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In Vitro Activity of Rifampin Alone and in Combination with Nafcillin and Vancomycin Against Pathogenic Strains of *Staphylococcus aureus*

CARMELITA U. TUAZON,* MELODY Y. C. LIN, AND JOHN N. SHEAGREN†

Department of Medicine, Division of Infectious Diseases, The George Washington University Medical Center, Washington, D.C. 20037

Received for publication 15 November 1977

Twenty strains of *Staphylococcus aureus* isolated from patients with endocarditis were examined in vitro for susceptibility to rifampin, nafcillin, and vancomycin and to combinations of rifampin with nafcillin or vancomycin. Minimum bactericidal concentrations of rifampin ranged from 0.0031 to 0.0125 μg/ml, of nafcillin ranged from 0.078 to 0.312 μg/ml, and of vancomycin ranged from 0.312 to 1.25 μg/ml. The combination of rifampin with nafcillin was synergistic for 12 strains; the combination of rifampin plus vancomycin was synergistic for 5 of the isolates.

Although penicillins resistant to penicillinase are currently the drugs of choice for treatment of *Staphylococcus aureus* infections, there is a need for more effective agents and/or therapeutic regimens. With limited clinical experiences (9), synergistic combinations of penicillinase-resistant penicillins with aminoglycoside (3, 11-14) offer some promise. Rifampin, which has high activity against *S. aureus* in vitro (5, 7), is another possibility for either a single or combination drug regimen (2, 4, 10). The current study deals with the activity of rifampin, nafcillin, and vancomycin, singly and in combination, against strains of *S. aureus* known to be pathogenic for man.

MATERIALS AND METHODS

Twenty strains of coagulase-positive *S. aureus* recently isolated from patients with endocarditis were studied. Nafcillin sodium, vancomycin hydrochloride, and rifampin were supplied by Wyeth Laboratories Inc., Eli Lilly and Co., and Ciba Pharmaceutical Co., respectively. Standard stock solutions of each antibiotic, prepared by dissolving the lyophilized powders in sterile solutions per the manufacturer's instructions, were stored at −70°C and thawed immediately before use. At the time of each study, drug dilutions were done in brain heart infusion (BHI) broth.

Minimal inhibitory concentrations and minimal bactericidal concentrations (MBCs) of nafcillin, vancomycin, and rifampin alone and in combination were determined in vitro using a microtiter checkerboard dilution method (8, 15). Nafcillin concentrations ranged from 2.5 to 0.02 μg/ml, vancomycin concentrations ranged from 5.0 to 0.039 μg/ml, and rifampin concentrations ranged from 0.01 to 0.0004 μg/ml. An inoculum of 50 μl of an overnight broth culture of the strain of *S. aureus* being tested, adjusted to contain approximately 5×10^6 organisms per ml assayed as colony-forming units per ml by optical density, was added to each appropriate well. Each plate was sealed with a clean plastic strip, and a pinhole vented each well. The minimal inhibitory concentration was read as the lowest concentration of antibiotic (in micrograms per milliliter) which allowed no visible growth. The MBC was read as the lowest concentration of drug in which there was no growth of the test organism after plating 10 μl of solution from each well and reincubating at 37°C for 18 to 24 h. Since the MBC was always within one dilution of the minimal inhibitory concentration, all data are based on MBCs.

Drug combinations were considered "fully synergistic" if inhibition occurred at one-fourth or less of the MBC for both drugs. If inhibition had occurred with concentrations exceeding the MBC of either drug, "antagonism" between the two antibiotics would have been demonstrated. "Partial synergism" was judged to have occurred when the MBC of one of the antibiotics in the combination was at least one-fourth of the MBC of that antibiotic alone, whereas the MBC of the second antibiotic was only one-half of the concentration when used alone. A one-half reduction in the MBC of both antibiotics in the combination was regarded as an "additive" effect. "Indifference" was observed if only one of the antibiotics demonstrated reduction in the MBC (1).

RESULTS

The MBCs of rifampin, nafcillin, and vancomycin alone or in combination for the 20 isolates are shown in Table 1. All isolates were sensitive to 0.0125 μg or less of rifampin per ml. The MBCs for nafcillin ranged from 0.078 to 0.312 μg/ml; for vancomycin, 0.312 to 0.25 μg/ml.

A total of 12 (60%) of 20 strains demonstrated

† Present address: Veterans Administration Hospital, Ann Arbor, MI 48105.

TABLE 1. *MBCs (μg/ml) of rifampin, nafcillin, and vancomycin alone and rifampin/nafcillin, rifampin/vancomycin combinations against 20 pathogenic strains of* S. aureus

Strain	Rifampin	Nafcillin	Vancomycin	Rifampin/nafcillin		Rifampin/vancomycin	
				MBC	Activity[a]	MBC	Activity[a]
1	0.0062	0.312	0.312	0.0016/0.156	PS	0.00039/0.312	I
2	0.0031	0.312	1.25	0.0004/0.156	PS	0.00039/0.625	PS
3	0.0125	0.156	1.25	0.0031/0.078	PS	0.00039/0.625	PS
4	0.0031	0.156	0.625	0.0004/0.156	I	0.00039/0.625	I
5	0.0062	0.156	1.25	0.0031/0.078	A	0.00039/1.25	I
6	0.0062	0.156	0.625	0.0031/0.156	I	0.00039/0.625	I
7	0.0031	0.156	1.25	0.0031/0.078	I	0.00039/0.625	PS
8	0.0062	0.312	1.25	0.0016/0.156	PS	0.00039/1.25	I
9	0.0031	0.156	1.25	0.0031/0.078	I	0.00039/1.25	I
10	0.0062	0.156	1.25	0.0031/0.078	A	0.00039/1.25	I
11	0.0031	0.156	1.25	0.0008/0.156	I	0.0031/0.625	I
12	0.0031	0.156	0.625	0.0008/0.156	I	0.0078/0.625	I
13	0.0062	0.156	0.625	0.0031/0.039	PS	0.00039/0.625	I
14	0.0031	0.156	1.25	0.0008/0.039	S	0.00039/1.25	I
15	0.0125	0.156	1.25	0.0031/0.078	PS	0.0015/0.625	PS
16	0.0031	0.156	0.625	0.0008/0.039	S	0.00039/0.625	I
17	0.0062	0.156	0.625	0.0016/0.039	S	0.00039/0.625	I
18	0.0031	0.156	1.25	0.0016/0.039	PS	0.00078/0.625	PS
19	0.0031	0.312	1.25	0.0008/0.156	PS	0.00039/1.25	I
20	0.0031	0.078	1.25	0.008/0.039	PS	0.0016/0.625	A

[a] Interpretation: S, synergy; PS, partial synergy; A, additive; I, indifferent.

some degree of synergy between rifampin and nafcillin: 3 (15%) of the isolates were fully synergistic, whereas 9 (45%) showed partial synergy (Table 1). An additive effect was shown by two (10%) and indifference was shown in six (30%) of the isolates.

The rifampin-vancomycin combination was partially synergistic in five (25%) and additive in one (5%) of the isolates. In 14 (70%) of the isolates, indifference was observed.

DISCUSSION

Recently, two groups have reported the successful therapy of *S. aureus* endocarditis with the combination of rifampin and erythromycin (10) and rifampin and vancomycin (4) in pediatric patients previously treated unsuccessfully with other agents. Experimental studies have suggested that the combination of oleandomycin and rifampin was synergistic against *S. aureus* bacteremia in rabbits (2). However, another study had shown antagonism between penicillin and rifampin both in vitro and in the rabbit endocarditis model (12).

Our present studies in vitro demonstrated susceptibility of all 20 pathogenic strains of *S. aureus* to rifampin, nafcillin, and vancomycin. The combination of rifampin and nafcillin seemed somewhat better than the combination of rifampin and vancomycin in that 60% of the strains demonstrated some degree of synergy. The rifampin-vancomycin combination was partially synergistic against about one-fourth of the isolates. Antagonism was not produced by either antibiotic combination.

Previous studies (6, 7) have suggested that rifampin might be useful in treating deep-seated, undrainable abscesses due to *S. aureus* and infections in patients with defective leukocyte-killing function. Those authors further suggested that because of early emergence of rifampin resistance (5), a second antibiotic should always be added. Our studies support the concept that rifampin might be useful in serious infections due to *S. aureus*. Clinical studies should be carried out to determine if clinical responses are enhanced by the addition of rifampin to nafcillin or vancomycin.

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