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In Vitro Activities of Rifapentine and Rifampin, Alone and in Combination with Six Other Antibiotics, Against Methicillin-Susceptible and Methicillin-Resistant Staphylococci of Different Species

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The antistaphylococcal activity of rifapentine, a new rifamycin SV derivative, was evaluated in vitro and compared with that of rifampin. A total of 313 staphylococcal strains freshly isolated from clinical material and including representatives of all currently recognized *Staphylococcus* species of human origin were used. The susceptibility to methicillin of all the test strains was determined preliminarily. Despite minor differences with some species, the MICs of rifapentine were found to be substantially similar to those of rifampin. Methicillin-resistant strains of all species were most resistant to rifapentine and rifampin than were their methicillin-susceptible counterparts. For most strains tested, the MBCs of both rifamycins exceeded by twofold the respective MICs. Both the checkerboard dilution and time-kill methods were used to determine the interactions of rifapentine or rifampin with six different antibiotics: cefamandole, vancomycin, teicoplanin, gentamicin, erythromycin, and fusidic acid. No significant differences between the two rifamycins in the combinations were observed against either methicillin-susceptible or methicillin-resistant strains. Minor differences were noted depending on the second antibiotic tested or the staphylococcal species examined. Antagonism was never observed, and indifference was the prevalent response. Cases of synergism were observed occasionally with the checkerboard method and slightly more often with the time-kill method.

Rifapentine (DL 473) has recently been developed as a semisynthetic derivative of rifamycin SV, differing from rifampin in the presence of a cyclopentyl group in place of a methyl group (3). Weight for weight, the in vitro activity of rifapentine against *Mycobacterium tuberculosis* has been found to be superior (3) or similar (25) to that of rifampin, depending on the technical procedure used; against other susceptible microorganisms, rifapentine was most often slightly less active than rifampin (3, 15). However, rifapentine has been shown to be less toxic than rifampin (3) and more effective in protecting mice experimentally infected with *M. tuberculosis* (3, 18) or *M. leprae* (16) and to have significant pharmacokinetic advantages over rifampin, including a longer half-life in both animals (3, 4) and human volunteers (6), a higher penetration into bone (11), and an apparently better uptake by human neutrophils (8).

This study was aimed at assessing the in vitro activity of rifapentine, alone and in combination with other antibiotics (cefamandole, vancomycin, teicoplanin, gentamicin, erythromycin, and fusidic acid), against freshly isolated clinical *Staphylococcus* strains, including all currently recognized methicillin-susceptible (MS) and methicillin-resistant (MR) species of human origin. Rifampin, whose antistaphylococcal activity is largely documented, was tested in parallel as a reference antibiotic throughout all experiments to better evaluate the results obtained with rifapentine.

MATERIALS AND METHODS

Bacterial strains. A total of 313 *Staphylococcus* strains, all freshly isolated from clinical material in our institute's diagnostic laboratory, were used. The isolates were identified on the basis of their lytic activity patterns (22) and other conventional tests (12). Of these strains, 112 were *Staphylococcus aureus*, 92 were *S. epidermidis*, and lower numbers represented each of eight other *Staphylococcus* species. All strains were tested for their susceptibility to methicillin by a standard agar diffusion method with Mueller-Hinton agar (Difco Laboratories, Detroit, Mich.) supplemented with 5% NaCl as the test medium and commercial disks containing 5 μg of the antibiotic (BBL Microbiology Systems, Cockeysville, Md.). The test plates were examined after 18 h of incubation at 37°C.

Antibiotics. Rifapentine, rifampin, teicoplanin, and erythromycin were supplied by Gruppo Lepetit, Milan, Italy; cefamandole and vancomycin were supplied by Eli Lilly Italia, Sesto Fiorentino, Italy; gentamicin was supplied by Essex, Milan, Italy; and fusidic acid was supplied by Sigma-Tau, Pomezia, Italy. Sterile stock solutions of the antibiotics (2 mg/ml) were prepared from standard reference powders in accordance with the instructions of the manufacturers and stored at −70°C before use.

Susceptibility tests. MICs were determined by the broth dilution method in microtiter trays (10) with Mueller-Hinton broth (Difco) as the test medium. Twofold dilutions of rifapentine or rifampin, prepared so as to obtain final concentrations ranging from 0.0006 to 10.24 μg/ml, were made with a hand-held multidilution device (Titertek; Flow Laboratories, Inc., McLean, Va.) that delivered 50 μl per well. Equal volumes of inoculum, prepared from log-phase cultures so as to obtain final concentrations of 10^5 CFU/ml, were dispensed by using automated equipment (Titertek Autodrop; Flow). After 18 h of incubation at 37°C, the MIC was read as the lowest concentration of antibiotic which allowed no visible growth.

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TABLE 1. Susceptibility of 188 MS and 125 MR *Staphylococcus* strains of 10 different species to rifapentine and rifampin

Species	Methicillin susceptibility	No. of strains tested	MIC (μg/ml) of:					
			Rifapentine			Rifampin		
			Range	50%	90%	Range	50%	90%
S. aureus	MS	63	0.005–>10.24	0.02	1.28	0.005–>10.24	0.01	0.08
	MR	49	0.005–>10.24	0.02	10.24	0.005–>10.24	0.01	>10.24
S. epidermidis	MS	52	0.0025–2.56	0.04	1.28	0.0012–2.56	0.01	0.64
	MR	40	0.005–>10.24	2.56	>10.24	0.0025–>10.24	0.64	>10.24
Other coagulase-negative species[a]	MS	73	0.005–2.56	0.02	1.28	0.005–2.56	0.02	1.28
	MR	36	0.005–>10.24	0.04	10.24	0.005–>10.24	0.04	10.24

[a] These strains included isolates of the species *S. capitis* (5 MS and 1 MR), *S. cohnii* (3 MS and 1 MR), *S. haemolyticus* (13 MS and 7 MR), *S. hominis* (22 MS and 15 MR), *S. saprophyticus* (14 MS and 3 MR), *S. simulans* (6 MS and 3 MR), *S. warneri* (5 MS and 4 MR), and *S. xylosus* (5 MS and 2 MR).

MBCs were determined by drawing five 10-μl samples from each of the wells showing no growth with a calibrated loop and streaking them on the surfaces of plates containing Mueller-Hinton agar (1). These plates were incubated at 37°C for 48 h. The MBC was read as the lowest concentration of antibiotic which resulted in ≤0.1% survival in the subculture.

Antibiotic combinations. Combinations of rifapentine or rifampin with six different antibiotics were assessed for activity by two methods, checkerboard titration and time-kill curves (13), with Mueller-Hinton broth as the test medium.

Checkerboard studies were performed in microtiter trays. In each well, 50 μl of antibiotics diluted in broth (25 μl of each drug at a concentration four times as great as the desired final concentration) was mixed with 50 μl of inoculum, prepared and dispensed as described above. Inoculated trays were incubated at 37°C for 18 h. Antibiotic interactions were interpreted as follows: synergism, when the MIC of both drugs was at least one-fourth of the MIC of each drug alone; antagonism, when inhibition occurred at concentrations exceeding the MIC of either drug; indifference, when results intermediate between synergism and antagonism were obtained.

Time-kill studies were performed by adding antibiotics to log-phase staphylococcal cultures diluted to 10^5 to 10^6 CFU/ml and growing in 500-ml flasks at 37°C. Rifapentine and rifampin were each used at a concentration one-fourth of its MBC, and the second antibiotic in the combination was used at one-half or one-eighth of its MBC (or its MIC for bacteriostatic agents). Just before the antibiotics were added (zero time) and at 4, 8, and 24 h, the viable numbers of organisms were enumerated with serial 10-fold dilutions plated on Mueller-Hinton agar. Antibiotic interactions were interpreted as synergistic or antagonistic if the antibiotic combination, as compared with the most effective single antibiotic, caused at least a 100-fold reduction or increase, respectively, in the CFU at 24 h. Intermediate results were interpreted as indifference.

RESULTS

MIC tests. The MIC range and the MICs of rifapentine and rifampin that were inhibitory to 50% (MIC_{50}) and 90% (MIC_{90}) of the strains of *S. aureus*, *S. epidermidis*, and the other species (subdivided according to their susceptibility to methicillin) are shown in Table 1.

With both drugs, MICs were distributed over a very wide range. For both MS and MR staphylococci tested, the MIC_{50}s of rifapentine were twofold higher overall than those of rifampin, as was the case for the MIC_{90}s of the two drugs. On the whole, however, the MICs of rifapentine were largely similar to those of rifampin. Moreover, the same strains for which higher MICs of rifapentine (≥2.56 μg/ml) were required most often required similarly high MICs of rifampin, indicating the occurrence of cross-resistance between the two antibiotics.

MS strains of all species were generally more susceptible than their MR counterparts to both rifapentine and rifampin. This was most evident in *S. epidermidis*, in which the MIC_{50}s of both rifamycins were sixfold higher for MR strains than for MS strains. Other differences in the susceptibilities of the individual species did not appear to be significant.

MBC tests. The MBCs were determined for 44 isolates for which the MICs of rifapentine and rifampin were ≤2.56 μg/ml. These isolates included at least one MS strain and

TABLE 2. In vitro interactions of rifapentine with six different antibiotics as assessed in 44 *Staphylococcus* strains by the checkerboard (CB) method and in 22 strains by the time-kill (TK) method

Species	No. of strains tested	Test method	No. of strains showing indicated reaction[a] to rifapentine plus:											
			Cefamandole		Vancomycin		Teicoplanin		Gentamicin		Erythromycin		Fusidic acid	
			SYN	IND	SYN	IND	SYN	IND	SYN	IND	SYN	IND	SYN	IND
S. aureus	10	CB	2	8	0	10	0	10	3	7	1	9	0	10
	4	TK	2	2	1	3	1	3	3	1	2	2	0	4
S. epidermidis	6	CB	0	6	0	6	0	6	1	5	0	6	2	4
	2	TK	0	2	0	2	1	1	1	1	0	2	2	0
Other coagulase-negative species	28	CB	2	26	0	28	1	27	1	27	1	27	2	26
	16	TK	5	11	1	15	1	15	1	15	3	13	2	14

[a] SYN, Synergism; IND, indifference.

TABLE 3. In vitro interactions of rifampin with six different antibiotics as assessed in 44 *Staphylococus* strains by the checkerboard (CB) method and in 22 strains by the time-kill (TK) method

Species	No. of strains tested	Test method	No. of strains showing indicated reaction[a] to rifampin plus:											
			Cefamandole		Vancomycin		Teicoplanin		Gentamicin		Erythromycin		Fusidic acid	
			SYN	IND	SYN	IND	SYN	IND	SYN	IND	SYN	IND	SYN	IND
S. aureus	10	CB	2	8	1	9	1	9	3	7	1	9	0	10
	4	TK	2	2	1	3	1	3	2	2	2	2	0	4
S. epidermidis	6	CB	0	6	0	6	0	6	1	5	1	5	1	5
	2	TK	0	2	0	2	1	1	1	1	1	1	2	0
Other coagulase-negative species	28	CB	2	26	0	28	0	28	2	26	0	28	2	26
	16	TK	4	12	1	15	1	15	2	14	3	13	2	14

[a] SYN, Synergism; IND, indifference.

one MR strain of each species. Significant differences in the MBC-to-MIC ratios were not observed either from species to species or from MS strains to MR strains. For over half of the strains examined, the MBCs of both rifamycins exceeded by twofold the respective MICs. On the whole, the MBC-to-MIC ratios ranged from 1 to 8 for rifapentine and from 2 to 16 for rifampin.

Combination tests. The interactions of rifapentine or rifampin with six different antibiotics (cefamandole, vancomycin, teicoplanin, gentamicin, erythromycin, and fusidic acid) were determined by both a microtiter checkerboard dilution method and a time-kill method. For the former method, the same 44 isolates used for the MBC determinations were tested. For the time-kill method, 22 isolates (2 MS strains and 2 MR strains of *S. aureus* and 1 MS strain and 1 MR strain of each of the other species) were tested. The results obtained are shown in Tables 2 and 3.

No case of antagonism was encountered. Indifference was the prevalent interaction of both rifapentine and rifampin with the other antibiotics tested. Synergism was occasionally observed when the checkerboard method was used but was more often encountered when the time-kill method was used. When the checkerboard method was used, the incidence of synergism in combinations containing rifapentine ranged from 0 isolates (rifapentine plus vancomycin) to 5 isolates (rifapentine plus gentamicin) out of the 44 staphylococci tested; in combinations containing rifampin, this incidence ranged from 1 isolate (rifampin plus vancomycin or teicoplanin) to 6 isolates (rifampin plus gentamicin). When the time-kill method was used, the incidence of synergism in combinations containing rifapentine ranged from 2 isolates (rifapentine plus vancomycin) to 7 isolates (rifapentine plus cefamandole) out of the 22 isolates tested; in combinations containing rifampin, this incidence ranged from 2 isolates (rifampin plus vancomycin) to 6 isolates (rifampin plus cefamandole or erythromycin). As determined by either method, synergistic interactions with the antibiotic combinations tested were relatively frequent in some species, including *S. aureus* and *S. epidermidis*, but they were not encountered in *S. capitis*, *S. cohnii*, and *S. warneri* strains.

DISCUSSION

Rifampin has been used much more in clinical practice recently for the treatment of nontuberculous infections, although the validity of this trend is still in question (7, 17). Against staphylococci, rifampin is known to be highly active in vitro, being inhibitory and bactericidal at concentrations far lower than those of virtually all other antistaphylococcal agents (5). In this study, the in vitro antistaphylococcal activity of the new rifamycin SV derivative rifapentine has been shown to be very similar to that of rifampin. MR strains proved less susceptible to rifapentine than MS strains; in this respect, the activity of rifapentine also substantially paralleled that of rifampin.

In *S. aureus*, the mutation rate toward resistance to rifapentine has been shown to be very similar to that to rifampin (3), indicating that rifapentine, like rifampin, should be administered in combination with another agent when used in the treatment of staphylococcal infections. The in vitro antistaphylococcal efficacy of antibiotic combinations containing rifampin has been variously evaluated in different surveys (2, 9, 14, 19–21, 23, 24). In this study, antagonism was never observed with either rifapentine or rifampin-containing combinations, either by the checkerboard method or by the time-kill method. The incidence of synergism varied depending on the test method used. However, experience with rifampin suggests that, with combinations containing highly lipid-soluble and intracellularly diffusible drugs, the occurrence of synergistic interactions in vitro is not essential for the in vivo efficacy of the combinations.

On the basis of these results, rifapentine could reasonably be expected to be at least as effective as rifampin in antibiotic combinations for the treatment of human staphylococcal infections. Its lower toxicity, longer half-life, and better tissue penetration may offer some therapeutic advantages over rifampin. Further clinical trials to check these expectations seem to be warranted.

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