Treatment of Experimental *Staphylococcus aureus* Abscesses: Comparison of Cefazolin, Cephalothin, Cefoxitin, and Cefamandole

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Cefazolin (CZ), cephalothin (CF), cefoxitin (CX), and cefamandole (CM) were evaluated in therapy of *Staphylococcus aureus* infection produced in perforated table tennis balls placed intraperitoneally in rabbits. Four weeks after placement of two balls in each rabbit, a beta-lactamase producing strain of *S. aureus* was injected into one of the balls. Twenty-four hours later therapy was initiated with 40 mg of CZ or 80 mg of CF, CX, or CM per kg intramuscularly every 6 h. After 24 h of treatment, the mean log_{10} colony-forming units per ml were 7.1 for CF, 6.7 for CZ, 6.5 for CX, and 7.2 for CM. After 72 h the mean log_{10} colony-forming units per ml were 5.0 for CZ, 4.1 for CF, 3.6 for CX, and 5.6 for CM. After 8 days, the titers were 1.6/ml for CZ, 1.0 for CF, 1.9 for CX, and 3.6 for CM. CZ serum levels were about double CF and CX levels and about two-thirds of CM levels. In sterile ball fluid CZ and CM levels were more than CF or CX concentrations. Concentrations of all four antibiotics were lower in infected balls.

Cefazolin (CZ) is much more susceptible to inactivation by *Staphylococcus aureus* beta-lactamase (cephalosporinase) than is cephalothin (CF) (6). This has raised doubts about the usefulness of CZ in staphylococcal endocarditis (1). In a previous publication from this laboratory (3), CZ and CF did not differ in rate of sterilization of vegetations in rabbits with endocarditis caused by a beta-lactamase-producing strain of *S. aureus*. Although it would have been of interest to determine the concentrations of antibiotic at the local site of infection, this is not feasible in a vegetation. A model using infection in a localized cavity, such as that in the present study, permits such measurements.

The purpose of this study was to determine the effectiveness of cephalosporins in treatment of infection in artificial cavities in rabbits caused by a beta-lactamase-producing strain of *S. aureus* and to measure the concentrations of the antibiotics in the cavities. CZ and CF, as well as two new cephalosporins, cefoxitin (CX) and cefamandole (CM), were studied.

**MATERIALS AND METHODS**

*S. aureus* strain. The strain of *S. aureus* used was isolated from a patient with endocarditis and was a producer of beta-lactamase (3). The minimal inhibitory concentrations of penicillin G, CZ, CF, CX, and CM were determined in Mueller-Hinton broth, using 0.5 ml of a 10^{-2} dilution of an 18-h culture added to each 0.5 ml of antibiotic dilution. The minimal inhibitory concentration was determined after 24 h of incubation at 37°C. CF and CM were supplied by Eli Lilly & Co. Research Laboratories (Indianapolis, Ind.). CZ was supplied by Smith Kline & French Laboratories (Philadelphia, Pa.), and CX was supplied by Merck Sharp & Dohme Research Laboratories (West Point, Pa.).

Stock cultures were made by incubating the strain in heart infusion broth (HIB) at 37°C for 24 h and storing 1-ml portions at −20°C. For each experiment a portion was subcultured into HIB, incubated at 37°C for 18 h, and diluted in HIB.

The concentrations of all antibiotics were assayed by an agar diffusion method, using paper disks (7). The assay strain for CZ, CF, and CM was *Bacillus subtilis* (ATCC 6633). The assay organism for CX was *S. aureus* MB-2786 (obtained from Merck Sharp & Dohme Research Laboratories). For concentrations in serum, the standard curve was determined with rabbit serum. For concentrations in ball fluid, the standard curve was determined with ball fluid.

Animal experiments. Female white New Zealand rabbits (West Jersey Biological Supply Farms, Wenonah, N.J.), weighing 2 to 2.7 kg each, were used in all experiments. Two table tennis balls, each perforated with 200 holes drilled with a 3/32-inch (0.24-cm) drill, were placed in the peritoneal cavity of a rabbit under intravenous sodium pentobarbital anesthesia according to the method of Gerdig et al. (4). The balls were fixed in place with sutures. After 4 weeks, 1 ml of fluid was aspirated percutaneously from each ball with a 21-gauge needle on a syringe and cultured in HIB. One milliliter of HIB containing 10^{6} *S. aureus* and 1.25% sterilized hog mucin was then injected into one of the two balls. After 24 h, therapy was initiated with 40 mg of CZ or 80 mg of CF, CX, or CM per kg intramuscularly every 6 h. Treatment was continued for 8 days.
After 24 h, 72 h, and 8 days of treatment, each time 2 h after administration of an antibiotic, 1 ml of fluid (containing 10 bacteria per ml) was aspirated from each ball percutaneously with a 21-gauge needle on a syringe. A 0.1 ml amount of the specimen was plated directly on sheep blood agar plates, and 0.1 ml was used for serial dilution in HIB and plating as previously described (2) for determination of numbers of bacteria. The remainder of the specimen was heated at 85°C for 30 s to inactivate beta-lactamase and then used for determination of the concentration of antibiotic. Heating at 85°C for 30 s did not decrease known concentrations of CZ, CF, CX, or CM placed in table tennis ball fluid.

For statistical purposes, sterile balls were considered as containing 10 bacteria per ml, as 10/ml was the smallest number that could be detected by the methods used.

Blood was taken from the ear veins at 0.5, 1, 2, and 4 h after the first injection of antibiotic, and the serum was removed for determination of antibiotic concentrations. The serum half-life of the antibiotic was calculated by the method of least squares (5). Student’s t test was used to determine significances.

RESULTS

In vitro studies. The minimal inhibitory concentrations for the S. aureus strain were >50 μg/ml for penicillin G, 0.78 μg/ml for CZ and CF, 3.1 μg/ml for CX, and 0.2 μg/ml for CM.

Animal experiments. At 4 weeks after placement of the table tennis balls (before injection of S. aureus), all table tennis ball fluid was sterile. Protein averaged 4.0 (range, 3.1 to 5.1) g/100 ml before infection and 4.2 (range, 3.5 to 4.9) g/100 ml after infection; over 90% of the protein was albumin.

After 24 h of treatment, the mean log_{10} colony-forming units ± standard error per ml were 7.1 ± 0.1 for CZ, 6.7 ± 0.3 for CF, 6.5 ± 0.2 for CX, and 7.2 ± 0.3 for CM, as compared with 7.1 ± 0.4 for untreated controls (Fig. 1). None of these titers is significantly different (P > 0.05 for all comparisons).

After 72 h of treatment, the mean log_{10} colony-forming units ± standard error per ml were 5.0 ± 0.4 for CZ, 4.1 ± 0.7 for CF, 3.6 ± 0.7 for CX, and 5.6 ± 0.9 for CM, as compared with 6.8 ± 0.3 for untreated controls. The titers for CZ, CF, and CX, but not CM, animals were significantly lower (P < 0.01) than control titers. There were no other significant differences.

After 8 days of treatment, the log_{10} colony-forming units ± standard error per ml were 1.6 ± 0.6 for CZ, 1.0 ± 0 for CF, 1.9 ± 0.4 for CX, and 3.6 ± 1.1 for CM, as compared with 6.3 ± 0.3 for untreated controls. The titers for CZ, CF, CX, and CM animals were significantly lower than control titers; (P < 0.01 for CZ, CF, and CX versus controls, and P < 0.05 for CM versus controls). The titers for CF animals were significantly lower (P < 0.05) than those for CX or CM animals. There were no other significant differences. There were no differences between control titers on days 1, 3, and 8 (P > 0.05).

Antibiotic serum levels. At 30 min, 1 h, and 2 h after the first injection of antibiotic, mean CZ levels were 141.3, 88.5, and 22.5 μg/ml, respectively (Fig. 2). Mean CF levels were 78.1, 27.8, and 7.2 μg/ml. Mean CX levels were 76.7, 40.7, and 9.5 μg/ml. Mean CM levels were 219.9,
122.4, and 19.1 μg/ml. At 4 h, mean levels were 2.6 μg/ml in the CM animals, but there were no measurable levels in most CZ, CF, and CX animals. Serum half-lives were 0.55 h for CZ, 0.54 h for CM, 0.49 h for CX, and 0.44 h for CF. In general, CZ levels were about double CF and CX levels and about two-thirds of CM levels.

**Antibiotic concentrations in ball fluid.**

Table 1 shows the mean concentrations of antibiotic ± standard error in ball fluid after 1, 3, and 8 days of therapy. In sterile ball fluid, CZ and CM tended to have the highest mean levels, which were more than double the mean concentrations of CF and CX. The concentrations of CZ in sterile fluid after 1 day of therapy were significantly higher (P < 0.05) than those after 8 days of therapy. Concentrations of CF, CX, and CM in sterile fluid did not differ after 1, 3, or 8 days of treatment (P > 0.05 for all comparisons).

Concentrations of all four antibiotics were lower in infected balls than in sterile balls. These differences were significant for CZ, CF, and CM, but not for CX (P < 0.05 for CZ, P < 0.01 for CF after 1 day of treatment, and P < 0.01 for CM after 3 days of treatment, all by t test for paired observations).

**DISCUSSION**

The effectiveness of CZ in *S. aureus* endocarditis has been questioned as a result of the in vitro demonstration of its inactivation by staphylococcal beta-lactamase (1, 6). In the present study, CZ was as effective as CF, CX, and CM in the sterilization of table tennis balls in rabbits infected with a beta-lactamase-producing strain of *S. aureus*. It is of interest that CF reduced bacterial titers more effectively than CX or CM, but not more effectively than CZ.

Doses of 40 mg of CZ per kg gave serum levels that were about double those of 80 mg of CF or CX per kg and about two-thirds those of 80 mg of CM per kg. For equivalent doses this would be a 4-fold advantage of blood levels of CZ over CF and CX and a 1.3-fold advantage of CZ over CM.

Antibiotic concentrations in sterile table tennis ball fluid were above the minimal inhibitory concentrations for all four antibiotics. However, mean levels in infected balls were slightly lower than the minimal inhibitory concentrations for CF and CX. The decrease in levels in infected fluid as compared with sterile fluid cannot be explained on the basis of protein binding, as there was little difference in protein content of infected versus noninfected balls. The highest levels in the sterile as well as infected table fluid were measured in the CM animals, but there were no measurable levels in most CZ, CF, and CX animals. Serum half-lives were 0.55 h for CZ, 0.54 h for CM, 0.49 h for CX, and 0.44 h for CF. In general, CZ levels were about double CF and CX levels and about two-thirds of CM levels.

**Table 1. Antibiotic concentrations in ball fluid 2 h after intramuscular injection**

<table>
<thead>
<tr>
<th>Ball fluid</th>
<th>Days of therapy</th>
<th>Mean concn (μg/ml), ± standard error, of:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>CZ</td>
</tr>
<tr>
<td>Sterile</td>
<td>1</td>
<td>14.7 ± 3.2</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>10.3 ± 2.6</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>7.6 ± 1.1</td>
</tr>
<tr>
<td>10⁶ S. aureus/ml</td>
<td>1</td>
<td>8.2 ± 3.2</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.7 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td></td>
</tr>
</tbody>
</table>

*— No observations.*
tennis balls were achieved by CZ, followed by CM, CX, and CF.

It has been demonstrated in studies from our laboratory (3) that the strain of *S. aureus* used slowly inactivates CZ. It seems clear that in this model, despite slow inactivation of CZ by beta-lactamase, penetration into the table tennis balls occurred fast enough to maintain high and effective antibiotic levels. The slow inactivation of CZ by staphylococcal beta-lactamase (2- to 3-h half-life [3]) seemed to be of little importance, because diffusion into the area of infection occurred rapidly enough to yield effective antibacterial concentrations. Inactivation of CZ at the site of infection in humans should be even less important a factor. The much longer serum half-life of 2 h in humans (5), as compared with about 30 min in rabbits, results in more sustained serum concentrations.

**ACKNOWLEDGMENT**

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**LITERATURE CITED**