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Bactericidal Activity and Killing Rate of Serum in Volunteers Receiving Teicoplanin Alone or in Combination with Oral or Intravenous Rifampin

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A total of 10 volunteers, in two groups of 5 each, received the following on separate days: group 1, 200 mg of teicoplanin intravenously (i.v.), 600 mg of rifampin orally, or teicoplanin-rifampin; group 2, 400 mg of teicoplanin i.v., 300 mg of rifampin i.v. in 60 min, or teicoplanin-rifampin. Blood samples were obtained before, at the end, and at 1 and 6 h after the administration of the antibiotics. Bactericidal activity in serum (SBA) was measured in microtiter plates against 20 clinical isolates (five strains each) of oxacillin-susceptible and -resistant *Staphylococcus aureus* and *Staphylococcus epidermidis*. The endpoint of the SBA corresponded to 99.9% killing. Killing rates were measured in serum obtained at 1 and 6 h. The concentrations of each antibiotic were measured by bioassay. The antibiotic concentrations in serum obtained at the peak and at 1 and 6 h after the end of administration were as follows: group 1, teicoplanin, 26, 15.6, and 8.4 mg/liter; rifampin, not determined, 8.3, and 3.8 mg/liter; group 2, teicoplanin, 66, 29.4, and 11.5 mg/liter; rifampin, 14.8, 3.8, and 1.2 mg/liter. Higher median SBAs were obtained after treatment with rifampin than after that with teicoplanin. No interaction was observed between rifampin and teicoplanin. This was confirmed by determination of the killing rate in serum. Teicoplanin killed more slowly than rifampin. The combination had the same killing rate as rifampin alone. Rifampin neither improved nor antagonized the bactericidal activity of teicoplanin, as determined by the SBAs or the rate of killing.

Gram-positive infections are still a major cause of morbidity and death (16). The treatment of infections in neutropenic patients usually consists of a combination of a β-lactam antibiotic and an aminoglycoside. However, the recent increase in the incidence of infections caused by methicillin-resistant staphylococci (11, 21) and the lack of efficacy of the new cephalosporins and penicillins against them have caused vancomycin to be required more frequently (10). This antibiotic is toxic, however, and intravenous (i.v.) administration and drug monitoring are required. Teicoplanin has recently been developed and has shown high in vitro activity against staphylococci (22); the cure rate has been in the range of 60 to 70% (8), which is similar to that which has been obtained with vancomycin in severe infections (10). Rifampin has been shown to increase the clinical response to oxacillin in patients with severe *Staphylococcus aureus* infections (18). This is probably due to the excellent intracellular penetration of rifampin (12) and to the prevention of the occurrence of resistance to rifampin by oxacillin. The combination of rifampin with vancomycin has been widely studied in vitro with variable and conflicting results, depending on the strains, inoculum, growth phase, methodology, and concentration of antibiotics used (3, 17, 17a, 19, 20, 23).

Using the killing curve method in broth, we have shown that the combination of teicoplanin-rifampin is more likely to be synergistic than the combination of vancomycin-rifampin (17a). Similar findings were reported by Varaldo et al. (19) and Tuazon and Miller (17).

The purpose of this investigation was to evaluate the efficacy of the combination of teicoplanin-rifampin against staphylococci as assessed by measuring the bactericidal activity in serum (SBA) and the rate of killing in serum (5).

MATERIALS AND METHODS

Volunteers. The protocol for this study was reviewed and approved by the Ethical Committee of the Institut Jules Bordet. Ten healthy human volunteers were included in the study. Exclusion criteria were as follows: abnormal renal (creatinine in serum, >1 mg/dl) or hepatic (bilirubin in serum, >1 mg/dl) functions; pregnancy; allergy; or intolerance to vancomycin, teicoplanin, or rifampin. A written informed consent was obtained from each volunteer before admission to the study.

Administration of antibiotics. The volunteers were randomly divided into two groups of five each. Volunteers in each group received the following drug regimens on separate days in a randomly allocated sequence and with at least a 48-h period of washout: group 1, 200 mg of teicoplanin i.v. by short infusion (15 min) in 50 ml of 5% dextrose in water, 600 mg of rifampin orally, teicoplanin-rifampin given 45 min before the initiation of teicoplanin infusion; group 2, 400 mg of teicoplanin i.v. by short infusion (15 min), 300 mg of rifampin i.v. given over 30 min in 250 ml of 5% dextrose in water, teicoplanin-rifampin, with rifampin infusion being initiated 15 min before the initiation of teicoplanin infusion. For the administration of the antibiotic combination, each antibiotic was infused in separate arms, and both infusions were completed simultaneously. The end of infusions was recorded as time zero. When rifampin was given orally, time zero corresponded to 1 h after administration. Blood samples were taken before administration, at time zero, and at 1

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and 6 h after the end of infusion. In the case of oral administration of rifampin alone, the second blood sample was not taken.

Test strains. For each group, five strains each of oxacillin-susceptible and -resistant *S. aureus* and oxacillin-susceptible and -resistant *Staphylococcus epidermidis* were selected for the study. The 20 strains were recently isolated from blood cultures at the Institut Jules Bordet. All strains were tested prior to the study for the absence of susceptibility to pooled human serum.

Susceptibility testing. MICs and MBCs were measured for all strains by microtiter serial dilutions in Mueller-Hinton broth supplemented with Ca and Mg ions (50 and 20 mg/liter, respectively) in the presence or absence of 50% human serum. The final inoculum in each well was 10^6 CFU/ml. Each test was done in duplicate. MBCs were determined by subculturing 10 μl from each well (final volume, 100 μl) on drug-free agar. Criteria for the MBC was a 99.9% killing of the original ioculum, with a theta value of 10 and a 5% error on initial inoculum determination and sampling volume by the method described by Pearson et al. (14); this corresponded to a cutoff number of regrowing colonies of 47.

SBS and SBA in serum. Bacteriostatic activity in serum (SBS) and SBA against all strains were measured for each serum sample taken at 1 and 6 h. Titration of serum was done in a microtiter system, with a 1:1 mixture of Mueller-Hinton broth–normal human serum used as the diluent (15). The inoculum concentration and sampling for bactericidal activity were as described above. Results are expressed as the median reciprocal SBS or SBA for each microbial species at a given time and drug regimen and as a percentage of serum samples with a reciprocal SBS or SBA of ≥1:8.

Serum assays. Teicoplanin and rifampin levels in serum were measured in each sample by the bioassay method described by Bennett et al. (4) with *Bacillus subtilis* spores 1904E (Merrell-Dow, Brussels, Belgium) for teicoplanin and *B. subtilis* spores 6633 (Difco Laboratories, Detroit, Mich.) for rifampin. The corresponding susceptibilities were 3 mg/liter for teicoplanin and 1 mg/liter for rifampin. The linearity range was 3 to 48 mg/liter for teicoplanin and 1 to 16 mg/liter for rifampin. Between-day coefficients of variation were 7.6% for teicoplanin (3, 6, 12, 24, and 48 mg/liter; tested 10 times) and 8.5% for rifampin (1, 2, 4, 8, and 16 mg/liter; tested 10 times).

Rate of killing in serum. All serum samples obtained at 1 and 6 h after the end of infusion were pooled for each different regimen and timing and were stored at −80°C. Each pool was then tested for killing rate. After a 1:2 dilution in supplemented Mueller-Hinton broth (final volume, 2 ml) time-kill curves were determined for all test strains (5). The bacteria concentration was 10^6 CFU/ml at time zero. All tubes were put on a rotator at 37°C and agitated throughout the experiment. Samples were taken at time zero and at 2, 4, 6, and 24 h with a 10-μl calibrated loop. Suitable dilutions were made and plated on Mueller-Hinton agar, and colonies were counted after overnight incubation.

Statistics. The calculated SBS was defined as the ratio between the serum concentration and the MIC measured in serum-broth. The calculated SBA was defined as the ratio between the concentration in serum and the MBC measured in serum-broth. A Spearman rank coefficient was calculated to study the correlation between the calculated and the observed SBS (or SBA). Comparisons between regimens were assessed by using the Wilcoxon matched pairs rank test. Significance was assessed by a two-tailed Student *t* test, and results of $P \leq 0.05$ were considered significant.

TABLE 1. Concentrations of teicoplanin and rifampin in serum after the administration of the individual drugs

Regimen (no. of volunteers)[a]	Mean concn (mg/liter) ± SD after:		
	0 h	1 h	6 h
Group 1			
Rifampin (5)	ND[b]	8.3 ± 5.1	3.8 ± 2.9
Teicoplanin (5)	26 ± 3.2	15.6 ± 1.5	8.4 ± 5.4
Group 2			
Rifampin (5)	14.8 ± 10.1	3.8 ± 0.5	1.2 ± 0.9
Teicoplanin (5)	66 ± 16.6	29.4 ± 4.2	11.5 ± 1.1

[a] Group 1, 200 mg of teicoplanin i.v., 600 mg of rifampin orally; group 2, 400 mg of teicoplanin i.v., 300 mg of rifampin i.v.
[b] ND, Not determined.

RESULTS

The concentrations of teicoplanin and rifampin in serum at time zero and at 1 and 6 h after dosing are given in Table 1. No antimicrobial activity was detected in the serum samples that were collected prior to the administration of any of the regimens tested.

Concentrations of rifampin in serum after 600 mg was given orally were twice as high as those obtained after 300 mg was given i.v. at the corresponding times after administration. A similar finding was obtained with teicoplanin after 400 mg was administered versus that after 200 mg was administered.

Teicoplanin MICs and MBCs for *S. epidermidis* were not affected by the presence of serum, but the MICs and MBCs for *S. aureus* were affected, especially those for oxacillin-resistant *S. aureus*, for which a fourfold increase in the MICs and MBCs was observed in the presence of serum. For oxacillin, the MICs were slightly higher for *S. aureus* with serum than without, and the MBCs were equal to or twice as high as the MIC. Rifampin MICs and MBCs were below 0.025 mg/liter with and without serum.

The relation between the dose of teicoplanin and rifampin and the resulting SBS are presented in Table 2. SBAs (data not shown) were equal to or twice as low as the SBSs. Doubling of the dose resulted in doubling of the corresponding titers. We observed a highly significant correlation between the calculated and the measured SBS and SBA by the Spearman rank correlation ($r \geq 0.88$; $P \leq 0.0001$). The combination of teicoplanin-rifampin produced an indifferent interaction, with the combination resulting in a SBS or SBA similar to that of the most active antibiotic, which was rifampin. This was confirmed by the study of the killing curves in serum (Fig. 1). The same was true for the two regimens tested. Rifampin had a higher killing rate than teicoplanin, and the combination of teicoplanin-rifampin had a similar killing rate against all strains tested.

DISCUSSION

Despite conflicting results on the in vitro interaction between vancomycin and rifampin, this combination has been found to have a synergistic effect in an animal model of chronic osteomyelitis caused by *S. aureus* (13). It was suggested that the resulting therapeutic efficacy was due to the killing of rifampin-resistant mutants by vancomycin. Moreover, rifampin has been shown to kill phagocytized *S. aureus* better than β-lactam antibiotics, which do not penetrate within the polymorphonuclear leukocytes (12).

There are only a few anecdotal reports of the clinical use of the combination of vancomycin-rifampin (1, 2, 6, 9, 18).

TABLE 2. Reciprocal SBS titers 1 and 6 h after the end of administration of teicoplanin with or without rifampin

Species (no. of strains)	Regimen[a]	Median SBS (% of serum samples ≥ 8) at the following times after administration:	
		1 h	6 h
Group 1			
S. aureus, oxacillin susceptible (5)	Teicoplanin	32 (100)	8 (84)
	Rifampin	2,048 (100)	1,024 (80)
	Combination	2,048 (100)	512 (100)
S. aureus, oxacillin resistant (5)	Teicoplanin	8 (64)	4 (20)
	Rifampin	2,048 (100)	512 (80)
	Combination	1,024 (100)	512 (100)
S. epidermidis, oxacillin susceptible (5)	Teicoplanin	8 (68)	8 (52)
	Rifampin	2,048 (100)	256 (80)
	Combination	2,048 (100)	512 (100)
S. epidermidis, oxacillin resistant (5)	Teicoplanin	8 (68)	4 (20)
	Rifampin	1,024 (100)	512 (80)
	Combination	2,048 (100)	1,024 (100)
Group 2			
S. aureus, oxacillin susceptible (5)	Teicoplanin	64 (100)	16 (100)
	Rifampin	512 (100)	128 (100)
	Combination	512 (100)	256 (100)
S. aureus, oxacillin resistant (5)	Teicoplanin	16 (68)	8 (56)
	Rifampin	512 (100)	128 (100)
	Combination	512 (100)	128 (100)
S. epidermidis, oxacillin susceptible (5)	Teicoplanin	16 (84)	4 (48)
	Rifampin	512 (100)	128 (100)
	Combination	512 (100)	128 (100)
S. epidermidis, oxacillin resistant (5)	Teicoplanin	16 (88)	8 (56)
	Rifampin	512 (100)	256 (100)
	Combination	512 (100)	256 (100)

[a] Combination indicates a combination of teicoplanin-rifampin.

We have successfully treated four patients with severe infections caused by *S. aureus* without the emergence of rifampin resistance (18). This is in contrast with the results of Acar et al. (1), who reported the emergence of rifampin resistance in 3 of 11 patients, or those of Karchmer et al. (9), who reported rifampin resistance in 1 of 8 patients with *S. epidermidis* prosthetic valve endocarditis.

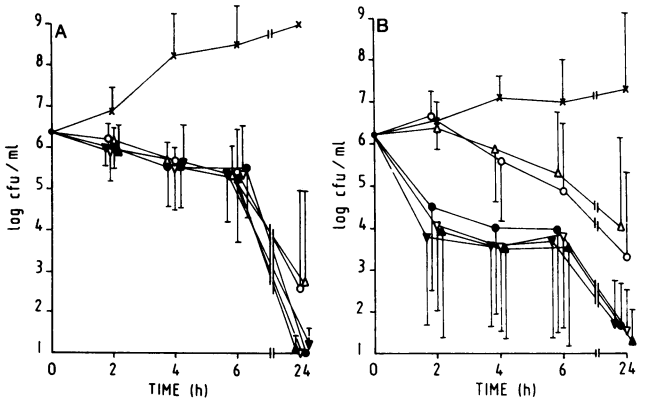

FIG. 1. Killing rate in the serum of volunteers receiving teicoplanin and rifampin alone and in combination (group 1 regimen) against staphylococci. Each point represents the mean value for five strains; vertical bars represent the standard deviation. (A) *S. aureus*; (B) *S. epidermidis*. Symbols: ×, control without antibiotic; ○, 1 h after 200 mg of teicoplanin i.v.; △, 6 h after 200 mg of teicoplanin i.v.; ▽, 2 h after 600 mg of rifampin orally; ●, 7 h after 600 mg of rifampin orally; ▲, 1 h after teicoplanin-rifampin (2 h after 600 mg of rifampin orally); ▼, 6 h after teicoplanin-rifampin (7 h after 600 mg of rifampin orally).

Teicoplanin has been shown to produce a synergistic effect more frequently when it is combined with rifampin than does vancomycin-rifampin (17, 17a, 20). Moreover, both rifampin and teicoplanin have been shown to kill *S. aureus* isolates that have been phagocytized by human polymorphonuclear leukocytes (7). The combination of teicoplanin-rifampin thus appears to be a potentially useful regimen for infections caused by oxacillin-resistant staphylococci.

In this study we have shown that the combination of teicoplanin-rifampin results in an additive or indifferent effect, as measured by the SBS or SBA or the killing rate. Because the concentration of teicoplanin or rifampin in serum was not measured when they were administered in combination, a possible pharmacological interaction between the two antibiotics has not been excluded. The failure to detect a synergistic effect between teicoplanin and rifampin might be due to the fact that rifampin was so active that any further improvement would be difficult to detect.

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