Treatment of Experimental *Staphylococcus aureus* Endocarditis: Comparison of Cephalothin, Cefazolin, and Methicillin

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The effectiveness of cefazolin in *Staphylococcus aureus* endocarditis has been questioned because of in vitro inactivation by staphylococcal beta-lactamase. Cefazolin, although inactivated in vitro by *S. aureus* beta-lactamase, was as effective as cephalothin in the treatment of left-sided *S. aureus* endocarditis in rabbits. Cefazolin (20 mg/kg every 6 or 8 h), cephalothin (40 mg/kg every 6 h), and methicillin (40 mg/kg every 6 h), administered intramuscularly, were compared in the treatment of left-sided endocarditis caused in rabbits by a highly penicillin-resistant strain of *S. aureus*. The three antibiotics were all effective in reducing titers in vegetations. However, at the dose used, methicillin reduced the titers more rapidly than cephalothin or cefazolin. Cefazolin concentrations in serum were about double those achieved with cephalothin or methicillin. However, cefazolin was only half as active as methicillin and one-eighth as active as cephalothin in vitro in a serum assay. The half life in serum of cefazolin, cephalothin, and methicillin were each about 30 min. Serum bactericidal activities of the three antibiotics were very similar.

Cefazolin (CZ) is much more susceptible to inactivation by *Staphylococcus aureus* beta-lactamase than cephalothin (CF) (7), raising doubts about the usefulness of CZ in staphylococcal endocarditis (2). This study compares the efficacy of CZ, CF, and methicillin (M) in the therapy of left-sided endocarditis in rabbits, caused by a highly penicillin-resistant, methicillin-susceptible strain of *S. aureus*, which rapidly inactivates CZ in vitro.

MATERIALS AND METHODS

Organism. A strain of *S. aureus* obtained from the blood of a patient with endocarditis was used in all experiments. The minimal inhibitory concentrations (MIC) and minimal bactericidal concentrations of CZ, CF, M, and penicillin G were determined by using an antibiotic dilution method in both heart infusion broth (HIB; Difco Laboratories, Detroit, Mich.) and pooled rabbit serum (Microbiological Associates, Inc., Bethesda, Md.). The antibiotics were diluted in twofold steps in tubes containing 0.5 ml of HIB or serum. The bacterial inoculum for each tube was 0.5 ml of a 10^{-2} dilution in HIB or serum of an 18-h HIB culture. The MIC was considered to be the lowest concentration of antibiotic that prevented turbidity after 24 h of incubation at 37°C. After the MIC was determined, 0.01 ml of each clear tube was streaked on the surface of a sheep blood agar plate with a sterile platinum loop. The minimal bactericidal concentration was the lowest concentration of antibiotic that resulted in no growth on the plate after 48 h at 37°C.

Stock cultures were made by incubating the organism in 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 or serum.

In vitro studies. The rate at which the *S. aureus* was killed was studied in flasks with HIB containing CZ, CF, or M. The inoculum in HIB was added to each flask and incubated at 37°C. The final concentrations of antibiotics were 20 µg/ml, and of *S. aureus* were 3×10^6 /ml. Samples were removed from the flasks at the start of the experiment and at 2, 4, 6, and 24 h. Each sample was serially diluted in 10-fold steps in HIB, and 0.1 ml of each dilution and 1 ml of undiluted sample were plated on the surfaces of blood agar plates. After incubation at 37°C for 48 h the numbers of colonies on the plates were counted and the colony-forming units (CFU) in the flasks were calculated.

The inactivation of CZ, CF, and M by beta-lactamase produced by the strain of *S. aureus* was studied in vitro as described by Regamey et al. (7). A flask containing HIB with 0.2 µg of CZ per ml (to induce beta-lactamase) and an inoculum of 10^8 *S. aureus* were incubated overnight at 37°C. The culture was then filtered through a disposable unit containing a 0.45-µm grid membrane (Nalgene Sybron Corp., Rochester, N.Y.). The assay was performed by using tubes containing 5 ml of the *S. aureus* filtrate or a sterile filtrate of HIB plus 5 ml of HIB containing 32 µg of CZ per ml, 38 µg of CF per ml, or 32 µg of M per ml at 37°C. Samples were taken at 0, 2, 4, 6, and 24 h and heated to 85°C for 30 s to inactivate beta-lactamase, and then the antibiotic concentration was assayed.

The concentrations of all antibiotics (CZ, CF, and M) were assayed by an agar diffusion method using paper disks (8). Serum bactericidal activity was determined by diluting serum in twofold steps in tubes containing 0.5 ml of normal rabbit serum and adding 0.5 ml of serum containing 10^6 CFU of an 18-h culture of the *S. aureus*. After incubation for 24 h at 37°C, 0.01 ml of the contents of each tube was plated on the surface of a blood agar plate. The maximal bactericidal dilution was the highest dilution of serum that resulted in less than five colonies on the plate after 48 h of incubation at 37°C. This represented at least a 99.9% kill (1).

Animal experiments. Female white New Zealand rabbits (West Jersey Biological Supply Farms, Wenonah, N.J.) weighing 2 to 2.7 kg each, were anesthetized, and the right carotid arteries were cannulated as previously described (4). One day later, each rabbit was inoculated by ear vein with 1 ml of HIB containing 2×10^6 *S. aureus*. Inoculated control animals were left untreated. In treatment groups, antibiotic therapy was initiated 18 h after infection; the regimens, all intramuscular, were CZ, 20 mg/kg every 6 or 8 h; CF, 40 mg/kg every 6 h; and M, 40 mg/kg every 6 h. After varying intervals of therapy, following a period of at least 6 h without treatment, the rabbits were anesthetized by intravenous injection of sodium pentobarbital. Right atrial blood specimens were obtained, and the aortic valve vegetations were excised and weighed. The vegetations weighed 0.02 to 0.4 g. After a 1:10 suspension of each vegetation was homogenized in HIB, the number of CFU was determined by serial dilution and plating techniques as previously described (3). In "sterile" vegetations, the number of CFU was calculated as $\log_{10}$ CFU 2, since the largest weight of vegetation plated was 0.01 g. Right atrial blood was cultured by plating 0.1 and 1 ml of blood on the surfaces of blood agar plates.

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 determinations of antibiotic concentrations and serum bactericidal activity. The serum half life of the antibiotic was calculated by the method of least squares (6). Student's *t*-test was used to determine significance.

RESULTS

The MICs in micrograms per milliliter for the *S. aureus* in HIB as compared with serum (HIB/serum) were 1.6/6.3 for CZ, 0.8/0.8 for CF, and 3.1/3.1 for M. The MIC of penicillin G against the organism was >50 $\mu\text{g/ml}$. The minimum bactericidal concentrations were identical to the MICs for CF and CZ but twofold higher than the MICs for M.

In vitro studies. Concentrations of 20 μg of CZ, CF, or M per ml resulted in an equally rapid decrease in CFU of *S. aureus*. After 6 h, the titers had decreased from $\log_{10}$ 6.5 to $\log_{10}$ 5.5, and by 24 h the titers were $\log_{10}$ 1.8, 1.5, and 1.3, respectively.

When a filtrate of the *S. aureus* was incu-

bated with the antibiotics, CZ was rapidly destroyed, whereas the activities of CF and M were relatively well preserved (Fig. 1). The concentration of CZ dropped from 16 $\mu\text{g/ml}$ to no measurable concentrations by 6 h. M activity did not change during 24 h of incubation, and CF activity dropped from 19 to 7 $\mu\text{g/ml}$ at 24 h. However, the decrease in CF activity in the culture filtrate (19 to 7 $\mu\text{g/ml}$) was not very different from that seen in the broth filtrate control (19 to 11 $\mu\text{g/ml}$).

Animal experiments. All 16 rabbits sacrificed 18 h after infection had endocarditis with mean $\log_{10}$ CFU $\pm$ standard deviation of 6.7 ± 1.8 *S. aureus* per g of vegetation. The mortality rate in untreated controls was 14% at 24 h and 57% at 48 h after infection. Of the rabbits treated with CZ, CF, or M starting 18 h after infection, 86% survived to the end of the experiment.

The number of *S. aureus* in the vegetations was similar for each of the treatment regimens after 1 day of treatment (Fig. 2). The mean $\log_{10}$ CFU $\pm$ standard deviation per g of vegetation in treated rabbits was 6.2 ± 2.6 for the 6-h CZ regimen (CZ-6), 6.9 ± 2.4 for the 8-h CZ regimen (CZ-8), 7.4 ± 2.7 for CF, and 5.3 ± 2.5 for M ($P > 0.05$ for all comparisons). At the time of sacrifice, right atrial blood cultures were positive for *S. aureus* in 6 of 10 CZ-6 rabbits, 3 of 9 CZ-8 rabbits, 3 of 7 CF rabbits, and 2 of 9 M rabbits in which blood was obtained.

After 3 days of therapy, the mean $\log_{10}$ CFU $\pm$ standard deviation per g of vegetation in rabbits was 4.2 ± 2.7 for CZ-6, 4.7 ± 2.8 for CZ-8, 4.5 ± 2.7 for CF, and 2.0 ± 0 for M ($P < 0.01$ for comparisons between M and each of the three other groups; all other comparisons were not significantly different). At time of

FIG. 1. Rate of decrease in concentrations of CZ, CF, and M when incubated at 37°C in (---) a culture filtrate of a beta-lactamase-producing strain of *S. aureus*, or (—) a filtrate of sterile broth.

FIG. 2. Rate of decrease of *S. aureus* in vegetations, expressed as $\log_{10}$ CFU/g after intramuscular administration of CZ (20 mg/kg) every 6 h, CZ (20 mg/kg) every 8 h, CF (40 mg/kg) every 6 h and M (40 mg/kg) every 6 h. Each point represents one vegetation.

sacrifice, right atrial blood cultures were sterile in all 10 M-treated rabbits. Blood cultures were positive in 2 of 13 CZ-6 rabbits, 2 of 13 CZ-8 rabbits, and 3 of 13 CF-treated rabbits.

After 5 days of antibiotic treatment, the mean $\log_{10}$ CFU $\pm$ standard deviation per g of vegetation in rabbits was 3.1 ± 2.2 for CZ-6, 3.3 ± 2.2 for CZ-8, 2.8 ± 1.8 for CF, and 2.0 ± 0 for M ($P > 0.05$ for all comparisons). Right atrial blood cultures from all 11 CF and 11 M rabbits were sterile. There was 1 positive culture in the 14 CZ-6 rabbits and 1 of 9 in CZ-8 rabbits.

Antibiotic serum levels. At 15 min, 30 min, 1 h, and 2 h after the first injection of antibiotic, mean CZ levels were 121.4, 77.4, 51.3, and 12.1 $\mu\text{g/ml}$, respectively (Fig. 3). Mean CF levels were 68.2, 34.8, 17.9, and 3.6 $\mu\text{g/ml}$, respectively. Mean M levels were 74.2, 48.0, 17.3, and 6.4 $\mu\text{g/ml}$, respectively. At 4 h, there were no measurable concentrations of CZ, CF, or M in most rabbits. The serum half lives were 0.50, 0.42, and 0.49 h for CZ, CF, and M, respectively.

Serum bactericidal activity. The maximal bactericidal dilutions of serum for CZ-treated rabbits ranged from 1:32 to 1:128 at 15 min with a median of 1:64. The ranges and medians were 1:16 to 1:32 at 30 min (median 1:32), 1:8 to 1:64 at 1 h (median 1:16), and <1:2 to 1:8 at 2 h (median 1:4), and were all <1:2 at 4 h.

The maximal bactericidal dilutions for CF ranged from 1:32 to 1:128 at 15 min (median 1:32), from 1:8 to 1:128 at 30 min (median 1:64), from 1:16 to 1:128 at 1 h (median 1:32), and from 1:4 to 1:16 at 2 h (median 1/16), and were <1:2 at 4 h.

The maximal bactericidal dilutions for M ranged from 1:8 to 1:128 at 15 min (median

FIG. 3. Concentrations of CZ, CF, and M in serum after intramuscular injection of 20 mg of CZ, 40 mg of CF, or 40 mg of M per kg. Each point represents the mean of four sera with the best straight lines drawn through the points.

1:32), from 1:16 to 1:64 at 30 min (median 1:32), from 1:4 to 1:16 at 1 h (median 1:4 to 1:8), from 1:2 to 1:4 at 2 h (median 1:2), and were all <1:2 at 4 h.

DISCUSSION

The effectiveness of CZ in *S. aureus* endocarditis has been questioned as a result of the in vitro demonstration of inactivation by staphylococcal beta-lactamase (2, 7). In the present study, CZ, although inactivated in vitro by *S. aureus* beta-lactamase, was as effective as CF

in the treatment of left-sided *S. aureus* endocarditis in rabbits. It is of interest that M reduced titers of *S. aureus* in the vegetations at a more rapid rate than CZ or CF (i.e., after 3 days of treatment, the mean log₁₀ CFU/g of vegetation was 4.7 for CZ-8, 4.2 for CZ-6, and 4.5 for CF, as compared with 2.0 for M). Also, after 3 days of therapy, all blood cultures from rabbits treated with M were sterile, whereas blood cultures were positive in some rabbits from each of the other antibiotic groups.

Although the serum concentrations of CZ were about twice those of CF and M with the doses used, CZ was only half as active as M and one-eighth as active as CF against the *S. aureus* in a serum assay. The half lives in serum of the three antibiotics were similar. The serum bactericidal activities of CZ, CF, and M against the strain of *S. aureus* were very similar with M holding little or no advantage at any time. Furthermore, M killed *S. aureus* in vitro at the same rate as CZ and CF. Therefore, there is no explanation for the faster bactericidal effect of M in the vegetations.

The present studies agree with an abstract recently published by A. I. Hartstein and R. E. Bryant (Clin. Res. 25:376A, 1977). They showed that the rate of killing of *S. aureus* in vitro did not differ for CZ and CF, providing that the concentrations of the antibiotics were maintained. When the relatively short half life of CZ (about 30 min) is taken into account, the relatively slow inactivation by staphylococcal beta-lactamase (i.e., half inactivated in 2 to 3 h) would seem to become less important. It appears that the frequent administration of the drug to maintain therapeutic serum levels and serum bactericidal activity is the most important factor. Although it is impossible to determine minute-to-minute concentrations of CZ in the vegetation (and thus to take diffusion of antibiotic into the vegetations and local destruction into account), the concentrations

achieved were apparently adequate for treatment of *S. aureus* endocarditis in rabbits.

Although the results of these studies in rabbits cannot be directly extrapolated to *S. aureus* endocarditis in humans, at least the daily doses of antibiotics used and blood levels achieved were similar to those in humans. The major difference is that CZ has a longer serum half life in humans (2h) (6) than in rabbits (30 min); CF and M have half lives of about 30 min, both in humans and in rabbits (5).

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