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Enhancement of Antistaphylococcal Activity of Nafcillin and Oxacillin by Sisomicin and Netilmicin

CHATRCHAI WATANAKUNAKORN* AND CHERYL GLOTZBECKER

Department of Internal Medicine (Infectious Diseases), University of Cincinnati College of Medicine, Cincinnati, Ohio 45267

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The in vitro activity of sisomicin, netilmicin, nafcillin, and oxacillin against 35 strains of *Staphylococcus aureus* isolated from blood cultures of patients with endocarditis or septicemia was studied. The effects of combinations of either of the two newer aminoglycosides and either of the two penicillinase-resistant penicillins on the killing of *S. aureus* were investigated. All *S. aureus* strains were susceptible to the four antibiotics. Enhancement of antistaphylococcal activity was demonstrated by the antibiotic combinations.

It has been demonstrated that aminoglycosides in general enhance the antistaphylococcal activity of penicillinase-resistant penicillins (6). Sisomicin and netilmicin are two new semisynthetic aminoglycosides closely related to gentamicin (2, 4, 5). In this investigation, we studied the in vitro activity of sisomicin and netilmicin against *Staphylococcus aureus* and the effects of the combinations of either sisomicin or netilmicin with nafcillin or oxacillin on the killing of *S. aureus*.

MATERIALS AND METHODS

S. aureus. Thirty-five strains of *S. aureus* isolated from blood cultures of 35 patients with endocarditis or septicemia were studied.

Antibiotic susceptibility test. The minimal inhibitory concentrations (MICs) of nafcillin, oxacillin, sisomicin, and netilmicin were determined by the ICS agar-dilution method (1). Mueller-Hinton agar was used. The inoculum was 0.002 ml of a 10^{-2} dilution of an overnight culture (approximately 10^3 to 10^4 organisms) delivered by a Steers replicator (3).

Test of enhancement of activity. The time-kill curve method was used. An overnight culture of *S. aureus* was diluted with Mueller-Hinton broth to give between 10^6 and 10^7 colony-forming units (CFU) per ml and incubated with different antibiotics in a water bath at 37°C. The final concentrations of each antibiotic were as follows: nafcillin, 10 μg/ml; oxa-

TABLE 1. *Antimicrobial susceptibility of 35 strains of S. aureus*

Antibiotic	No. of strains with MIC (μg/ml) at:				
	0.06	0.125	0.25	0.5	1
Nafcillin			34		1
Oxacillin		1	23	9	2
Sisomicin	4	13	8		
Netilmicin			9	12	14

TABLE 2. *Comparisons of the effectiveness of antibiotic combinations with that of each antibiotic alone in the killing of 35 strains of S. aureus*

Antibiotic combination	No. of strains showing decrease in CFU (×$\log_{10}$) at:								
	6 h			24 h			48 h		
	<1	1-2	>2	<1	1-2	>2	<1	1-2	>2
Nafcillin + sisomicin									
Compared with nafcillin	0	0	35	2	2	31	4	11	20
Compared with sisomicin	33	2	0	20	11	4	12	11	12
Nafcillin + netilmicin									
Compared with nafcillin	0	19	16	2	13	20	12	16	7
Compared with netilmicin	34	1	0	20	8	7	4	15	16
Oxacillin + sisomicin									
Compared with oxacillin	0	0	35	2	9	24	6	11	18
Compared with sisomicin	33	2	0	19	8	8	10	13	12
Oxacillin + netilmicin									
Compared with oxacillin	0	20	15	6	17	12	19	11	5
Compared with netilmicin	35	0	0	17	11	7	9	8	18

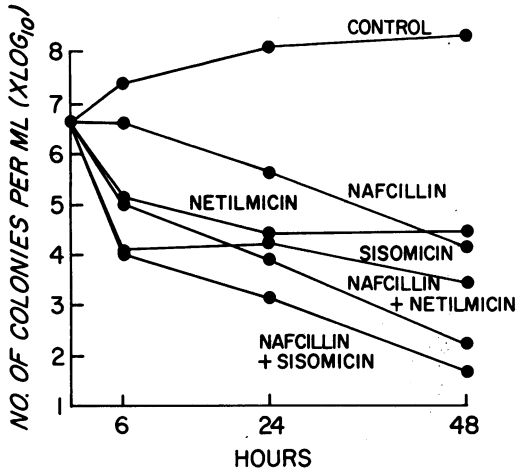

FIG. 1. *Enhancement of killing of a strain of S. aureus by nafcillin-sisomicin and nafcillin-netilmicin combinations.*

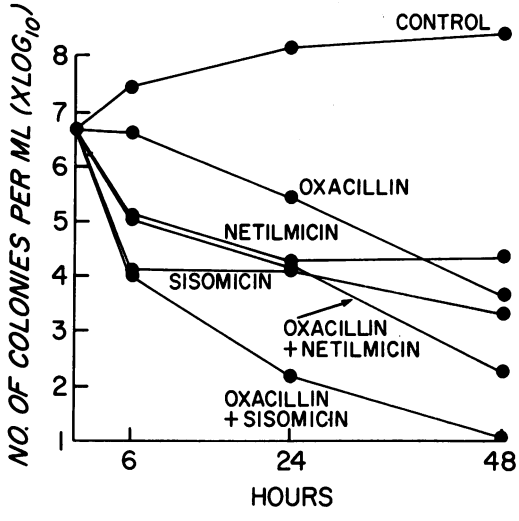

FIG. 2. *Enhancement of killing of a strain of S. aureus by oxacillin-sisomicin and oxacillin-netilmicin combinations.*

cillin, 10 μg/ml; sisomicin, 1 μg/ml; netilmicin, 1 μg/ml; oxacillin, 10 μg/ml, plus sisomicin, 1 μg/ml; and oxacillin, 10 μg/ml, plus netilmicin, 1 μg/ml. A culture with no antibiotic was set up as a control. At 6, 24, and 48 h of incubation, viable CFU were enumerated by 10-fold dilutions and the pour plate technique with mannitol salt agar. Colonies were counted after incubation at 37°C for 48 h.

RESULTS AND DISCUSSION

The MICs of nafcillin, oxacillin, sisomicin, and netilmicin for the 35 strains of *S. aureus* are listed in Table 1. All of the strains were susceptible to the antibiotics tested. Sisomicin was more active than netilmicin against *S. aureus*.

TABLE 3. *Effect of single antibiotics and antibiotic combinations on the viability of 35 strains of S. aureus*

Single antibiotics or combination	No. of strains with ≤100 viable CFU/ml at:		
	6 h	24 h	48 h
Nafcillin	0	0	0
Oxacillin	0	0	0
Sisomicin	0	1	4
Netilmicin	0	0	0
Nafcillin + sisomicin	0	8	27
Nafcillin + netilmicin	0	0	8
Oxacillin + sisomicin	0	9	21
Oxacillin + netilmicin	0	0	7

Comparisons of the effectiveness of antibiotic combinations with that of each antibiotic alone in the killing of 35 strains of *S. aureus* are presented in Table 2. When the results from antibiotic combinations were compared with those from nafcillin or oxacillin alone, significantly enhanced killing was demonstrated against all strains at 6 h and against the majority of strains at 24 and 48 h. On the other hand, when comparisons were made of the results from antibiotic combinations and those from sisomicin or netilmicin alone, a significant decrease in viable CFU was demonstrated against only one to two strains at 6 h but against increasing numbers at 24 and 48 h. Figures 1 and 2 show typical results.

Another way of assessing the enhancement of activity is by comparing the viable CFU at 6, 24, and 48 h. Table 3 shows the number of strains with ≤100 viable CFU per ml in different antibiotic systems. With nafcillin and oxacillin alone; at no time was there decrease of viable CFU to ≤100 per ml. With the combinations, especially nafcillin plus sisomicin and oxacillin plus sisomicin, significant numbers of strains showed ≤100 viable CFU per ml at 48 h.

The results of this study demonstrate that both sisomicin and netilmicin are active against *S. aureus* and that both of these new semisynthetic aminoglycosides enhance the killing of *S. aureus* by nafcillin and oxacillin.

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