Enhancement of the Effects of Anti-Staphylococcal Antibiotics by Aminoglycosides

CHATRCHAI WATANAKUNAKORN AND CHERYL GLOTZBECKER

Department of Internal Medicine, University of Cincinnati College of Medicine, Cincinnati, Ohio 45267

Received for publication 20 August 1974

The in vitro activity of seven anti-staphylococcal antibiotics alone and in combination with four aminoglycoside antibiotics against 35 clinical isolates of *Staphylococcus aureus* from blood cultures of patients with endocarditis or septicemia was studied. The combination of nafcillin-gentamicin or nafcillin-tobramycin when compared with nafcillin alone killed significantly more *S. aureus* at 6 h for 33 of 35 isolates. A significant decrease in viable colony-forming units at 24 and 48 h was demonstrated for a smaller number of isolates. Using the agar dilution antibiotic susceptibility test, significant enhancement of activity was demonstrated against almost all isolates when cephalothin, cefazolin, or clindamycin was combined with gentamicin, tobramycin, or kanamycin. The combination of nafcillin or vancomycin with gentamicin, tobramycin, or kanamycin also showed enhanced activity against the majority of isolates. Oxacillin or methicillin when combined with the aminoglycosides showed enhancement of activity against the least number of isolates. Streptomycin was the least effective of the four aminoglycoside antibiotics studied.

The morbidity and mortality of serious infections due to *Staphylococcus aureus*, such as septicemia and endocarditis, remain high despite the availability of antibiotics (7, 12, 13). Before methicillin was introduced, antibiotic combinations were often used to treat such serious staphylococcal infections (13). Since the semisynthetic penicillinase-resistant penicillins and cephalosporins became available, these antibiotics alone have been recommended by many authorities and generally accepted as the therapy of choice for serious infections due to penicillin-resistant *S. aureus* (3, 4, 6). However, in a major textbook on infectious diseases published recently, the combination of nafcillin and gentamicin was recommended for the therapy of staphylococcal endocarditis (5).

In this investigation, we studied the in vitro activity of seven anti-staphylococcal antibiotics alone and in combination with four aminoglycoside antibiotics against 35 clinical isolates of *S. aureus* from blood cultures of patients with endocarditis or septicemia.

MATERIALS AND METHODS

S. aureus. Thirty-five clinical isolates of *S. aureus* from blood cultures of 35 patients with endocarditis or septicemia were studied. Thirty isolates produced penicillinase and five isolates were non-penicillinase producers.

Antibiotics. The following antibiotics were used: sodium nafcillin (Wyeth Laboratories, Philadelphia,

Pa.); sodium oxacillin, sodium methicillin, and kanamycin sulfate (Bristol Laboratories, Syracuse, N.Y.); sodium cephalothin, cefazolin sodium, vancomycin hydrochloride, and tobramycin sulfate (Eli Lilly & Co., Indianapolis, Ind.); clindamycin phosphate (Upjohn Co., Kalamazoo, Mich.); gentamicin sulfate (Schering Corporation, Bloomfield, N.J.); streptomycin sulfate (Premo Pharmaceutical Laboratories, Inc., South Hackensack, N.J.).

Bacterial killing by nafcillin. Eight of the 35 clinical isolates of *S. aureus* were used to study the effects of three different concentrations of nafcillin on the viability of these microorganisms at time intervals. An overnight culture was diluted with Mueller-Hinton broth to give between 10^6 and 10^7 colony-forming units/ml and incubated with nafcillin in the final concentrations of 5, 10, and 20 $\mu\text{g/ml}$. At 6, 24, and 48 h of incubation in a water bath at 37 C, viable colony-forming units were enumerated by 10-fold dilutions and the pour-plate technique. Penicillinase (Neutropen, Riker Laboratories, Northridge, Calif.) (10,000 U) was added to each plate containing 10^6 and 10^{-1} dilutions before agar was poured (2). Colonies were counted after incubation at 37 C for 48 h.

Bacterial killing by nafcillin-gentamicin and nafcillin-tobramycin. All 35 isolates were used in this study. An overnight culture was diluted with Mueller-Hinton broth to give between 10^6 and 10^7 colony-forming units/ml and incubated with nafcillin, gentamicin, and tobramycin singly and in combinations in a water bath at 37 C. The final concentrations were nafcillin, 10 $\mu\text{g/ml}$; gentamicin, 1 $\mu\text{g/ml}$; tobramycin, 1 $\mu\text{g/ml}$; nafcillin, 10 $\mu\text{g/ml}$ with gentamicin, 1 $\mu\text{g/ml}$; and nafcillin, 10 $\mu\text{g/ml}$ with tobramycin, 1 $\mu\text{g/ml}$. At 6, 24, and 48 h of incubation, viable

colony-forming units were enumerated by 10-fold dilutions and the pour-plate technique. Penicillinase (10,000 U) was added to each plate of 10^0 and 10^{-1} dilutions of cultures containing nafcillin before agar was poured. Magnesium sulfate (40% solution 0.5 ml) was added to each plate of 10^0 and 10^{-1} dilutions of cultures containing gentamicin or tobramycin before agar was poured. Colonies were counted after incubation at 37 C for 48 h.

MIC. The agar dilution technique was employed to determine the minimal inhibitory concentration (MIC) of the 11 antibiotics for the 35 isolates. Mueller-Hinton agar (Difco Laboratories, Detroit, Mich.) was used. The inoculum was 0.002 ml of an undiluted overnight culture, delivered by a Steers replicator (8). The results were read after incubation at 37 C for 48 h.

For the study of antibiotic combinations, the antibiotics were divided into two groups. Group I consisted of nafcillin, oxacillin, methicillin, cephalothin, cefazolin, vancomycin, and clindamycin. Group II consisted of gentamicin, tobramycin, kanamycin, and streptomycin. Group II antibiotics in concentrations of 5, 2.5, and 1.25 $\mu\text{g/ml}$ (gentamicin and tobramycin) and 20, 10, and 5 $\mu\text{g/ml}$ (kanamycin and streptomycin) were added to serial two-fold dilutions of antibiotics in Group I. The MIC of the antibiotic combinations was determined and compared with the MIC of Group I antibiotics alone. Significant enhancement of anti-staphylococcal activity was defined as a four-fold or more decrease in the MIC of Group I antibiotics, as a result of the addition of an aminoglycoside at a particular concentration.

RESULTS

Bacterial killing by nafcillin. The effects of three different concentrations of nafcillin on the viability of eight clinical isolates of *S. aureus* are depicted in Fig. 1. There was essentially no difference among results obtained from concentrations of 5, 10, and 20 $\mu\text{g/ml}$.

Bacterial killing by nafcillin-gentamicin and nafcillin-tobramycin. The combination of nafcillin-gentamicin or nafcillin-tobramycin when compared with each antibiotic alone exerted at least equal and frequently more bacterial killing at all time intervals studied. The comparison of the effectiveness of killing of *S. aureus* by antibiotic combinations and each antibiotic alone is presented in Table 1.

At 6 h, the antibiotic combinations killed significantly more organisms for 33 of the 35 isolates of *S. aureus* when compared with nafcillin alone. Figures 2 and 3 represent two examples. In contrast, when a comparison was made with gentamicin or tobramycin alone, the antibiotic combinations did not exert significant increases in bacterial killing for a majority of isolates (Table 1; Fig. 2). However, for some isolates, nafcillin-gentamicin and nafcillin-tobramycin exerted significant increases in kill-

FIG. 1. Effects of three different concentrations of nafcillin on the viability of eight clinical isolates of *S. aureus*. The ranges with the means of the number of viable colony-forming units at time intervals are shown.

ing when compared with gentamicin or tobramycin alone (Fig. 3).

At 24 and 48 h, a different situation existed. When the killing by the antibiotic combinations was compared with that of nafcillin alone, significant decrease in colony counts was demonstrated for a smaller number of isolates (Table 1). Figure 2 is an example of a nonsignificant decrease and Fig. 3 a significant decrease in colony counts at 24 h when the antibiotic combinations were compared with nafcillin alone. On the other hand, when comparisons of antibiotic combinations were made with gentamicin or tobramycin alone, significant and, in most instances, marked decreases in colony counts were shown for almost all isolates (Table 1) as exemplified by Fig. 2 and 3.

MIC and effects of antibiotic combinations. The MIC of 11 antibiotics for the 35 isolates of *S. aureus* is shown in Table 2. There was no methicillin-resistant *S. aureus*. The isolates tested were less susceptible to cefazolin than cephalothin. Tobramycin was the most active and streptomycin the least active aminoglycoside.

Data on the enhancement of anti-staphylococcal activity due to the addition of Group II antibiotics to Group I antibiotics are shown in Table 3. Significant enhancement of activity was demonstrated against almost all isolates when cephalothin, cefazolin, or clindamycin was combined with gentamicin, tobramycin, or kanamycin. The combination of nafcillin or vancomycin with gentamicin, tobramycin, or kanamycin also showed enhanced activity against the majority of isolates. Oxacillin or methicillin when combined with the aminoglycosides showed enhancement of activity

TABLE 1. Comparison of the effectiveness of killing of 35 isolates of *S. aureus* by antibiotic combinations and each antibiotic alone

Antibiotic being compared	Magnitude of decrease in colony count ($\times \log_{10}$) by nafcillin-gentamicin and nafcillin-tobramycin													
	6 h				24 h					48 h				
	<1	1-2	2-3	3-4	<1	1-2	2-3	3-4	>4	<1	1-2	2-3	3-4	>4
Nafcillin	2 ^a	20	12	1	17	11	6	0	1	18	12	3	1	1
Gentamicin	27	6	2	0	2	6	5	11	11	0	1	4	7	23
Nafcillin	2	16	15	2	9	16	7	3	0	22	6	2	3	2
Tobramycin	30	4	1	0	3	1	6	8	17	3	1	1	3	27

^a Number of isolates. FIG. 2. Enhancement of killing of a clinical isolate of *S. aureus* by nafcillin-gentamicin and nafcillin-tobramycin.

against the least number of isolates. Streptomycin was the least effective of the four aminoglycoside antibiotics studied.

DISCUSSION

Although antibiotic therapy has improved the survival rate considerably during the era of the penicillins and cephalosporins, staphylococcal endocarditis continues to be a very serious infection with high mortality, especially in the elderly (11, 12). Early diagnosis and prompt institution of antibiotic therapy are important, but more efficacious antibiotic treatment regimens must continuously be sought. Contrary to the current recommendation of using a single antibiotic by many authorities (3, 4, 6), Hoeprich advocated the combination of gentamicin with penicillin (for penicillin-susceptible *S. aureus*) and gentamicin with nafcillin or cloxacillin (for penicillin-resistant *S. aureus*) in the therapy of staphylococcal endocarditis (5). However, there have been no clinical data to support such recommendations.

Except for a few preliminary reports in abstract forms, there has not been much informa-

FIG. 3. Enhancement of killing of a clinical isolate of *S. aureus* by nafcillin-gentamicin and nafcillin-tobramycin.

tion regarding the comparative in vitro efficacy of the combination of the penicillins and gentamicin versus the penicillins alone against *S. aureus*. It is clear from our data that increasing the concentration of nafcillin from 5 to 20 µg/ml did not improve the rate or magnitude of killing of *S. aureus*. However, when compared with nafcillin alone, the combinations of nafcillin-gentamicin and nafcillin-tobramycin significantly increased the numbers of organisms killed during the first 6 h of incubation. Similar findings were reported by N. H. Steigoigel, J. I. Casey, and B. J. Heeter (Clin. Res. 21:976, 1973). When the magnitude of killing at 6 h by the antibiotic combinations was compared with gentamicin or tobramycin alone, there was no significant difference for the majority of *S. aureus* tested. This is in contrast to the synergistic action of penicillin or vancomycin in combination with gentamicin against enterococci (9, 10). At 24 and 48 h, for the majority of *S. aureus*, the magnitude of bacterial killing

TABLE 2. MIC of antibiotics for 35 isolates of *S. aureus*

Antibiotics	MIC (μ g/ml)												
	0.15	0.31	0.62	1.25	2.5	5.0	10	20	40	80	160	320	>320
Nafcillin		2 ^a	15	18									
Oxacillin		12	14	4	5								
Methicillin				8	27								
Cephalothin		1	12	14	6	2							
Cefazolin			4	8	9	1	4	6	1	2			
Vancomycin				6	10	13	6						
Clindamycin	24	10						1					
Tobramycin					1	26	8						
Gentamicin						7	20	8					
Kanamycin								15	12	8			
Streptomycin								17	8	4	0	6	

^a Number of isolates susceptible at a given concentration.

TABLE 3. Enhancement of the effects of anti-staphylococcal antibiotics by aminoglycosides on 35 isolates of *S. aureus*

Group I antibiotic	No. of isolates with $\geq$ fourfold decrease in MIC of group I antibiotic											
	+ Gentamicin (μ g/ml)			+ Tobramycin (μ g/ml)			+ Kanamycin (μ g/ml)			+ Streptomycin (μ g/ml)		
	5	2.5	1.25	5	2.5	1.25	20	10	5	20	10	5
Nafcillin	25	15	3	30	21	13	25	13	14	14	12	5
Vancomycin	29	9	6	28	10	4	19	1	2	6	5	0
Oxacillin	16	11	6	22	13	9	25	12	5	9	8	1
Methicillin	16	1	0	8	0	0	8	0	0	3	0	0
Cephalothin	35	26	14	29	24	22	35	30	26	19	14	10
Cefazolin	35	30	26	34	31	25	34	29	28	24	23	21
Clindamycin	34	32	16	31	22	12	33	28	6	1	5	0

did not differ significantly when the antibiotic combinations were compared with nafcillin alone. This again is in marked contrast to enterococci (9, 10). It appears that the addition of gentamicin or tobramycin in a relatively low concentration to nafcillin significantly improved the early killing of *S. aureus* due to the rapid bactericidal action of these aminoglycosides.

Using the agar dilution antibiotic susceptibility test with a heavy inoculum, we compared the in vitro activity of seven anti-staphylococcal antibiotics alone and in combination with four aminoglycosides. There was enhancement of anti-staphylococcal activity for a large number of drug combinations. Less uniform enhancement of activity was observed when oxacillin or methicillin was combined with the aminoglycosides. Streptomycin was the least effective aminoglycoside, a finding also reported in a much smaller study by M. J. Carter and N. Miller (Abstr. Annu. Meet. Amer. Soc. Microbiol. 1973, M88, p. 88).

Recently, encouraging preliminary results regarding the therapy with combinations of penicillins and the newer aminoglycosides in experi-

mental staphylococcal infection in mice were reported (1) (R. Greenman, R. Steigbigel, and J. Remington, Clin. Res. 22:185A, 1974). With these and the in vitro results reported here, it seems that the comparative efficacy of anti-staphylococcal antibiotics alone and in combination with the newer aminoglycosides should be studied in experimental staphylococcal endocarditis. If positive results are obtained, controlled clinical trials are warranted.

ACKNOWLEDGMENTS

This investigation was supported by Public Health Service research grant AI-09755 from the National Institute of Allergy and Infectious Diseases and by the Morton Hamburger Memorial Fund.

ADDENDUM IN PROOF

After this manuscript was submitted, M. A. Sande reported (Prog. Abstr. Intersci. Conf. Antimicrob. Ag. Chemother., 14th, San Francisco, Calif., Abstr. 76, 1974) that *S. aureus* was eradicated sooner with nafcillin plus gentamicin than with nafcillin alone from vegetations of experimental endocarditis in rabbits.

LITERATURE CITED

- Campos, M. E., S. Rabinovich, and I. M. Smith. 1974.

- Therapy of experimental staphylococcal infection with antibiotic combinations. *J. Lab. Clin. Med.* **83**:241-248.
2. Carleton, J., and M. Hamburger. 1963. Unmasking of false-negative blood cultures in patients receiving new penicillins. *J. Amer. Med. Ass.* **186**:157-159.
 3. Cluff, L. 1974. Bacterial endocarditis, p. 761-765. *In* M. M. Wintrobe, G. W. Thorn, R. D. Adams, E. Braunwald, K. J. Isselbacher, R. G. Petersdorf (ed.), *Harrison's principles of internal medicine*, 7th ed. McGraw Hill Book Co., New York.
 4. Hamburger, M. 1964. Treatment of bacterial endocarditis. *Mod. Treat.* **1**:1003-1015.
 5. Hoeprich, P. D. 1972. Infective endocarditis, p. 1045-1060. *In* P. D. Hoeprich (ed.), *Infectious diseases*. Harper and Row, Publishers, Inc., Hagerstown, Md.
 6. Rabinovich, S., I. M. Smith, and L. E. January. 1968. The changing pattern of bacterial endocarditis. *Med. Clin. N. Amer.* **52**:1091-1101.
 7. Smith, I. M., and A. B. Vickers. 1960. Natural history of 338 treated and untreated patients with Staphylococcal septicemia (1936-1955). *Lancet* **1**:1318-1322.
 8. Steers, E., E. L. Foltz, and B. S. Graves. 1959. An inocula replicating apparatus for routine testing of bacterial susceptibility to antibiotics. *Antibiot. Chemother.* **9**:307-311.
 9. Watanakunakorn, C. 1971. Penicillin combined with gentamicin or streptomycin: synergism against enterococci. *J. Infect. Dis.* **124**:581-586.
 10. Watanakunakorn, C., and C. Bakie. 1973. Synergism of vancomycin-gentamicin and vancomycin-streptomycin against enterococci. *Antimicrob. Ag. Chemother.* **4**:120-124.
 11. Watanakunakorn, C., and J. S. Tan. 1973. Diagnostic difficulties of staphylococcal endocarditis in geriatric patients. *Geriatrics* **28** (May):168-173.
 12. Watanakunakorn, C., J. S. Tan, and J. P. Phair. 1973. Some salient features of *Staphylococcus aureus* endocarditis. *Amer. J. Med.* **54**:473-481.
 13. Wilson, R., and M. Hamburger. 1957. Fifteen years' experience with staphylococcus septicemia in a large city hospital. *Amer. J. Med.* **22**:437-457.