Comparison of Ceforanide, Cefazolin, Methicillin, and Nafcillin in *Staphylococcus aureus* Endocarditis Therapy in Rabbits

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Ceforanide (30 mg/kg) administered every 12 h, cefazolin (20 mg/kg) administered every 8 h and methicillin or nafcillin (40 mg/kg) administered every 6 h were equally effective in reducing the number of *Staphylococcus aureus* in vegetations in rabbits with endocarditis. These treatments were more effective than methicillin or nafcillin administered every 12 h. Ceforanide produced higher peak concentrations and greater bactericidal activity in serum than the other drugs and had the longest half-life (5.8 h, compared with 0.4 to 0.8 h for the other agents).

In previous studies in this laboratory cefazolin, methicillin, and nafcillin were effective in reducing the numbers of *Staphylococcus aureus* in vegetations in rabbits with endocarditis (3, 4). Ceforanide, a new semisynthetic cephalosporin which is about one-fourth as active as cefazolin in vitro, produces high and sustained serum concentrations and has a prolonged half-life (1, 8).

The purpose of this study was to compare the effectiveness of ceforanide administered at 12-h intervals with that of cefazolin, methicillin, and nafcillin in the treatment of experimental *S. aureus* endocarditis.

**MATERIALS AND METHODS**

**Organism.** A beta-lactamase-producing strain of *S. aureus* (3, 4) obtained from the blood of a patient with endocarditis was used in all experiments. The minimal inhibitory concentrations of ceforanide, cefazolin, methicillin, and nafcillin were determined using an antibiotic dilution method in Mueller-Hinton broth (MHB). The antibiotics were diluted in twofold steps in tubes containing 0.5 ml of MHB. The bacterial inoculum for each tube was 0.5 ml of a 10⁻² dilution in MHB of an 18-h MHB culture. The minimal inhibitory concentration was considered to be the lowest concentration of antibiotic that prevented turbidity after 24 h of incubation at 37°C.

Stock cultures were made by incubating the organism in MHB at 37°C for 24 h, and 1-ml samples were stored at −20°C. For each experiment a sample was subcultured into MHB, incubated at 37°C for 18 h, and diluted in MHB or pooled rabbit serum (Microbiological Associates).

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

**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 artery of each was cannulated as previously described (5). A polyethylene catheter was left in place throughout the experiment. After 1 day, each rabbit was inoculated in the ear vein with 1 ml of MHB containing 2 × 10⁶ *S. aureus* cells. Inoculated control animals were left untreated. In treatment groups, antibiotic therapy was begun 24 h after infection. The regimens were as follows: ceforanide (30 mg/kg) administered every 12 h; cefazolin (20 mg/kg) administered every 8 h; and methicillin or nafcillin (40 mg/kg) administered every 6 or 12 h. After varying intervals of therapy, and after a period of at least 6 h without treatment, the rabbits were sacrificed by intravenous injection of sodium pentobarbital. The chest of each rabbit was opened, 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 MHB, the number of CFU was determined by serial dilution and plating techniques, as previously described (2). The number of CFU in "sterile" vegetations was expressed as log₁₀ 2 CFU, as the largest weight of vegetation plated was 0.01 g.

Blood was taken from the ear veins of four rabbits at 15 and 20 min and 1, 2, and 4 h after the first injection of each antibiotic, and the serum was removed. Specimens were stored at −20°C until they were assayed for serum levels and bactericidal activity.

The concentrations of all antibiotics were assayed by an agar diffusion method, using paper disks (9). Serum bactericidal activity was determined by diluting the serum in twofold steps in tubes containing 0.5 ml of normal rabbit serum and adding 0.5 ml of serum containing 10⁷ CFU of an 18-h MHB culture of *S. aureus*. After incubation for 24 h at 37°C, 0.1 ml from each tube was plated onto the surface of a blood agar
plate. The maximal bactericidal dilution (MBD) was the highest dilution of serum that resulted in less than five colonies on a plate after 48 h of incubation at 37°C. The serum half-life of the antibiotic was calculated by using the least-squares method (7). The Student t test was used to determine significance.

RESULTS

The minimal inhibitory concentrations for S. aureus in MHB were 3.1 μg/ml for ceftazolin, 0.4 μg/ml for ceftazolin, 1.6 μg/ml for methicillin, and 0.4 μg/ml for nafcillin.

In vitro studies. Concentrations of 20 μg of ceforanide per ml, 20 μg of ceftazolin per ml, 20 μg of methicillin per ml, and 20 μg of nafcillin per ml in MHB resulted in a rapid decrease in S. aureus CFU. After 6 h the titers decreased from $\log_{10} 7.2$ to $\log_{10} 6.0$, 5.4, 5.4 and 5.2 CFU/ml, respectively, and by 24 h the titers were $\log_{10} 4.1$, 4.8, 4.0, and 3.0 CFU/ml, respectively. When 20 μg concentrations of ceforanide, ceftazolin, methicillin, and nafcillin were studied in serum, the titers of S. aureus decreased from $\log_{10} 7.2$ to $\log_{10} 5.6, 5.5, 4.3$ and 5.2 CFU/ml, respectively, after 6 h, and to $\log_{10} 4.3, 4.2, 1.3$, and 2.1 CFU/ml, respectively, after 24 h.

Animal experiments. The mortality rate in 224 rabbits was 13% by 24 h after infection, at which time therapy was started. Seven rabbits sacrificed 24 h after infection (just before onset of therapy) all had endocarditis, with $\log_{10} 7.7$ ± 1.4 CFU of S. aureus per g of vegetation (mean ± standard deviation). Each treatment group contained 26 to 34 rabbits. The mortality rates in these groups ranged from 13 to 26% during the first 24 h after the start of therapy and then decreased to 4 to 14% during the next 24 h. Subsequently, there was no mortality. The mortality rates in the different groups did not differ significantly.

As Fig. 1 shows, the numbers of S. aureus in the vegetations were similar for each of the treatment regimens after 1 day of treatment. The values were $\log_{10} 3.8 \pm 1.2$ CFU/g of vegetation (mean ± standard deviation) for rabbits treated with ceforanide every 12 h, $\log_{10} 5.3 \pm 1.8$ CFU/g for rabbits treated with ceftazolin every 12 h, $\log_{10} 5.3 \pm 1.6$ CFU/g for rabbits treated with methicillin every 6 h, $\log_{10} 5.3 \pm 2.2$ CFU/g for rabbits treated with nafcillin every 6 h, $\log_{10} 6.5 \pm 2.1$ CFU/g for rabbits treated with methicillin every 12 h, and $\log_{10} 5.0 \pm 1.5$ CFU/g for rabbits treated with nafcillin every 12 h ($P < 0.05$ for ceforanide versus methicillin every 12 h; $P > 0.05$ for all other comparisons).

After 3 days of therapy, the numbers of S. aureus in the vegetations were $\log_{10} 2.9 \pm 1.9$ CFU/g for rabbits treated with ceforanide, $\log_{10} 4.6 \pm 2.6$ CFU/g for rabbits treated with ceftazolin, $\log_{10} 2.4 \pm 0.9$ CFU/g for rabbits treated with methicillin every 6 h, $\log_{10} 4.5 \pm 2.0$ CFU/g for rabbits treated with nafcillin every 6 h, $\log_{10} 3.8 \pm 2.3$ CFU/g for rabbits treated with methicillin every 12 h, and $\log_{10} 2.8 \pm 1.6$ CFU/g for rabbits treated with nafcillin every 12 h ($P < 0.05$ for methicillin administered every 6 h compared with nafcillin administered every 6 h and ceftazolin administered every 8 h; all other comparisons were not significantly different).

![Fig. 1. Rate of decrease of S. aureus in vegetations after intramuscular administration of 30 mg of ceforanide (CR) per kg every 12 h, 20 mg of ceftazolin (CZ) per kg every 8 h, 40 mg of methicillin per kg every 6 h (M6), 40 mg of methicillin per kg every 12 h (M12), 40 mg of nafcillin per kg every 6 h (N6), and 40 mg of nafcillin per kg every 12 h (N12). Each point represents one vegetation from one rabbit.](http://aac.asm.org/Downloaded from http://aac.asm.org/)
After 5 days of antibiotic treatment, the numbers of *S. aureus* cells in the vegetations were log$_{10}$ 2.0 ± 0 CFU/g for rabbits treated with ceforanide, log$_{10}$ 2.9 ± 2.2 CFU/g for rabbits treated with cefazolin, log$_{10}$ 2.0 ± 0 CFU/g for rabbits treated with methicillin every 6 h, log$_{10}$ 2.1 ± 0.4 CFU/g for rabbits treated with nafcillin every 6 h, log$_{10}$ 4.3 ± 2.6 CFU/g for rabbits treated with methicillin every 12 h, and log$_{10}$ 2.5 ± 0.7 CFU/g for rabbits treated with nafcillin every 12 h (P < 0.05 for the comparisons of methicillin administered every 12 h with ceforanide, methicillin administered every 6 h and nafcillin administered every 6 h; P < 0.05 for comparisons of ceforanide and methicillin administered every 6 h with nafcillin administered every 12 h; P > 0.05 for all other comparisons).

**Antibiotic serum levels.** After the first injection of ceforanide, mean antibiotic levels obtained at 15 and 30 min and 1, 2, and 4 h were 201.5, 294.8, 287.1, 222.6, and 156.4 µg/ml, respectively. Mean cefazolin levels obtained at 0.5, 1, and 2 h were 136.7, 71.6, and 15.1 µg/ml, respectively. Mean methicillin levels obtained at 15 and 30 min and 1 and 2 h were 92.8, 67.6, 22.4, and 3.6 µg/ml, respectively. Mean nafcillin levels obtained at 15 and 30 min and 1 and 2 h were 41.0, 35.6, 19.3, and 9.5 µg/ml, respectively. At 4 h there were no measurable concentrations of cefazolin, methicillin, and nafcillin in most of the rabbits. The serum half-lives of ceforanide, cefazolin, methicillin, and nafcillin were 5.8, 0.47, 0.5, and 0.4 h, respectively.

**Antibacterial activity in serum.** After an injection of each antibiotic, three or four sera were periodically assayed for antibacterial activity by determining the MBD. The MBDs for ceforanide were more than 1:64 at 15 and 30 min, 1:8 to 1:64 at 1 h, 1:16 to 1:64 at 2 h, and 1:32 to 1:64 at 4 h. The MBDs for cefazolin were more than 1:64 at 30 and 60 min and 1:64 at 2 h. The MBDs for methicillin were 1:64 at 15 min and more than 1:64 at 30 min and 1 h, and they ranged from 1:4 to more than 1:64 at 2 h. The MBDs for nafcillin ranged from 1:8 to 1:32 at 15 min and 1:2 to 1:16 at 0.5, 1, and 2 h.

**DISCUSSION**

In this study, ceforanide administered intramuscularly every 12 h was effective in the therapy of rabbits with *S. aureus* endocarditis. After 1, 3, or 5 days of treatment, comparisons between ceforanide and methicillin or nafcillin administered every 6 h and cefazolin administered every 8 h showed no significant differences. After 1 day of treatment ceforanide was more effective in reducing numbers of *S. aureus* cells in vegetations than methicillin given every 12 h, and after 5 days of treatment ceforanide was significantly more effective than methicillin or nafcillin administered every 12 h. Methicillin administered every 6 h was more effective in reducing titers in vegetations at 3 days than nafcillin administered every 6 h or cefazolin administered every 8 h. Although methicillin administered every 12 h decreased the number of *S. aureus* cells in vegetations, this was generally the least effective regimen.

Van Ness et al. (M. M. Van Ness, W. M. Scheld, and M. A. Sande, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 19th, Boston, Mass., abstr. no. 1059, 1979) also studied ceforanide in the rabbit model of *S. aureus* endocarditis. Utilizing the same dose of ceforanide as in the present study (30 mg/kg administered every 12 h), these authors demonstrated a decrease in vegetation titers from a mean of log$_{10}$ 9.8 to a mean of log$_{10}$ 3.9 after 3 days of therapy. This was more effective than cephalothin at 30 mg/kg administered every 6 h or at 60 mg/kg administered every 12 h. However, the *S. aureus* strain used by these investigators was atypical in that it was extremely susceptible to ceforanide (i.e., it was inhibited by only 0.5 µg of ceforanide per ml). The strain used in the present study required 3.1 µg of ceforanide per ml for inhibition. This is in the usual range reported (1.6 µg/ml to inhibit 50% of non-beta-lactamase-producing strains and 3.1 µg/ml to inhibit 50% of beta-lactamase-producing strains) (1).

In the present study ceforanide gave the highest serum concentrations and had a much longer half-life than the other agents. This explains the fact that, in contrast to rabbits treated with the other agents, only rabbits treated with ceforanide still had high bactericidal titers (i.e., 1:32 to 1:64) in their sera at 4 h after injection. However, it should be noted that cefazolin has a much longer serum half-life in humans than in rabbits (2 compared with 0.5 h) (8), whereas ceforanide, methicillin, and nafcillin have comparable half-lives in humans and rabbits (i.e., 3, 0.5, and 0.5 h, compared with 5, 0.4 and 0.8 h, respectively) (6, 9).

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**LITERATURE CITED**

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