by Student's paired *t* test).

TABLE 3. Comparison of rates of bacterial killing

Drug(s) (concn [μg/ml])	6-h killing rate ($\log_{10}$ CFU/ml per h; mean ± SEM
Nafcillin (40)	−0.24 ± 0.05[a]
Nafcillin-rifampin	−0.11 ± 0.04
Vancomycin (30)	−0.23 ± 0.04[a]
Vancomycin-rifampin	−0.11 ± 0.02
Teicoplanin (5)	−0.25 ± 0.05[a]
Teicoplanin-rifampin	−0.08 ± 0.02
Clindamycin (10)	−0.11 ± 0.02[a]
Clindamycin-rifampin	−0.23 ± 0.03
Erythromycin (5)	−0.12 ± 0.03[a]
Erythromycin-rifampin	−0.24 ± 0.04
Trimethoprim (5)	−0.16 ± 0.06
Trimethoprim-rifampin	−0.09 ± 0.02
Ciprofloxacin (5)	−0.44 ± 0.03[a]
Ciprofloxacin-rifampin	−0.07 ± 0.01
Pefloxacin (5)	−0.37 ± 0.05[a]
Pefloxacin-rifampin	−0.03 ± 0.02
Rifampin (5)	−0.13 ± 0.01
Control	+0.32 ± 0.01

[a] $P < 0.02$ compared with the drug in combination with rifampin.

Time kill curves. Twenty-four-hour synergism was only found for two strains when rifampin was combined with clindamycin, trimethoprim, or erythromycin. Indifference or antagonism was observed for all other combinations tested (data not shown).

As expected, rifampin-resistant organisms emerged when rifampin was the only drug present. All of the combinations tested prevented this except trimethoprim-rifampin, with which rifampin-resistant organisms were found for two of the strains tested.

When killing rates were compared by paired *t* test (Table 3), the addition of rifampin to nafcillin, vancomycin, teicoplanin, pefloxacin, or ciprofloxacin markedly reduced the killing rate ($P < 0.02$). In contrast, the addition of rifampin to clindamycin or erythromycin increased the rate of bacterial killing over the first 6 h ($P < 0.01$). There was no difference in killing rates between trimethoprim and trimethoprim-rifampin.

DISCUSSION

In this study, when rifampin was used in combination with nafcillin, vancomycin, or teicoplanin, antimicrobial activity against *S. aureus* either was substantially reduced or remained the same. In the case of nafcillin, bactericidal activity as measured by SBT was abolished by the addition of rifampin. These results are consistent with those reported by others for a variety of in vitro susceptibility tests (1, 15, 18).

This apparent loss of bactericidal activity may not be reflected in vivo and probably is not important clinically. Van Der Anwera et al. (17) have reported that oxacillin-rifampin is an effective combination. We have previously shown that the combination rifampin-cloxacillin can be more effective than cloxacillin alone in reducing bacterial vegeta-

tion titers in experimental *S. aureus* endocarditis in rabbits, even though the combination often results in lower peak SBTs in serum than those achieved with cloxacillin alone (20).

The fact that rifampin added to a regimen of nafcillin, vancomycin, or teicoplanin is likely to result in an SBT of <1:8 should be borne in mind before such a test is used to monitor bactericidal activity for therapy of endocarditis or osteomyelitis. The decision to add yet another antimicrobial agent or to discontinue rifampin should be made on clinical grounds rather than in an attempt to achieve an SBT of ≥1:8.

In vitro antagonism between cell wall-directed antimicrobial agents and rifampin has slowed its acceptance for the treatment of staphylococcal infections. Even though rifampin may offer an advantage in the treatment of staphylococcal abscesses (9), osteomyelitis (10, 11), foreign body infections (14), and disseminated infections (1, 5, 6, 20), clinicians are reluctant to add a drug that appears to have an antagonistic effect in vitro. Thus, we sought other potential combinations that might not express this characteristic but still prevent the emergence of rifampin-resistant organisms.

In our present study, SBTs and killing rates both indicated that the addition of rifampin to the bacteriostatic agents clindamycin and erythromycin resulted in a bactericidal combination that was better than the single agents. The addition of rifampin to trimethoprim was also effective, but rifampin resistance emerged. Ciprofloxacin and pefloxacin have excellent antistaphylococcal activity (3, 4, 16), and the addition of rifampin to these two quinolones resulted in SBTs similar to those of the single agents. In light of these encouraging results, further in vitro and in vivo studies with all of these nonantagonistic combinations are warranted.

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