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Treatment of Experimental Endocarditis Due to Penicillin-Resistant *Streptococcus pneumoniae*

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Using two strains of pneumococci for which MICs of penicillin were 1 and 4 μg/ml, those of cefotaxime were 0.01 and 0.5 μg/ml, and those of teicoplanin were 0.01 and 0.1 μg/ml, we studied the efficacy of different dosages of penicillin, cefotaxime, and teicoplanin in the treatment of experimental pneumococcal endocarditis in rabbits. Animals treated with dosages of penicillin G procaine needed to achieve levels in serum near the MIC for pneumococci showed a significant reduction in $\log_{10}$ CFU per gram of vegetation, as compared with the control ($P < 0.001$), although only 20% of the animals showed sterile vegetations. When levels of penicillin in serum were in the range of three- to fourfold the MIC, a greater reduction in $\log_{10}$ CFU per gram of vegetation was seen, and 88% of the animals showed sterile vegetations. Only the regimen of penicillin that provided concentrations in serum above the MIC throughout the interval between two doses provided constant sterilization of the cardiac vegetations. Dosages of cefotaxime and teicoplanin selected to achieve concentrations in serum equivalent to that obtained in humans during treatment resulted in levels of antimicrobial agents in serum hundreds or thousands of times higher than the MICs for the infecting strains. In terms of antimicrobial efficacy, cefotaxime and teicoplanin were equivalent to regimens with high dosages of penicillin.

Pneumococcal infection continues to be an important cause of morbidity and mortality. Death rates for bacteremic pneumococcal pneumonia remain regrettably high, particularly in the elderly and in patients with a chronic underlying disease (15). Once thought invariable, the susceptibility of *Streptococcus pneumoniae* to penicillin is no longer guaranteed. As a matter of fact, physicians and microbiologists in the 1990s must face the increasing challenge of resistant pneumococci all over the world (11). Recent surveys indicate that the prevalence of antimicrobial resistance among pneumococci is increasing in Spain and other European countries and Israel (1, 13). In the United States, resistance of *S. pneumoniae* to penicillin has been observed among 5 to 10% of isolates (12) but, depending on the geographic area, rates as high as 26% have been found (1, 2, 12). Worrisome is the finding that 70% of penicillin-resistant strains show multiple resistance to non-beta-lactam antibiotics (13).

The optimal treatment for bacteremic infections caused by resistant pneumococci is unknown, and the role of penicillin remains controversial (11, 12). There is currently a scarcity of data for guiding antimicrobial therapy of resistant pneumococcal infections, and no clear-cut recommendation can be made on the basis of prospective studies (12). Using the experimental model of infectious endocarditis, we have assessed the therapeutic efficacy of different dosages of penicillin and other antimicrobial agents against moderately and highly resistant *S. pneumoniae* in vivo.

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MATERIALS AND METHODS

In vitro studies. Two strains of *S. pneumoniae* isolated from patients with bacteremic pneumonia were used; strain A was moderately resistant to penicillin, while strain B exhibited high resistance to penicillin. These strains were selected to assess the impact of resistance on the activity of different dosages of penicillin in vivo. The microorganisms were stored frozen at −40°C in skim milk until tested for MICs by a microdilution method with cation-supplemented Mueller-Hinton broth containing 5% whole defibrinated sheep blood (5). Broth cultures in the log phase of growth were used to prepare inocula of 5.5×10^5 CFU of pneumococci per ml, which were inoculated into serial twofold dilutions of antimicrobial agents. Wells containing inocula in serially diluted concentrations of antimicrobial agents were incubated for 18 to 24 h at 35°C in room air. The MIC was defined as the lowest concentration of antimicrobial agent in broth not allowing the visible growth of pneumococci.

The rate of killing induced by the antimicrobial agents used in the experimental design was determined by the time-kill method with inocula of 10^5 to 10^6 CFU of pneumococci per ml (19). Tests were performed in triplicate, and results are expressed as mean values.

Animal studies. Experimental aortic valve endocarditis was established in New Zealand White rabbits (weight, 2 to 3 kg) by modifications of the method described by Garrison and Friedman (9). In brief, animals were anesthetized with a mixture of ketamine and xylazine injected intramuscularly (i.m.). An incision was made in the neck, and the right carotid artery was exposed. The artery was ligated distally, and a sterile polyethylene catheter was inserted into the artery through a small incision and advanced proximally across the aortic valve into the left ventricle. A pressure-sensitive monitoring device was attached to the distal end of the catheter to ensure that the catheter tip crossed the aortic valve and entered the left ventricle. The end of the catheter was sealed and tied to the carotid artery, and the wound was closed over the catheter with surgical clips. The catheter was left in place throughout the experiment.

At 24 h after the insertion of the catheter, 1 ml of broth containing 10^7 to 10^8 CFU of *S. pneumoniae* per ml was injected into a peripheral ear vein. The presence of endocar-

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ditis was confirmed by a blood culture yielding pneumococci obtained before the initiation of antimicrobial therapy.

Antimicrobial therapy was started 24 h after the intravenous injection of *S. pneumoniae*. Animals were placed into treatment groups as follows. For strain A of *S. pneumoniae*, (i) the control included 10 animals that received no antimicrobial therapy; (ii) penicillin G procaine at 25,000 U/kg of body weight was administered twice daily i.m. to 10 animals; (iii) penicillin G procaine at 150,000 U/kg was given twice daily i.m. to 14 animals; (iv) cefotaxime at 25 mg/kg was administered i.m. three times daily to 14 animals; and (v) teicoplanin at 10 mg/kg was given i.m. twice daily to 10 animals. For strain B of *S. pneumoniae*, (i) the control included 9 animals that received no antimicrobial treatment; (ii) penicillin G procaine at 150,000 U/kg was administered i.m. twice daily to 14 animals; (iii) penicillin G procaine at 300,000 U/kg was given i.m. twice daily to 13 animals; (iv) cefotaxime at 50 mg/kg was administered i.m. three times daily to 10 animals; and (v) teicoplanin at 30 mg/kg was given i.m. twice daily to 10 animals.

Antimicrobial therapy was given for 3 days. At the end of treatment and at least 12 h after the administration of the last dose of antibiotics, animals were sacrificed by intravenous injection of sodium pentobarbital. The chest was opened, the heart was excised and opened, and the aortic valve vegetations were removed aseptically. To determine whether and to what degree endocardial infection was present, the entire valve and vegetations were weighed and homogenized in 0.9 ml of Mueller-Hinton broth by use of a stomacher. The homogenate was then placed in a tube containing 9 ml of broth and 0.1 ml of penicillinase (10^6 U). The number of CFU of *S. pneumoniae* per gram of vegetation was quantitated by a pour plate method with Mueller-Hinton agar supplemented with 5% whole defibrinated sheep blood. The results are expressed as $\log_{10}$ CFU of *S. pneumoniae* per gram of valve vegetation. Sterile vegetations were considered to have ≤ 1 $\log_{10}$ CFU/g.

Measurement of concentrations of antimicrobial agents in serum. Pharmacokinetic studies were performed with uninfected control animals to estimate dosages of penicillin needed to obtain levels of this antibiotic in the range of the MIC and three- to fourfold the MIC for the infecting organism. Blood samples were obtained on day 2 of therapy from rabbits with endocarditis 1 h after the administration of antibiotics and just before the next dose, to measure the peak and trough levels of the antimicrobial agents. Concentrations of penicillin, cefotaxime, and teicoplanin in serum were measured by a bioassay (7). Specifically, a modified agar well diffusion method was used to measure penicillin concentrations in serum from blood samples drawn from a rabbit ear (4). The accuracy of this method has been considered consistently better than 10% (error ± 10%) (4).

Analysis of results. Differences in mean $\log_{10}$ CFU of pneumococci per gram of vegetation were analyzed statistically by the Kruskall-Wallis test and the Wilcoxon two-sample test, and the results were corrected for multiple comparisons.

RESULTS

The MICs determined for the two strains of *S. pneumoniae* by the microdilution method were as follows. For strain A, the penicillin MIC was 1 μg/ml, the cefotaxime MIC was 0.01 μg/ml, and the teicoplanin MIC was 0.01 μg/ml. For strain B, the penicillin MIC was 4 μg/ml, the cefotaxime MIC was 0.5 μg/ml, and the teicoplanin MIC was 0.1 μg/ml. Figures 1 and 2 show the results of studies of in vitro killing by the antibiotics used in the experimental design. For strain A, all antibiotics produced a rapid bactericidal effect. Although the bactericidal

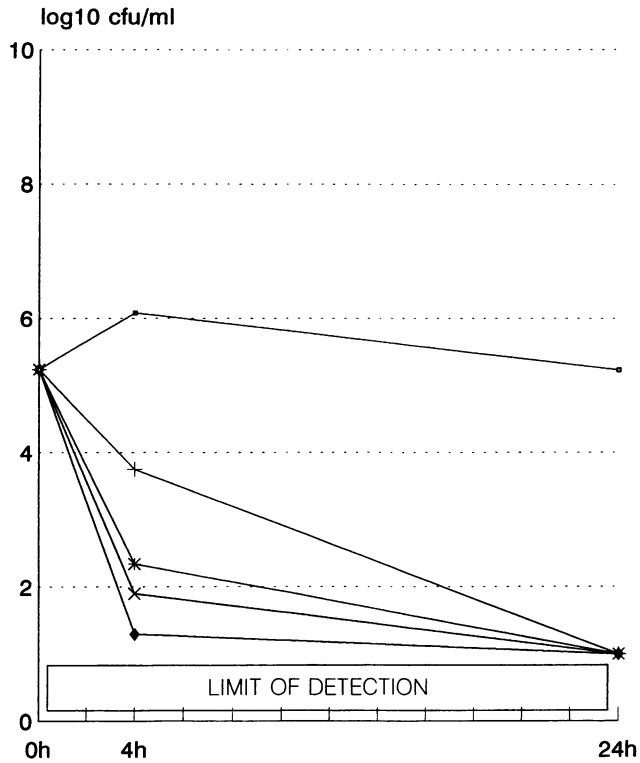

FIG. 1. Kinetics of killing of *S. pneumoniae* moderately resistant to penicillin (strain A; MIC, 1 μg/ml). Symbols: □, control; *, penicillin at 4 μg/ml; ◆, teicoplanin at 5 μg/ml; +, penicillin at 1 μg/ml; ×, cefotaxime at 45 μg/ml.

effect produced by penicillin at a concentration equivalent to the MIC at 4 h was smaller than that produced by penicillin at a higher concentration, after 24 h of incubation the bactericidal activities at both dosages seemed the same. For strain B, only penicillin at a concentration threefold the MIC, cefotaxime, and teicoplanin produced a decline in the number of pneumococci per milliliter to the limits of detection after 24 h of incubation.

The course of untreated experimental pneumococcal endocarditis in rabbits was that of an acute infection with continuous bacteremia, meningitis with quadriparesis and stiffness of the neck, and mortality rates near 75% within 72 h of inoculation. All the antimicrobial regimens used were able to suppress early mortality.

Tables 1 and 2 show the results of the treatment of experimental endocarditis caused by strain A and strain B of *S. pneumoniae*, respectively. Peak and trough concentrations of antimicrobial agents in serum are also shown. For strain A, a dosage of penicillin that achieved a level in serum equivalent to the MIC had a significant although minor effect on the number of organisms in vegetations. On the other hand, a dosage that achieved a higher level in serum, i.e., on the order of fourfold the MIC for the infecting strain, had a greater bactericidal effect. There were no significant differences between the results of treatment with the high dosage of penicillin and the results obtained with cefotaxime or teicoplanin therapy.

For strain B, penicillin at a concentration near or equivalent to the MIC also produced a significant reduction in the number of organisms in cardiac vegetations although, as seen in the strain A experiment, the bactericidal effect was significantly greater with the higher dosage. A concentration of penicillin

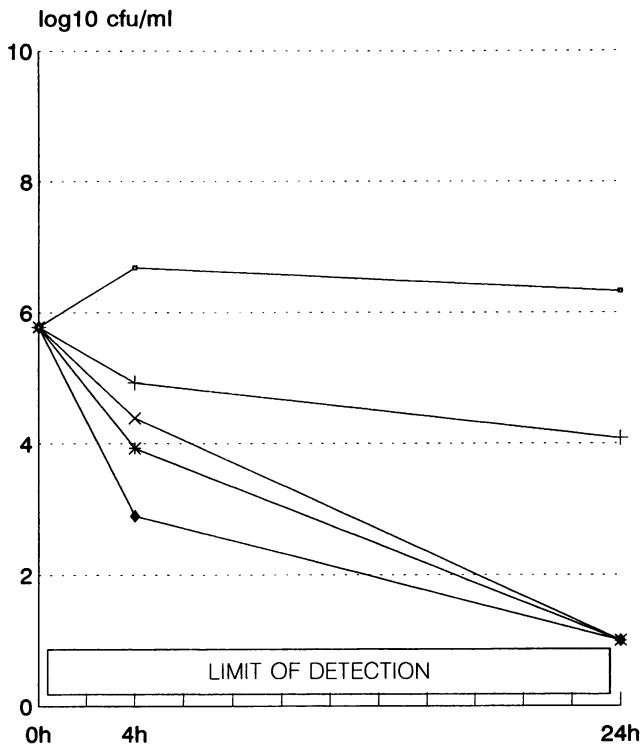

FIG. 2. Kinetics of killing of *S. pneumoniae* highly resistant to penicillin (strain B; MIC, 4 μg/ml). Symbols: □, control; *, penicillin at 12 μg/ml; ◆, teicoplanin at 10 μg/ml; +, penicillin at 4 μg/ml; ×, cefotaxime at 100 μg/ml.

three- to fourfold the MIC for the infecting strain produced sterile vegetations in all the treated animals. Equivalent results were observed for animals treated with cefotaxime and teicoplanin.

Overall, although in both experiments animals treated with dosages of penicillin that achieved levels in serum near the MIC showed a significant reduction in $\log_{10}$ CFU per gram of vegetation, only 20% of the rabbits had sterile vegetations. When levels of penicillin in serum were approximately three- to fourfold the MIC, antimicrobial efficacy reached a higher level, and 88% of the animals showed sterile vegetations.

DISCUSSION

In addition to host factors, the success of therapy with penicillin of pneumococcal infections caused by resistant strains may depend on the degree of resistance and the site of infection. In cases of meningitis, suboptimal concentrations of penicillin in the cerebrospinal fluid, despite the administration of high dosages of the antibiotic, result in a very high rate of failure, particularly when the MIC for the infecting strain is ≥1 μg/ml (18); hence, a consensus report has recommended treatment with cefotaxime or vancomycin for such infections (14). However, penicillin therapy may still be useful for patients with other bacteremic pneumococcal infections outside the central nervous system.

We have used the experimental model of endocarditis, a model characterized by continuous bacteremia, which is considered to be a rigorous test of antimicrobial efficacy, to assess the bactericidal activity of different dosages of penicillin and other antibiotics against *S. pneumoniae* with different levels of resistance to penicillin. In addition, because of the recent emergence of a growing number of cases of endocarditis caused by penicillin-resistant pneumococci (14a), the results of this therapeutic model may be of some interest for guiding therapy for such infections.

For the strains used in this experimental design, the magnitude of killing induced by penicillin in vitro seemed to be dose dependent, with the higher concentrations producing a greater effect after 4 h of incubation. For strain A, dosages of penicillin equal to the MIC induced complete bacteriolysis at 24 h.

The kinetics of killing observed in the in vitro studies accurately predicted the results of the treatment of experimental pneumococcal endocarditis. In this experimental model, penicillin showed concentration-dependent killing of pneumococci in cardiac vegetations. Concentrations of penicillin in serum near the MIC for the infecting strain produced a significant reduction in the number of organisms in cardiac vegetations. On the other hand, levels of penicillin in serum approximately three- to fourfold the MIC produced a profound reduction in the number of organisms in the vegetations, and most animals showed negative cultures. Recently, Barry et al., using a model of acute otitis in gerbils, showed that increasing doses of amoxicillin relative to the MICs were able to clear middle ear infections produced by penicillin-resistant pneumococci (3). These observations suggest that the amount of penicillin in the blood and the amount delivered to the site of infection are of paramount importance in determining the efficacy of penicillin against resistant *S. pneumoniae* (17) and are in agreement with the results of the original studies done 45 years ago by Eagle, who showed that concentrations of penicillin in serum twofold or higher than the MIC for the infecting organism were enough to promote the cure of pneumococcal pneumonia (6). These observations also provide an experimental explanation for the scarcity of reports that have documented the response to treatment with penicillin in patients with bacteremic pneumonia caused by moderately resistant pneumococci (15).

TABLE 1. Results of treatment of experimental endocarditis caused by *S. pneumoniae* moderately resistant to penicillin (strain A; MIC, 1 μg/ml)

Antibiotic	No. of animals tested	No. (%) of animals with sterile vegetations	Drug concn (μg/ml) (mean ± SD)		$\log_{10}$ CFU/g of vegetation (mean ± SD)
			Peak	Trough	
None (control)	10	0			9.9 ± 0.8[a]
Penicillin G procaine (25,000 U/kg every 12 h)	10	0	1.32 ± 0.6	<0.5	8.1 ± 0.1[a]
Penicillin G procaine (150,000 U/kg every 12 h)	14	10 (71)	3.7 ± 1.7	<0.5	1.6 ± 1.3[a]
Cefotaxime (25 mg/kg every 8 h)	14	9 (64)	45 ± 1.7	<6.2	1.9 ± 1.3[a]
Teicoplanin (10 mg/kg every 12 h)	10	10 (100)	5 ± 0.5	3 ± 0.4	≤1[a]

[a] $P < 0.001$.

TABLE 2. Results of treatment of experimental endocarditis caused by *S. pneumoniae* highly resistant to penicillin (strain B; MIC, 4 μg/ml)

Antibiotic	No. of animals tested	No. (%) of animals with sterile vegetations	Drug concn (μg/ml) (mean ± SD)		Log_{10} CFU/g of vegetation (mean ± SD)
			Peak	Trough	
None (control)	9	0			9.5 ± 0.2[a]
Penicillin G procaine (150,000 U/kg every 12 h)	14	5 (36)	3.72 ± 0.8	<0.5	4.6 ± 0.8[a]
Penicillin G procaine (300,000 U/kg every 12 h)	13	13 (100)	12.5 ± 2.1	6 ± 0.5	≤1[a]
Cefotaxime (50 mg/kg every 8 h)	10	10 (100)	104 ± 5.2	<6.2	≤1[a]
Teicoplanin (30 mg/kg every 12 h)	10	10 (100)	10.9 ± 1.9	3.1	≤1[a]

[a] $P < 0.001$.

In addition to the peak levels of penicillin in serum, the time during which levels in blood remain above the MIC for the infecting strain may be critical in determining the success of the antimicrobial regimen. In fact, although dosages of penicillin that produced levels in serum above the MIC for only 4 to 6 h (10, 16) resulted in a significant reduction in the number of microorganisms and eventually in the sterilization of vegetations, only dosages of penicillin that produced concentrations in blood above the MIC throughout the interval between two doses resulted in a consistent sterilization of cardiac vegetations. This phenomenon, observed for strain B in animals treated with a high-dosage penicillin regimen, was also observed in both groups of animals treated with teicoplanin, an antibiotic that has a long elimination half-life and that produced levels in serum above the MICs for the infecting strains throughout the interval between doses.

Both cefotaxime and teicoplanin showed rapid bactericidal activity against resistant pneumococci. As shown by others (8), teicoplanin resulted in a reduction in the viability of resistant pneumococci of 3.0 to 4.0 log_{10} CFU/ml within 4 h of exposure. In vivo, concentrations of teicoplanin that can be easily obtained during its administration to humans produced a uniform sterilization of vegetations. It must be taken into account, however, that the dosages of cefotaxime and teicoplanin selected for these experiments produced concentrations in serum hundreds or thousands of times higher than the MICs for the respective strains.

Although these observations cannot be extrapolated directly to the clinical arena, they suggest a role for penicillin in the therapy of bacteremic pneumococcal infections outside the central nervous system. In addition, we believe that clinical trials of the efficacy of teicoplanin in the treatment of infections caused by pneumococci resistant to penicillin are warranted.

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