Correlation of In Vitro Activities of Cephalothin and Ceftazidime with Their Efficacies in the Treatment of Staphylococcus aureus Endocarditis in Rabbits

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Rabbits with Staphylococcus aureus endocarditis were treated with cephalothin or ceftazidime to determine whether differences in in vitro activity would result in differences in in vivo efficacy. Antibiotics were administered in doses equivalent to maximum recommended human doses, and results of laboratory tests to predict antimicrobial efficacy were determined during treatment. Cephalothin and ceftazidime MICs for the challenge strain were 0.25 and 0.5 μg/ml, respectively. MBCs were 32 and >128 μg/ml, respectively. With peak sera, laboratory results (means) for cephalothin and ceftazidime were as follows: ratios of concentration in serum to MIC, 300 and 16; ratios of concentration in serum to MBC, 4.8 and <1; bacteriostatic antibacterial activity titers in serum, 1:256 and 1:16; and bactericidal antibacterial activity titers in serum, 1:16 and 1:4, respectively. Trough sera contained little or no measurable antibiotic and had no antibacterial activity. Both cephalothin and ceftazidime were efficacious in the treatment of infected rabbits. There were no statistically significant differences in efficacy as defined by survival, eradication of bacteremia, or sterilization of cardiac vegetations. Results of laboratory tests which quantitated antimicrobial activity did not correlate with efficacy, either independent of antibiotic or adjusted for antibiotic. Despite their lesser in vitro activities, the new cephalosporins may be equivalent to the older cephalosporins for treating staphylococcal infections in humans, when administered in maximum recommended doses.

A number of new cephalosporins and cephalosporin-like antibiotics have recently become available for use. They have greater in vitro activities than the older derivatives against gram-negative and anaerobic bacteria, but their activities against gram-positive organisms, such as Staphylococcus aureus, are considerably less. MICs of the new cephalosporins for S. aureus are typically 2 to 8 μg/ml, whereas MICs of older cephalosporins are 0.25 to 0.5 μg/ml. Because of these differences in MICs, the newer cephalosporins have been considered less desirable as therapeutic agents for treating staphylococcal infections even though they may reach concentrations in excess of the higher MICs in serum or other body fluids (7, 19, 26).

This study was designed to compare the efficacy of a new cephalosporin, ceftazidime, with that of the prototype cephalosporin, cephalothin, in the treatment of staphylococcal endocarditis in a rabbit model. Antibiotics were administered in dose regimens equivalent to those used in humans for treating serious infections. Efficacy was defined in terms of survival, eradication of bacteremia, and sterilization of cardiac vegetations. During treatment, peak and trough antibiotic concentrations in serum and bacteriostatic and bactericidal antibacterial activity (ABA) titers were measured, and results were correlated with therapeutic efficacy.

MATERIALS AND METHODS

Bacterial strain. A strain of S. aureus (ID 02794) isolated from a patient with endocarditis was used for all studies. MICs of cephalothin (Eli Lilly & Co., Indianapolis, Ind.) and ceftazidime (Glaxo, Research Triangle Park, N.C.) were 0.5 and 8 μg/ml, respectively. MBCs were 32 and >128 μg/ml, respectively.

Antibiotic administration. Antibiotic doses were as follows: cephalothin, 60 mg/kg of body weight per 6 h intramuscularly; and ceftazidime, 40 mg/kg of body weight per 8 h intramuscularly. Preliminary studies indicated that peak concentrations of cephalothin in serum occurred 15 min after injection; the elimination half-life was 0.5 h by the method of least squares (17). Peak concentrations of ceftazidime in serum occurred 45 min after injection; the elimination half-life was 0.9 h. One milliliter of sterile 0.9% saline injected intramuscularly every 6 h was used as a placebo control.

Animal studies. The procedure for producing endocarditis in rabbits has been described previously (12a). Male New Zealand white rabbits (Johnson’s Bunny Ranch, Wilkinson, Ind.) weighing 2 to 3 kg each were studied in groups of 7 to 11. After the rabbits were anesthetized with 50 mg of ketamine hydrochloride (Bristol-Myers Laboratories, Syracuse, N.Y.) and 8 mg of xylocine (Cutter Laboratories, Shawnee, Kans.) per kg of body weight intramuscularly, their right carotid arteries were cannulated with sterile polyethylene catheters (Clay Adams, Parsippany, N.J.); catheters were then advanced to aortic valves. Three days later, 1 ml of Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) containing 10⁶ CFU of the challenge strain was injected into an ear vein of each rabbit. Blood cultures were prepared 18 to 22 h after challenge, after which animals were randomly assigned to one of the three treatment groups. The first day of treatment was considered to be day 1 of the experiment. Blood cultures were prepared on days 2, 3, and 4 just before the next dose of antibiotic. On day 2, blood samples were obtained just before dosage and 15 and 45 min thereafter for recipients of cephalothin and ceftazidime for measurements of concentrations of these...
agents and ABA titers. On day 7, 8 h after the last antibiotic dose, blood samples were obtained for culture and measurement of concentrations of antibiotic in serum. Surviving animals were sacrificed by intracardial injection of 1 ml of sodium pentobarbital (W. A. Butler Co., Columbus, Ohio). After either death during the experiment or sacrifice, aortic vegetations were removed aseptically, weighed, and cultured.

Quantitative cultures. Quantitative blood cultures were performed by inoculating 0.1-ml portions of undiluted specimens and serial 10-fold dilutions of those specimens into Mueller-Hinton (Difco Laboratories, Detroit, Mich.) agar pour plates. Aortic vegetations were homogenized in 2 ml of sterile 0.9% saline; 0.1-ml portions of each undiluted homogenate and serial 10-fold dilutions of those specimens were cultured as described above.

Antibiotic concentrations in serum. Antibiotic assays were performed in triplicate by the filter paper disk diffusion method (22). Standard curves were constructed with heat-inactivated (56°C for 30 min) pooled rabbit sera as diluent. For cefalothin, antibiotic medium 1 (Difco) and 0.1% *Staphylococcus* aureus spore suspension (Difco) were used. For ceftazidime, antibiotic medium 2 (Difco) and a 0.25% suspension of *Escherichia coli ATCC 25922* from a 3-h broth culture were used. Minimal sensitivities were 0.3 and 0.9 μg/ml for cefalothin and ceftazidime, respectively.

ABA titers. Bacteriostatic and bactericidal ABA titers were determined in both Trypticase soy broth and heat-inactivated pooled normal rabbit sera by a microdilution method; the initial inoculum was 10^3 CFU/ml, and the bactericidal endpoint indicated a ≥99.9% kill (12a).

Analysis of results. Rabbits were excluded from evaluation if pretreatment (day 1) blood cultures were sterile or if cardiac vegetations were not present at sacrifice. For the various treatment regimens, the statistical significance of differences in percent survival, percent sterilization of blood cultures, and percent sterilization of cardiac vegetations were analyzed by the Fisher exact test. Quantitative blood cultures on specific days of the experiment were compared by analysis of variance and the Newman-Keuls after-test. Eradication of bacteremia versus time was analyzed by Cox regression analysis. Quantitative vegetation cultures among cefalothin- and ceftazidime-treated rabbits which were sacrificed on day 7 were compared by Kruskal-Wallis one-way analysis of variance. Various laboratory test results were correlated with quantitative blood and vegetation cultures by linear regression analysis. For all statistical analyses, results for which *P* > 0.05 were considered not significant.

RESULTS

Comparative efficacy of cefalothin, ceftazidime, and saline. There were 66 assessable rabbits. Survival rates were: 100% (25 of 25) for the cefalothin group, 85.2% (23 of 27) for the ceftazidime group, and 21.4% (3 of 14) for the control group. Two ceftazidime-treated rabbits died from unknown causes on day 5 or 6 with sterile blood cultures and vegetations; they were considered nonsurvivors. Differences in survival rates between either cephalosporin and saline were significant (*P* < 0.0001), but differences between cefalothin and ceftazidime were not significant.

The eradication of bacteremia in surviving rabbits treated with cefalothin, ceftazidime, or saline is shown in Table 1. Before treatment (day 1), there were no significant differences among the three treatment groups in numbers of organisms in the circulating blood. Thereafter, bacteremias declined more rapidly in rabbits given either cephalosporin than in rabbits given saline (*P* < 0.01). Bacterial clearances by cefalothin and ceftazidime were equivalent (*P* > 0.05).

The sterilization of cardiac vegetations in rabbits sacrificed on day 7 is shown in Table 2. Both cephalosporins were more effective than saline (*P* < 0.01). Cefalothin and ceftazidime were equivalent (*P* > 0.05). Cardiac vegetations from the 11 saline-treated and 2 ceftazidime-treated rabbits that died from infection averaged 8.6 ± 0.2 and 10.1 ± 1.1 (standard error) CFU (log₁₀) per vegetation and per g of vegetation, respectively.

Laboratory tests. The peak concentrations of antibiotic in serum, ratios of peak concentration in serum to MIC and MBC, and ABA titers in rabbits treated with cefalothin or ceftazidime are shown in Table 3. Peak concentrations of both antibiotics in serum were similar, but ratios of mean concentration in serum to MIC were ca. 18-fold higher with cefalothin than with ceftazidime. Mean bacteriostatic ABA titers were correspondingly higher with cefalothin (ca. 1:256) than with ceftazidime (1:16). Cefalothin and ceftazidime were bacteriostatic in both serum and broth. Ratios of mean concentration in serum to MBC were 4.8 for cefalothin and <1 for ceftazidime. Mean bacteriostatic ABA titers were correspondingly higher with cefalothin (ca. 1:16) than with ceftazidime (ca. 1:4) in both serum and broth. Both antibiotics demonstrated greater bactericidal activity in serum than we anticipated from the calculated ratios. There were no statistically significant correlations between any of the variables.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of sterile/live rabbits (%)</th>
<th>Mean CFU (log₁₀) ± SE</th>
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<tr>
<td></td>
<td>Per vegetation</td>
<td>Per g of vegetation</td>
</tr>
<tr>
<td>Cefalothin</td>
<td>21/25 (84)</td>
<td>1.1 ± 0.5</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>18/25 (72)</td>
<td>1.2 ± 0.4</td>
</tr>
<tr>
<td>Saline (control)</td>
<td>1/3 (33)</td>
<td>6.6 ± 2.6</td>
</tr>
</tbody>
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* Two ceftazidime-treated rabbits which died on day 5 or 6 with sterile vegetation and blood cultures are included.
the laboratory test results and survival, eradication of bacteremia, or sterilization of vegetations independent of antibi-otic or adjusted for antibiotic. All trough (days 2 and 7) cephalothin concentrations in serum were lower than the minimal sensitivity of the assay, and all trough ceftazidime ABAs were <1:2. Of the trough ceftazidime concentrations in serum, 64% were lower than the minimal sensitivity of the assay, and all trough ceftazi-dime ABAs were <1:2.

**DISCUSSION**

Selection of an antimicrobial agent for treatment of a specific infection is frequently based on differences in in vitro activities of possible agents against the causative pathogen. Early studies indicated that a favorable therapeutic outcome could be anticipated if the MIC of the agent was low, usually <3 µg/ml (1, 9), and if the concentration observed in serum exceeded the MIC for the infecting strain (8, 20). A current recommendation is that the ratio of peak concentration in serum to MIC should be ≥8 for optimal therapeutic efficacy (11). Studies in both human infections (12, 15, 21) and rabbit endocarditis (5) have shown that ratios of peak concentration in serum to MIC of ≥8 and peak bacteriostatic ABA titers of ≥1:8 are associated with a favorable therapeutic outcome. In the present study, ceftazidime was as effective as cephalothin in the treatment of staphylococcal endocarditis in rabbits, despite the fact that cephalothin was ca. 16-fold more active than ceftazidime as defined by comparative MICs, ratios of concentration in serum to MIC, and bacteriostatic ABA titers. For both antibiotics, however, ratios of mean peak concentration in serum to MIC were >16, and mean peak bacteriostatic ABA titers were ≥1:16. With lower doses, the differences in relative activities of these antibiotics may have been reflected in differences in efficacy.

Although the relationship between concentrations of antimicrobial agents achievable in serum and MICs has been frequently used to predict clinical outcome, a bactericidal antimicrobial agent-organism relationship is generally believed to be preferable to a bacteriostatic antimicrobial agent-organism relationship, particularly in the treatment of difficult-to-eradicate infections such as endocarditis. Bactericidal activity has generally been defined by modifying MIC and ABA tests to determine endpoints at which organisms are not merely inhibited but are reduced in number to ≤99.9% of the inoculum (2). Although endocarditis studies in both humans (4, 13, 14, 16) and rabbits (3, 5, 23) have shown that ratios of peak concentration in serum to MBC of ≥8 and bactericidal ABA titers of ≥1:8 are associated with a favorable therapeutic outcome, the validity of laboratory tests which define bactericidal activity to predict efficacy has been seriously questioned in both human (6) and rabbit endocarditis (12a). In the present study, the ratio of mean peak cephalothin concentration in serum to MBC was 4.8 and the mean peak bactericidal ABA titer was ca. 1:16, whereas with ceftazidime, the corresponding mean values were <1 and 1:4. The observed difference in bactericidal activity, like the difference in bacteriostatic activity, was not reflected in a difference in efficacy, and the results of this study did not support the contention that a ratio of peak concentration in serum to MBC of ≥8 or a peak bactericidal ABA titer of ≥1:8 is an important determinant of therapeutic outcome.

Although relationships between peak concentrations in serum and MICs or MBCs and peak bacteriostatic or bactericidal ABAs are usually determined to predict antimicrobial efficacy, the first laboratory tests employed for this purpose were performed with sera with trough concentrations of antibiotic to ensure that concentrations in serum were continuously inhibitory (24, 25). In the present study, favorable results were observed despite the absence of antibacterial activity in sera with trough concentrations of antibiotic. Similar results have been observed in studies of both human infections (15) and rabbit endocarditis (3, 5, 12a), and they are consistent with the concept that trough concentrations may fall below MICs or MBCs as long as dosage intervals are short enough to prevent a series of relapsing infections (10, 18).

Although this study included only one strain of S. aureus, it indicated that the new cephalosporins, despite their lesser in vitro activities, may be equivalent to the older cephalosporins in the treatment of staphylococcal infections when they are given in maximum recommended doses. They should be useful for treating mixed infections or infections of unknown etiology in which S. aureus is a potentially significant pathogen. However, cephalothin must still be considered the preferential antistaphylococcal agent because of its established clinical efficacy and lower cost.

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**LITERATURE CITED**


