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Influence of the Developmental State of Valvular Lesions on the Antimicrobial Activity of Cefotaxime in Experimental Enterococcal Infections

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Cefotaxime has little antimicrobial activity in vitro against most strains of enterococci, as measured by conventional MICs and MBCs. However, the MICs of cefotaxime against many enterococci are markedly reduced by the addition of serum to the test medium. To assess the relevance of this observation in vivo, we examined the efficacy of cefotaxime in experimental *Streptococcus faecalis* endocarditis. Since response to antimicrobial agents may vary with the degree of vegetation development, therapeutic efficacy was assessed both in rabbits with newly formed vegetations and in rabbits with well-developed endocardial lesions. Peak serum levels of cefotaxime (50.1 ± 20.0 μg/ml) exceeded the MIC in medium supplemented with serum (4 μg/ml), but not in Mueller-Hinton broth alone (>64 μg/ml). After 4 days of therapy, animals with newly formed lesions (therapy initiated 1 h after infection, transvalvular catheters removed) had lower mean vegetation bacterial titers than did untreated controls. Among animals with mature vegetations (therapy initiated 12 h after infection, catheters indwelling), the rate of mortality was significantly reduced by cefotaxime therapy. However, no difference in vegetation titers was observed. Thus, cefotaxime demonstrated antienterococcal activity within newly formed vegetations, but did not inhibit bacterial proliferation within well-established vegetations.

The activity in vitro of the broad-spectrum cephalosporins against most strains of enterococci is poor when assessed by conventional techniques. However, the interpretation of in vitro results may be difficult, since the outcome may vary when the test conditions are altered. For example, the MICs and MBCs of many antimicrobial agents may be modified by the addition of serum to the test medium, although usually only to a modest extent (14). Recent studies in vitro indicate that the MIC of cefotaxime against enterococci is markedly reduced when the test medium is supplemented with 5% lysed blood (18). These results suggest cefotaxime could have antienterococcal properties in vivo that are not detected by conventional assays in vitro. In addition, the clinical observation that enterococcal superinfection is rare with cefotaxime therapy, in contrast to moxalactam (20), also suggests possible activity in vivo.

To examine this possibility, the therapeutic efficacy of cefotaxime was assessed in rabbits with experimental enterococcal endocarditis. Since previous studies have shown that newly colonized valves may respond more rapidly to antimicrobial agents than mature vegetations, and that antimicrobial response may be retarded by the presence of a transvalvular catheter, two models of endocarditis were employed (1, 11, 13). In the first model, the susceptibility of newly colonized aortic valves to antimicrobial therapy in the absence of a catheter was evaluated. In the second model, mature cardiac vegetations were allowed to develop before therapy was initiated, and catheters were left in place throughout therapy. Thus, the first model was designed to detect the presence of any antienterococcal activity, whereas the second model evaluated activity in the setting of a more firmly established infection. The mortality rate and bacterial vegetation titers were used as measures of antimicrobial activity in vivo.

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MATERIALS AND METHODS

Test organism. *Streptococcus faecalis* strain 122, isolated from blood samples of a patient at San Francisco General Hospital with diagnosed enterococcal endocarditis, was selected for evaluation of in vitro sensitivities and for the inoculation of test animals. Stock cultures of this organism were prepared by coating sterile glass beads with an overnight growth of strain 122 in Mueller-Hinton broth, followed by freezing to −70°C. Before each experiment, 10 ml of Mueller-Hinton broth was inoculated with one glass bead.

Production of endocarditis. In the early lesion group, nonbacterial thrombotic endocarditis was established in 43 2-kg New Zealand rabbits by placement of a sterile polyethylene catheter across the aortic valve (12). After 1 h, 2×10^7 CFU of strain 122 in the early stationary phase of growth were injected through the catheter, which was then immediately withdrawn. The inoculum size was confirmed by plating serial 10-fold dilutions of the inoculum onto blood agar plates; colony counts were performed after 48 h of incubation at 35°C. Antimicrobial therapy was initiated 1 h after inoculation. In the mature lesion group, nonbacterial thrombotic endocarditis was established in 56 rabbits. After 24 h, animals were inoculated with 2×10^7 CFU of strain 122. Antibiotic therapy was initiated 12 h after inoculation. Unlike the early lesion group, the transvalvular catheters were left in place throughout the treatment period.

Administration of antibiotics. In the early lesion group, 14 rabbits received cefotaxime (100 mg/kg, intramuscularly [i.m.] every 6 h [q 6 h]), 8 were treated with ampicillin (100 mg/kg i.m. q 6 h), 8 received ampicillin (100 mg/kg i.m. q 6 h) in combination with gentamicin (3 mg/kg i.m. q 6 h), and 10

served as untreated controls. Six additional animals were sacrificed 1 h after inoculation to determine the number of bacteria in the vegetations at the onset of therapy. In the mature lesion study, 32 rabbits were randomly assigned to ampicillin (100 mg/kg i.m. q 6 h, 10 animals), ampicillin plus gentamicin (100 mg/kg plus 3 mg/kg i.m. q 6 h, 10 animals), or cefotaxime (100 mg/kg i.m. q 6 h, 12 animals). Nineteen rabbits served as untreated controls. Five additional animals were sacrificed 12 h after receiving the bacterial inoculum to ascertain the mean vegetation titer at the onset of therapy.

All antibiotics were given for a total of 18 doses per animal. Doses were selected to achieve serum levels equivalent to those considered therapeutic in humans (5, 7, 17). On day 3 of therapy blood samples were obtained 1 h and 6 h after drug administration for the determination of peak and trough antibiotic concentrations in the serum, as measured by an agar diffusion bioassay (19). A strain of *Bacillus globigii* was used to measure cefotaxime and ampicillin concentrations, and a strain of *Staphylococcus epidermidis* (ATCC 27626) served to assay gentamicin levels. Agar containing 75,000 U of penicillinase (Difco Laboratories) per ml was used to permit measurement of gentamicin concentrations in serum from rabbits treated with both ampicillin and gentamicin. To inactivate gentamicin, 50 mg of cellulose phosphate was combined with 0.5 ml of serum, vortexed, and centrifuged at 200 × *g* for 5 min. The supernatant was used to determine ampicillin levels (16).

In vitro measurement of antibiotic susceptibilities. The MIC and MBC of each antibiotic were determined in (i) Mueller-Hinton broth, (ii) a 50% mixture of broth and human serum, or (iii) 50% broth and serum inactivated by heating at 56°C for 30 min. Identical studies were performed with rabbit serum. An inoculum of 10^5 CFU of strain 122 (in the logarithmic phase of growth), per ml was used for each assay. The MIC was defined as the lowest concentration of antibiotic that prevented turbidity in the culture media after 24 h of incubation. A volume of 0.01 ml was subcultured from each clear tube onto a blood agar plate and incubated at 35°C for 24 h. Colonies were counted, and the MBC was defined as the concentration that killed 99.9% of the inoculum (19). Three additional strains of *S. faecalis* were similarly tested.

Evaluation of efficacy in vivo. After 108 h of treatment, surviving rabbits were sacrificed with intravenous pentobarbital 6 h after the last antibiotic dose. The aortic valves were excised aseptically, weighed, and then homogenized in a tissue grinder (Polytron; Brinkmann Instruments). Serial 10-fold dilutions of the homogenate were made in saline and plated onto blood agar. After 48 h of incubation at 35°C, colony counts were performed and expressed as the number of organisms per gram of tissue. Valves from rabbits sacrificed at the onset of therapy were processed similarly. Animals that died during therapy were refrigerated at 5°C within 6 h of death, to prevent bacterial overgrowth. Valves from these animals were processed as described above.

Statistical methods. The statistical significance of differences in mean vegetation titers was determined by Scheffe's test for multiple comparisons. The Fisher exact test was used to evaluate the significance of differences in mortality.

RESULTS

In vitro sensitivities. The MIC and MBC of cefotaxime for strain 122 were both greater than 128 μg/ml, when measured in Mueller-Hinton broth. When grown in broth combined with human serum, the MIC and MBC were reduced to 4 and 32 μg/ml, respectively. The addition of rabbit serum to the test medium effected a similar reduction in the MIC of cefotaxime, but failed to influence the MBC (MIC, 4 μg/ml; MBC, >128 μg/ml). In contrast, supplementation of MHB with human or rabbit serum produced no significant change in the MICs and MBCs of ampicillin (MIC, 1 μg/ml; MBC, 4 μg/ml) or gentamicin (MIC, 16 μg/ml; MBC, 64 μg/ml). The three additional strains of *S. faecalis* that were tested showed similar results.

Assessment of therapy in vivo. Animals treated with cefotaxime had a mean peak serum antibiotic concentration ± standard deviation of 50.1 ± 20.0 μg/ml and a mean trough concentration of 1.8 ± 1.9 μg/ml. For rabbits treated with ampicillin, the peak and trough serum concentrations were 61.7 ± 24.9 and 0.7 ± 2.0 μg/ml, respectively. Gentamicin-treated animals had peak and trough serum concentrations of 6.7 ± 2.2 and 0.5 ± 0.4 μg/ml, respectively.

Cefotaxime inhibited bacterial growth within newly formed cardiac vegetations (Table 1). In recently colonized valves (early lesion), the mean bacterial titer in the vegetations (mean ± standard deviation $\log_{10}$ CFU per gram) of animals receiving cefotaxime (4.90 ± 2.35) was significantly lower than that of controls (7.38 ± 1.94, $P < 0.05$). However, bacterial titers in cefotaxime-treated animals were significantly higher than titers in animals treated with either ampicillin alone (2.97 ± 1.40) or the combination of ampicillin and gentamicin (3.00 ± 1.98, $0.05 < P < 0.10$). The antimicrobial activity of cefotaxime was markedly diminished in animals with mature vegetations and indwelling transaortic valve catheters. In contrast to treatment of early lesions, cefotaxime failed to even retard the growth of bacteria within the vegetations (mean $\log_{10}$ titer = 8.19 ± 0.56, *P* not significant in comparison to controls), despite peak serum concentrations of cefotaxime that exceeded its MICs and MBCs in serum. The combination of ampicillin and gentamicin was most effective in lowering the number of bacteria within vegetations; the mean bacterial $\log_{10}$ titer after 4 days of therapy was 3.37 ± 1.32 versus 8.45 ± 1.85 for controls ($P \leq 0.01$). Ampicillin alone produced a static or inhibitory effect, with a mean $\log_{10}$ titer of 4.90 ± 0.92 as compared with bacterial log titers of 6.5 ± 1.0 at the onset of therapy (*P* not significant).

TABLE 1. Effect of antimicrobial therapy on the rate of mortality and the number of bacteria per gram of valve tissue

Lesions	Treatment	No. dead/ total animals	Titer (mean $\log_{10}$ ± SD)
Early	1 h after infection	0/6	4.08 ± 1.06[a]
	Control (4 days after inoculation)	0/13	7.38 ± 1.94
	Cefotaxime	1/14	4.90 ± 2.35[b]
	Ampicillin	0/8	2.97 ± 1.40[c]
	Ampicillin and gentamicin	0/8	3.00 ± 1.98[c]
Well developed	12 h after infection	0/5	6.50 ± 1.00
	Control (4 days after inoculation)	13/19	8.45 ± 1.85
	Cefotaxime	2/12[b]	8.19 ± 0.56
	Ampicillin	0/10[b]	4.90 ± 0.92[b]
	Ampicillin and gentamicin	0/10[b]	3.37 ± 1.32[d]

[a] $P < 0.05$ compared with cefotaxime or controls at 4 days.
[b] $P < 0.05$ compared with controls.
[c] $0.05 < P < 0.10$, cefotaxime versus ampicillin (± gentamicin).
[d] $P < 0.05$ compared with all other regimens.

The effect of cefotaxime therapy on mortality rate could only be assessed in animals with mature lesions, since a total of only one death occurred in any of the animals with early lesions, despite the high bacterial titers in vegetations of untreated controls. In animals with well-established infections (mature vegetations), mortality was markedly reduced in treated animals as compared with controls; 13 of 19 (68%) control animals died within 4 days of infection compared with only 2 of 12 animals (17%) receiving cefotaxime ($P < 0.02$). No deaths occurred among animals that received either ampicillin alone or ampicillin in combination with gentamicin. No statistical difference in mortality was detected between the individual treatment groups, despite the higher vegetation titers in animals receiving cefotaxime.

DISCUSSION

The rabbit model of endocarditis provides a means of assessing antimicrobial therapy in a controlled, in vivo setting. In general, past work has shown it to be predictive of antibiotic response in human endocarditis (3). In addition, the model can facilitate the investigation of more subtle aspects of antimicrobial activity, such as the discrimination in vivo of bacteriostatic agents from bactericidal agents or the effect of subinhibitory concentrations of antibiotics on bacterial virulence (4, 6, 9, 15). To assess the relevance of the recently observed activity in vitro of cefotaxime against *S. faecalis*, we used two rabbit models of endocarditis to detect and quantify the antienterococcal properties in vivo of cefotaxime.

Our results confirm the previously described activity in vitro of cefotaxime; both human and rabbit serum significantly reduced the concentration of cefotaxime required to inhibit growth in vitro of the enterococcal strains tested. The mechanism for this phenomenon is unknown. Recent studies in vitro indicate that a serum alpha-2 globulin may be responsible for the observed reduction in MICs (8). The effect on bactericidal activity was less dramatic. The addition of human serum to the test medium did lower the MBC to 32 μg/ml. However, rabbit serum had no apparent effect on the MBC of cefotaxime (>128 μg/ml) at the concentration tested (50%).

When evaluated in vivo, cefotaxime also exhibited antienterococcal properties. In animals with recently colonized aortic valves, in which vegetations are poorly developed and bacterial titers are low (4.08 ± 1.06) at the onset of therapy, cefotaxime retarded bacterial proliferation within the vegetation. However, it was less effective than either ampicillin alone or the combination of ampicillin and gentamicin. Interestingly, combination therapy was no more effective than ampicillin alone. The observed responses to either cefotaxime or ampicillin alone suggest that the early lesion may be singularly susceptible to antimicrobial agents, including those that are not bactericidal in vitro.

In well-established disease, cefotaxime proved totally ineffective in controlling bacterial growth within the vegetations, in contrast to ampicillin (which was bacteriostatic) or the bactericidal regimen of ampicillin in combination with gentamicin. The failure of cefotaxime to inhibit enterococcal growth within mature endocardial vegetations is not readily explained. Possible mechanisms for this lack of efficacy include the inability of either cefotaxime or its serum antimicrobial cofactor to penetrate well-established vegetations, drug or cofactor inactivation within the vegetation, or diminished susceptibility secondary to bacterial metabolic inactivity (2, 6). In addition, since cefotaxime failed to demonstrate any bactericidal activity in vitro when tested either in broth alone or in rabbit serum-supplemented medium, it is possible that antimicrobial agents that are not bactericidal in vitro may not be capable of inhibiting bacterial proliferation within well-developed vegetations.

While cefotaxime failed to retard bacterial growth in this setting, it did reduce the rate of mortality in rabbits with well-established vegetations. The observed reduction in mortality in the absence of a direct effect on vegetation titers is not readily explained. Additional studies (data not presented) indicate that levels of bacteremia may be lower in cefotaxime-treated animals, as compared with untreated controls. Conceivably, cefotaxime may attenuate the course of endocarditis by reducing the level of bacteremia or the incidence and severity of sepsis or by decreasing the frequency of secondary peripheral infection.

Although these results indicate that cefotaxime will not be effective in the treatment of enterococcal endocarditis, it may be adequate to prevent the emergence of enterococci as a cause of superinfection. Although at least one broad-spectrum cephalosporin has been associated with enterococcal superinfection, this has not been reported with use of cefotaxime (10, 20, 21). The ability of cefotaxime to inhibit enterococcal growth in vivo under certain conditions may help explain this observation.

These results reaffirm the concept that antimicrobial efficacy in vivo is determined by multiple factors. Although conventional assays of antibiotic susceptibility are generally adequate predictors of therapeutic outcome, both host factors and the degree to which an infection has evolved may influence the antimicrobial response in vivo. In addition these data support the suggestion of Reimer et al. that in vitro sensitivity tests conducted with serum-enriched medium may more accurately predict the antimicrobial activity of some drugs in vivo (14).

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