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In Vitro Interaction between Rifampin and Clindamycin against Pathogenic Coagulase-Negative Staphylococci

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The MICs and MBCs for 90% of strains tested (MIC_{90} and MBC_{90}, respectively) of rifampin for 75 clinical isolates of pathogenic coagulase-negative staphylococci (PCNS) were 0.03 and 0.25 μg/ml, respectively, while the MIC_{90} and MBC_{90} of clindamycin were both >25 μg/ml. Although no synergy between rifampin and clindamycin was found among the 15 strains studied by the checkerboard method, 6 of 12 selected strains showed synergy by the kill-curve method. No antagonism was observed by either method. All 30 strains rapidly developed resistance to rifampin in vitro, and this could be prevented by the simultaneous presence of 1.0 μg of clindamycin per ml in the 24 methicillin-susceptible PCNS strains. The synergy between rifampin and clindamycin observed in vitro for some strains of PCNS, together with the prevention of emergence of resistance to rifampin by clindamycin, suggests that this antibiotic combination may be useful for the treatment of infections caused by methicillin-susceptible PCNS.

The role of pathogenic coagulase-negative staphylococci (PCNS) in human infections is well established, particularly in infections of implanted foreign bodies such as intravascular catheters, cerebrospinal fluid shunts, and prosthetic valves (11, 27). Until recently, β-lactamase-stable penicillins were regarded as the treatment of choice for such infections. However, up to two-thirds of PCNS strains are currently resistant to methicillin (1, 7, 18). Therefore, vancomycin is generally recommended for such resistant PCNS (12). However, the cure rate has ranged from 60 to 80% (12, 13), probably because of the relatively high vancomycin MBC for these bacteria (MBC for 50% of strains tested [MBC_{50}], 6.3 μg/ml) (1, 11). In addition, vancomycin-resistant PCNS strains have recently been reported (24, 27). Because current therapeutic alternatives to vancomycin are limited, it seems prudent to identify potential alternative antibiotic strategies for the treatment of PCNS infections.

Rifampin is one of the most active antistaphylococcal antibiotics and achieves therapeutic concentrations in a wide range of tissues such as leukocytes and body fluids, including cerebrospinal fluid (5, 23). However, rapid emergence of rifampin resistance when the drug is used alone has limited the use of this drug except in combination with another effective antistaphylococcal agent (1, 18). The purpose of the present study was to evaluate the in vitro interaction between rifampin and clindamycin against PCNS strains.

Seventy-five PCNS isolated from cerebrospinal fluid (n = 71) or blood (n = 4) of patients, including six methicillin-resistant strains, were tested. The organisms were identified as coagulase-negative staphylococci by typical colony appearance on sheep blood agar, by Gram stain, and by failure to coagulate rabbit plasma. When a 1-μg oxacillin disk was used, methicillin resistance was defined as a zone size of ≤10 mm and methicillin susceptibility was defined as a zone size of ≥13 mm.

MICs and MBCs were determined by the microdilution method as recommended by the National Committee for Clinical Laboratory Standards (20), with variations recommended by Thornsberry and McDougal (25). The final bacterial inoculum was approximately 5×10^5 CFU/ml. The MIC-2000 (Dynatech Laboratories, Inc., Alexandria, Va.) was used to inoculate plates containing Mueller-Hinton broth and rifampin (powder; CIBA-GEIGY Corp., Summit, N.J.) at concentrations ranging from 0.0005 to 1.0 μg/ml or clindamycin (hydrochloride powder; The Upjohn Co., Kalamazoo, Mich.) at concentrations ranging from 0.0125 to 25 μg/ml. Following 18 to 24 h of incubation at 35°C, the MICs were determined as described in other studies (21) and each well was subcultured to determine MBCs by adhering to recommendations made by Pearson et al. (22). Table 1 summarizes the MICs and MBCs of rifampin and clindamycin for the 75 PCNS strains tested. Five of the six methicillin-resistant PCNS strains were highly susceptible to rifampin (MIC, ≤0.004 μg/ml; MBC, ≤0.016 μg/ml). Fifty percent of all strains were susceptible to low concentrations of clindamycin (MIC, ≤0.1 μg/ml; MBC, ≤1.5 μg/ml), while most of the remaining strains, including the six that were methicillin resistant, were highly resistant to clindamycin (MIC and MBC, >25 μg/ml). Such a susceptibility pattern has been previously observed by others (1, 6, 7).

By using the checkerboard method, 15 PCNS strains were tested for in vitro synergy between rifampin and clindamycin. Preparation and inoculation of bacteria were the same as for the MIC determinations except that the microdilution wells contained rifampin concentrations ranging from 0.0005 to 0.032 μg/ml on one axis and clindamycin concentrations of 0.006 to 1.6 μg/ml on the other axis. Following inoculation with the MIC-2000, plates were incubated for 18 to 24 h at 35°C and the fractional inhibitory concentration (FIC) of each drug was determined as described by others (9, 29). Synergy was defined by using the following FIC index: $(FIC_{Rif} + FIC_{Clin}) \leq 0.5$ (9, 14). The antibiotic interaction was interpreted as additive if the FIC index equaled 1 (14), indifferent if there was no change in the concentrations needed to inhibit the strain by combining the two antibiotics, and antagonistic if the FIC index was >4.0.

Of the 15 strains (all methicillin susceptible) tested by the checkerboard method, 12 were also studied for synergy between the two antibiotics by the time kill-curve method as previously described (9). The final concentration of each

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TABLE 1. Rifampin and clindamycin activity against 75 PCNS

Type of strain (*n*)	Drug	MIC (μg/ml)[a]			MBC (μg/ml)[a]		
		Range	50%	90%	Range	50%	90%
Methicillin susceptible (69)	Rifampin	0.002–>1	0.004	0.03	0.002–>1	0.016	0.25
	Clindamycin	0.05–>25	0.1	>25	0.1–>25	1.5	>25
Methicillin resistant (6)	Rifampin	0.002–>1	0.002	0.004	0.004–>1	0.008	0.016
	Clindamycin	>25	>25	>25	>25	>25	>25

[a] 50% and 90%, MIC or MBC for 50 and 90% of strains, respectively.

antibiotic studied individually was one-half of the previously determined MBC. When rifampin and clindamycin were tested in combination, one-fourth the MBC of each drug was used (9). In addition, clindamycin alone (at one-half the previously determined MIC) was used for each strain to document lack of effect on the growth curve. Tubes were incubated at 35°C, and 0.1-ml samples for quantitative culture were obtained at 0, 6, and 24 h. Synergy was defined as a ≥200-fold decrease in CFU per milliliter with a combination when compared with levels for the more active constituent used alone at one-half the MBC (9). Additive effect was defined as a 100- to 200-fold decline in CFU per milliliter with the combination in comparison with the more active drug alone. Indifference was defined as no change in CFU per milliliter with the combination compared with levels with the more active drug alone. The lowest detectable number of organisms was 10 CFU/ml. When the checkerboard method was used, the combination of rifampin and clindamycin was not synergistic for any of the 15 PCNS strains tested (Table 2). Four strains (24%) showed indifference, while 11 demonstrated an additive effect. No antagonism was observed. In contrast, when the kill-curve method was used, the combination of rifampin and clindamycin was synergistic against 6 of 12 PCNS strains tested. For an additional three strains, the combination was additive. No antagonism was found.

Thirty PCNS strains were tested for development of rifampin resistance in vitro. A 1-ml sample of bacteria at 10^9 CFU/ml was plated on Mueller-Hinton agar containing 0.25 μg of rifampin per ml, and 1 ml was plated on medium containing 0.25 μg of rifampin and 1 μg of clindamycin per ml. Following 48 h of incubation at 35°C, at which time growth was noted, up to five colonies were subcultured separately on blood agar in the absence of rifampin and clindamycin. The MIC and MBC of rifampin for these isolates were redetermined as described above. In all, of 30 PCNS strains grown on Mueller-Hinton agar containing 0.25 μg of rifampin per ml, resistance to rifampin developed, with a mutation rate ranging from 1×10^{-8} to $>5 \times 10^{-6}$. All mutant strains tested demonstrated a rifampin MIC of >50 μg/ml. The emergence of such rifampin resistance was prevented in 24 of 30 strains (80%) by the presence of 1.0 μg of clindamycin per ml in the medium. Of these 24 PCNS strains, 15 manifested a clindamycin MBC of >1 μg/ml. For the remaining six parent strains, which were methicillin and clindamycin resistant, the addition of clindamycin to rifampin failed to prevent emergence of rifampin resistance.

TABLE 2. In vitro interaction between rifampin and clindamycin against PCNS

Result	No. of strains	
	Checkerboard	Kill-curve
Synergy	0	6
Additive	11	3
Indifference	4	3
Antagonism	0	0

Rapid in vivo (17) and in vitro (19) emergence of rifampin-resistant staphylococci after exposure to rifampin alone can be prevented by combining rifampin with another effective antistaphylococcal agent (17). An additional potential advantage of a combination is synergistic activity against staphylococci (26). Several studies investigating the bactericidal interaction between rifampin and a β-lactam antibiotic or vancomycin against *Staphylococcus aureus* have yielded conflicting results, but most indicate indifference or antagonism (3, 26, 28). Using the checkerboard method, Zinner et al. (29) and Archer et al. (2) found little evidence of in vitro synergy between rifampin and β-lactam antibiotics or vancomycin against PCNS strains. Archer et al. (2) and Lowy et al. (15), using the time-kill curve method with methicillin-resistant *Staphylococcus epidermidis* strains, found little evidence of synergy between rifampin and cephalothin, vancomycin, or nafcillin. In none of these studies was antagonism observed, and most demonstrated that the addition of a second antibiotic to rifampin prevented the emergence of rifampin-resistant mutants.

Clindamycin is an effective antistaphylococcal agent, concentrates within leukocytes, and induces bacterial resistance only slowly (4). Few studies have investigated the in vitro interaction between rifampin and clindamycin against staphylococci. Ho and Klempner (10) found synergistic bactericidal activity in 5 of 15 (33%) of *S. aureus* strains by the kill-curve method. The remaining 10 strains showed enhanced killing which did not fulfill their definition of synergy. By using the kill-curve method for 10 methicillin-susceptible *S. aureus* strains, Hackbarth et al. showed enhanced killing rates by the combination of rifampin and clindamycin, and the combination prevented the emergence of rifampin-resistant mutant strains in vitro (8). In the present study, this combination was synergistic against 6 of 12 PCNS strains (50%) by the kill-curve method, but no synergy was observed by the checkerboard assay. Discrepancies between the two methods have been observed frequently (3, 21). Our observation of no antagonism by either method and our finding that clindamycin prevented the emergence of rifampin resistance in vitro in all methicillin-susceptible PCNS strains suggest that this combination may be clinically useful against infections caused by methicillin-susceptible PCNS strains. However, recent reports of emergence of rifampin resistance among *S. epidermidis* strains in vivo, despite the addition of another effective antistaphylococcal agent capable of preventing this phenomenon in vitro, are disturbing (2, 11). Therefore, close monitoring for emergence of rifampin resistance among PCNS during combination therapy is necessary.

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