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Antibiotic Therapy of Experimental *Staphylococcus epidermidis* Endocarditis

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Endocarditis was produced in rabbits with a methicillin-resistant *Staphylococcus epidermidis* isolate. Subpopulations resistant to other semisynthetic penicillins and cephalosporins were detected in the isolate. Their presence was probably responsible for the increase in minimum bactericidal concentrations and minimum inhibitory concentrations when tests with high inocula, rather than low inocula were pursued. Rabbits were treated for either 2 or 7 days with nafcillin, cephalothin, cefamandole, vancomycin, rifampin, or gentamicin. Spontaneous death was uncommon in either controls (84% survival) or treated animals (80 to 94% survival). There was no significant difference in the number of bacteria in vegetations of rabbits treated for 7 days with cephalothin, cefamandole, nafcillin, or no antibiotic (control). There was a significant reduction in total bacteria in vegetations of rabbits given vancomycin, gentamicin, or rifampin for 7 days as compared with cephalothin, cefamandole, nafcillin or control. Gentamicin or rifampin sterilized significantly more vegetations after 7 days than cephalothin, cefamandole, nafcillin, or control; rifampin was more effective in sterilizing vegetations than either gentamycin or vancomycin after 2 days. Mutants resistant to 10 μg of rifampin per ml comprised the total bacterial population cultured from vegetations of 2 of 17 rabbits treated with this antibiotic for 7 days; there was no change in the susceptibility of vegetation isolates to other antibiotics. Rifampin, vancomycin, or gentamicin may prove to be more effective in humans than cephalosporins or semisynthetic penicillins in the treatment of methicillin-resistant *S. epidermidis* endocarditis.

The development of prosthetic valve endocarditis (PVE) is one of the most serious complications after the placement of an artificial heart valve. Mortality rates of over 60% have been reported, and many of the survivors have required removal of the infected prosthesis with reimplantation of a new valve for eradication of the infection (10, 12). *Staphylococcus epidermidis* is the most common organism causing PVE, being responsible for over one-third of the cases (6). In vitro studies have demonstrated that over 60% of the SE isolates from cases of PVE contain subpopulations resistant to methicillin as well as to other penicillinase-resistant penicillins and cephalosporins (2). The presence of these subpopulations may be responsible for the observed failure of medical therapy in cases of PVE. Gentamicin, rifampin, and vancomycin have shown promise in vitro (2) and rifampin in vivo (4, 8) against *S. epidermidis*; these antibiotics may represent an improvement over current therapy. To compare the efficacy of single antibiotics in the treatment of *S. epidermidis* PVE, a rabbit model of experimental endocarditis was used. The following study evaluated the relative therapeutic efficacy of these antibiotics as assessed by a reduction in bacterial density and the selection of resistant bacterial populations in infected vegetations.

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MATERIALS AND METHODS

Production of endocarditis. Endocarditis was produced by a modification of previously described methods (3). A 19-gauge polyethelene catheter (Deseret Co., Salt Lake City, Utah) was introduced through the carotid artery into the left ventricle of 2-kg New Zealand white rabbits. The catheter, with its inner metal guide wire, was left in place for the entire study period so as to more closely simulate a rigid intracardiac prosthesis. Seventy-two hours after catheterization, the animals were challenged intravenously with a 1-ml suspension containing an average of $10^{10.1}$ (range, $10^{9.7}$ to $10^{10.5}$) colony-forming units (CFU) of a methicillin-resistant *S. epidermidis*. Antibiotic therapy was initiated 4 days after the bacterial challenge. Some infected untreated control rabbits were sacrificed at the beginning of therapy; the rest of the control animals were sacrificed at the same time as treated animals, after either 2 or 7 days of therapy. Any animal dying before the end of therapy was

autopsied as well. To minimize the effect of circulating antibiotics, the last antibiotic dose was given at least 12 h before sacrifice. Any vegetation material present in the aortic leaflets, around the catheter, or on the myocardium was resected, weighed wet, homogenized, and serially diluted in normal saline. The diluted material was plated on duplicate Mueller-Hinton agar plates (BBL Microbiology Systems, Cockeysville, Md.) with and without added antibiotics to detect the presence of antibiotic-resistant bacteria.

Antibiotic administration. The following antibiotics were administered to rabbits for either 2 or 7 days: gentamicin sulfate (Schering Co., Kenilworth, N.J.), vancomycin hydrochloride (Eli Lilly Co., Indianapolis, Ind.), rifampin (Dow Pharmaceuticals, Indianapolis, Ind.), cefamandole nafate (Eli Lilly), cephalothin sodium (Eli Lilly), and nafcillin sodium (Wyeth Laboratories, Philadelphia, Pa.). All antibiotics were given intramuscularly except for vancomycin which was injected intravenously. Rifampin and vancomycin were administered every 12 h whereas gentamicin, nafcillin, cefamandole, and cephalothin were given every 8 h.

Organism. The methicillin-resistant *S. epidermidis* used was a penicillinase-producing isolate obtained from a patient with sepsis secondary to an infected dialysis shunt (kindly supplied by Frank Lowy, Montefiore Hospital, New York, N.Y.). The organism was identified as *S. epidermidis* by its ability to ferment glucose and its failure to either coagulate rabbit plasma or use mannitol aerobically or anerobically. This organism had antibiotic susceptibility characteristics representative of methicillin-resistant *S. epidermidis* isolates from patients with PVE (2) and was chosen because it more reliably produced endocarditis in experimental animals than other isolates tested.

In vitro testing. The minimum inhibitory and bactericidal concentrations of the methicillin-resistant *S. epidermidis* to gentamicin, rifampin, vancomycin, nafcillin, cefamandole, and cephalothin were determined in triplicate by the microtiter method with inoculum sizes of 10^5 and 10^7 CFU/ml with Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.) as the diluent. Each minimal inhibitory concentration was read after 24 h of incubation at 37°C, and then 0.01 ml was subcultured from wells containing the lower inoculum or 0.001 ml was subcultured from wells containing the higher inoculum. After 48 h of incubation at 37°C, 99.9% kill was determined as the lowest antibiotic concentration yielding no growth on subculture of the wells containing the lower inoculum and <10 colonies from wells containing the higher inoculum.

The presence of resistant subpopulations in the challenge isolate was detected by plating 10^9 organisms grown overnight in Mueller-Hinton broth on Mueller-Hinton agar plates containing twofold dilutions of the antibiotic to be studied. Antibiotic concentrations ranged from the minimal inhibitory concentrations for the respective antibiotics to 100 μg/ml. Plates were incubated at 37°C for 72 h. Resistant subpopulations in the vegetations of both treated and untreated rabbits were sought by spreading 0.1 ml of homogenized vegetation on agar plates containing the antibiotic which that rabbit had received as therapy. The following concentrations were used for testing isolates from infected vegetations: nafcillin, cephalothin, cefamandole, or vancomycin, 20 μg/ml; rifampin, 10 μg/ml; and gentamicin, 5 μg/ml.

Antibiotic serum concentrations were measured by the agar disk diffusion assay with either *Sarcina lutea* ATCC 9341 (rifampin) or *Bacillus subtilis* ATCC 6633 (gentamicin, vancomycin, nafcillin, cephalothin, and cefamandole) as the indicator organism as previously described (2). Serum samples for antibiotic concentration were obtained on day 3 of therapy at intervals after the administration of the drugs as shown in Table 2.

Serum samples for measuring bactericidal titers were also obtained on day 3 of therapy at the peak of antibiotic activity. This was 0.5 h after the administration of cephalothin, cefamandole, nafcillin, vancomycin, or gentamicin and 2 h after the dose of rifampin. Titers were determined in triplicate by the microtiter twofold dilution method with inoculum sizes of 10^5 and 10^7 CFU/ml with rabbit serum as the diluent. Subculture of 0.01 ml from microtiter wells containing the lower inoculum and 0.001 ml from the wells with the higher inoculum to Mueller-Hinton agar was performed after incubation for 24 h at 37°C. The bactericidal titers (99.9% kill) were read as the highest dilution of serum that resulted in no growth on agar after 48 h of incubation for the lower inoculum or <10 colonies on agar for the higher inoculum.

RESULTS

The minimum inhibitory concentrations and minimum bactericidal concentrations of the antibiotics against the *S. epidermidis* isolate at the two inoculum sizes are shown in Table 1. Serial plating of the organism on agar containing increasing concentrations of antibiotics revealed the presence of subpopulations resistant to 100 μg of methicillin or nafcillin per ml and to 50 μg of cephalothin or cefamandole per ml. Mutants resistant to rifampin were present at a low frequency (5×10^{-7}). No colonies were detected at concentrations of gentamicin or vancomycin greater than the minimum inhibitory concentrations. The presence of resistant subpopulations was reflected in a fourfold or greater decrease in susceptibility of the isolate to nafcillin or the

TABLE 1. *Antibiotic susceptibility of the challenge S. epidermidis isolate*

Antibiotic	MIC[a]		MBC[a]	
	10^{5b}	10^7	10^5	10^7
Gentamicin	<0.01	<0.01	<0.01	<0.01
Rifampin	0.05	0.1	0.2	0.4
Vancomycin	3.1	6.3	3.1	6.3
Nafcillin	3.1	12.5	3.1	25.0
Cefamandole	3.1	6.3	6.3	25.0
Cephalothin	1.6	6.3	3.1	12.5

[a] MIC, Minimum inhibitory concentration (micrograms per milliliter); MBC, minimum bactericidal concentration (micrograms per milliliter).

[b] Inoculum size (CFU per milliliter).

cephalosporins at the high as compared with the low inoculum, whereas there was no more than a twofold difference due to inoculum size with rifampin, gentamicin, or vancomycin.

Table 2 shows the antibiotic dosages and mean serum antibiotic concentrations at the times indicated after injection. These serum levels are similar to those obtained in humans.

A total of 158 rabbits were included in the study. The mean weight of the vegetations for all the groups treated for 7 days was 133 mg. There was no difference between the vegetation weights in control and any treatment group ($P > 0.05$, Student's t test). The median (range) of CFU per gram in the controls at the initiation of therapy was $10^{7.9}$ ($10^{6.0}$ to $10^{10.4}$). Gross inspection of the heart demonstrated that infected vegetations were bulky, friable, and, in many cases, occluding the outflow tract. The infectious process was not confined to the valve leaflets; both the valve ring and myocardium were also involved.

The percentage of control rabbits surviving the 11 days between challenge and sacrifice was 84%. The survival of treated rabbits was similar, varying from 80% (nafcillin) to 92% (gentamicin). There were no statistically significant differences in survival among these groups ($P > 0.05$, Fisher's exact test).

The effect of 7 days of therapy on bacterial density in infected vegetations is illustrated in Fig. 1. There was no significant difference between the number of bacteria in vegetations of control rabbits and those given either nafcillin, cephalothin, or cefamandole ($P > 0.05$; Wilcoxon rank sum test). There was a significant reduction in the number of CFU of *S. epidermidis* per gram of vegetation in rabbits treated with either

TABLE 2. *Antibiotic dose and serum levels in rabbits given therapy for experimental S. epidermidis endocarditis*

Antibiotic	Dose (mg/kg)	Antibiotic serum concn (μ/ml) at intervals (h) after antibiotic dose:				
		0.5	2	4	6	12
Gentamicin	3.5	9.5 ± 3.2[a]	2.4 ± 1.7	<0.6	ND[b]	ND
Rifampin	20	ND	11.0 ± 3.5	ND	ND	1.73 ± 0.73
Vancomycin	30	42.4 ± 14.4	22.5 ± 1.4	3.2 ± 0.63	<3.0	ND
Nafcillin	100	34.1 ± 12.6	6.2 ± 1.2	<1.0	ND	ND
Cefamandole	30	45.6 ± 16.5	10.3 ± 1.5	<0.5	ND	ND
Cephalothin	90	30.8 ± 5.5	6.4 ± 0.78	<0.8	ND	ND

[a] Mean ± standard deviation.
[b] ND, Not done.

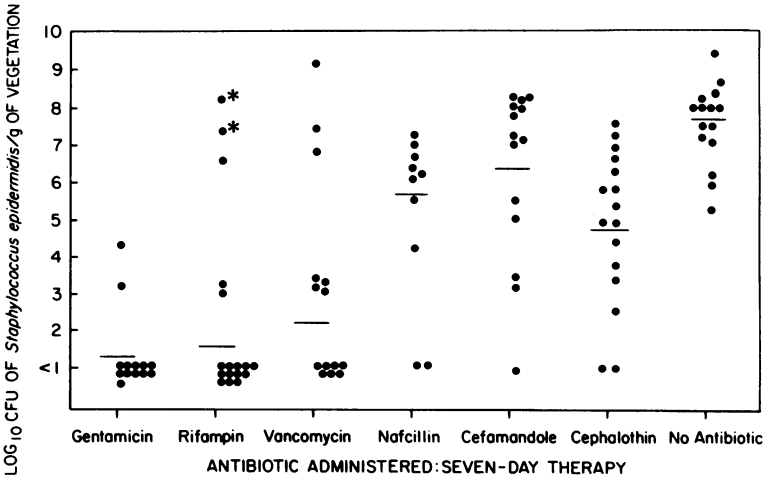

FIG. 1. *The $\log_{10}$ CFU of S. epidermidis in aortic valve vegetations of rabbits treated for 7 days with various antibiotics. The horizontal line in each treatment group is the mean $\log_{10}$ CFU per gram. The asterisks denote populations resistant to 10 μg of rifampin per ml. Each dot represents all of the vegetation tissue from one rabbit.*

vancomycin, gentamicin, or rifampin in comparison with rabbits given nafcillin, cephalothin, cefamandole, or no antibiotic ($P < 0.05$, Wilcoxon rank sum test). The percentage of rabbits with sterile vegetations (<10 CFU/g) was significantly greater in those animals given either rifampin (71% sterile) or gentamicin (85%) than in those receiving nafcillin (20%) cephalothin (13%), cefamandole (7%), or no antibiotic (0%; $P < 0.01$, Fisher's exact test). There were significantly more rabbits with sterile vegetations which received vancomycin (50% sterile) than cefamandole or cephalothin ($P < 0.05$) but not nafcillin. A high density ($\geq 10^5$ *S. epidermidis*/g) of bacteria was also significantly more frequent in rabbits treated with no antibiotic (21/21, 100%), nafcillin (7/10, 70%), cefamandole (11/14, 85%), or cephalothin (11/16, 69%) than in those receiving vancomycin (3/14, 21%), rifampin (3/17, 18%), or gentamicin (0/13, 0%; $P < 0.05$, Fisher's exact test).

In two out of three rabbits treated for 7 days with rifampin and with refractory infection, the total bacterial population in the vegetation was resistant to 10 μg of the antibiotic per ml. Rabbits receiving nafcillin had resistant subpopulations in vegetations but no subpopulations resistant to gentamicin, vancomycin, cephalothin, or cefamandole were detected. However, subpopulations resistant to nafcillin occurred with the same frequency in vegetations that they did in the challenge inoculum.

The median and range of the serum bactericidal titers are shown in Table 3. Rabbits treated with either gentamicin or rifampin demonstrated the highest median titers. Although a median peak titer of at least 1:8 was seen in rabbits treated with cephalothin, cefamandole, or nafcillin at the lower inoculum, a high inoculum resulted in a fall in titer of at least three dilutions for every rabbit. This fall in titer was not seen in rabbits treated with rifampin, gentamicin, or vancomycin.

A group of 39 rabbits was treated with either gentamicin, rifampin, or vancomycin for 2 days instead of 7 days. The dose, routes of administration, and infection schedules were similar to those given in the 7-day therapy course. The results of 2-day therapy are shown in Fig. 2. Rifampin was associated with a significant reduction in the number of *S. epidermidis* in vegetation tissue ($P < 0.05$, Wilcoxon rank sum test) and with a significant incidence of vegetation sterilization ($P < 0.05$; Fisher's exact test) as compared with either controls, vancomycin, or gentamicin. There was no difference between the controls and the animals treated with either gentamicin or vancomycin.

TABLE 3. *Serum bactericidal titers in rabbits with S. epidermidis endocarditis*

Antibiotic	Median peak serum bactericidal titer (range)	
	10^{5a}	10^7
Gentamicin	32 (8–64)	16 (8–32)
Rifampin	64 (8–128)	32 (8–64)
Vancomycin	16 (4–32)	8 (4–8)
Nafcillin	16 (8–32)	2 (<2–4)
Cefamandole	16 (6–32)	2 (<2–4)
Cephalothin	16 (16–32)	<2 (<2–4)

[a] Inoculum size (CFU per milliliter).

DISCUSSION

The animal model of *S. epidermidis* endocarditis used in this study simulated human PVE in several ways. First, a rigid catheter was left in place for the entire period, producing continuous trauma to the valve ring and endocardium and possibly interfering with normal local host defenses. This simulated the constant abrasion of perivalvular tissue by the rigid prosthetic valve sewing ring. Second, there was a low incidence of spontaneous deaths in both controls and treated animals, even though therapy was delayed for 4 days after challenge. This is similar to the subacute nature of *S. epidermidis* PVE in humans and contrasts with the high early mortality seen in experimental *Staphylococcus aureus* or *Streptococcus sanguis* endocarditis (9, 11). Third, the gross pathological findings were in agreement with human PVE where vegeta-

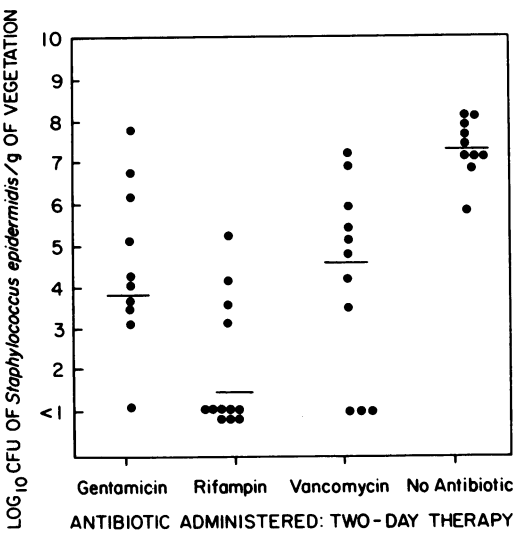

FIG. 2. *The $\log_{10}$ CFU of S. epidermidis in aortic valve vegetations of rabbits treated for 2 days with various antibiotics. The horizontal line in each treatment group is the mean $\log_{10}$ CFU per gram. Each dot represents all of the vegetation tissue from one rabbit.*

tions are large, friable, and have a tendency to invade the valve ring and myocardium (5). Thus, results of therapy in this model may be applicable to human *S. epidermidis* PVE.

Gentamicin, rifampin, or vancomycin given for 7 days were significantly more effective than nafcillin or cephalosporins in reducing the number of bacteria in vegetation tissue. These findings are in agreement with in vitro data showing that these three agents are the most active antibiotics against methicillin-resistant *S. epidermidis* and that nafcillin selects resistant subpopulations while the bacteria are exposed to the drug (2). The reasons for failure of therapy with cephalosporins are not clear. There are several possible explanations. First, there was a long interval between cephalosporin doses with unmeasurable antibiotic levels. However, there were equally long antibiotic-free intervals in animals given gentamicin or vancomycin yet therapy with these antibiotics was successful. Second, subpopulations resistant to cephalothin and cefamandole were present in the challenge isolate. Even though no cephalosporin-resistant subpopulations were detected in infected vegetations after therapy, it is possible that resistant subpopulations were selected during exposure to the drug and reverted to the parent phenotype when the drug concentration in the serum decreased. The reversion of methicillin-resistant *S. epidermidis* isolates to their parent phenotype occurs after selection of semisynthetic penicillin-resistant subpopulations if the inducing antibiotic is removed (2).

Mutants highly resistant to rifampin emerge rapidly when *S. epidermidis* are incubated in rifampin-containing broth (2). In our study, two out of three rabbits with persistent infection after rifampin therapy had endocarditis with rifampin-resistant mutants. However, these were the only 2 of 17 rabbits treated with rifampin for 7 days which had resistant organisms after therapy. The low incidence of rifampin-resistant *S. epidermidis* endocarditis may suggest either that mutants are less virulent than the wild isolate or that the number of organisms in the vegetations was low enough so that mutants occurring at a low frequency would not be selected by therapy. It has been shown in vitro that rifampin-resistant *S. epidermidis* mutants are found at a frequency of approximately one per 10^7 bacteria (2). This low rifampin resistance frequency was also found in our challenge isolate.

Treatment for only 2 days showed that rifampin was more rapidly effective than either gentamicin or vancomycin; 8 of 12 (67%) animals receiving rifampin had sterile vegetations after 48 h whereas only 3 of 11 (27%) rabbits treated with vancomycin and 1 of 10 (10%) given gentamicin had sterile vegetations. The greater efficacy of rifampin was possibly due to the low minimum bactericidal concentration of this antibiotic for *S. epidermidis*, the persistence of therapeutic rifampin blood levels (mean, 1.7 μg/ml) 12 h after a dose, and the ready diffusability of rifampin into tissue and body fluids (1, 7).

Rabbits treated with gentamicin or rifampin had the highest median peak serum bactericidal titers at both low and high inoculum sizes, and this correlated with therapeutic efficacy. Although all the treatment groups had median titers of 1:8 at the lower bacterial inoculum size, rabbits given the cephalosporins or nafcillin had at least an eightfold decrease in titer when serum was incubated with the higher inoculum of *S. epidermidis* such that no rabbit had a peak bactericidal titer of >1:4. Serum bactericidal titers in humans with *S. epidermidis* PVE should probably be performed with both a high and a low inoculum size to minimize this inoculum effect.

Because higher serum antibiotic levels can be maintained in humans by more frequent dosing than was possible in this study, our results showed only relative antibiotic efficacy for the treatment of methicillin-resistant *S. epidermidis* endocarditis. However, in view of the superiority demonstrated by gentamicin, rifampin, or vancomycin over nafcillin or cephalosporins, these antibiotics should be considered for initial therapy in the treatment of human *S. epidermidis* PVE. This is particularly true in view of the dismal results obtained in humans by using conventional therapy and the encouraging early successes achieved with the use of rifampin for treating patients with *S. epidermidis* PVE (4, 8).

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