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Ciprofloxacin Therapy of Experimental Endocarditis Caused by Methicillin-Susceptible or Methicillin-Resistant *Staphylococcus aureus*

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Ciprofloxacin was more effective ($P < 0.01$) than either imipenem or nafcillin therapy of experimental methicillin-susceptible *Staphylococcus aureus* endocarditis in rabbits after 2 or 3 days of treatment. There was no significant difference between results of treatment of methicillin-susceptible *S. aureus* experimental endocarditis with ciprofloxacin and results with the combination of nafcillin and gentamicin. Ciprofloxacin was more effective ($P < 0.01$) than vancomycin therapy of experimental methicillin-resistant *S. aureus* endocarditis after 3 days of treatment. After 5 days of treatment, there was no significant difference between the results of treatment of experimental methicillin-resistant *S. aureus* endocarditis with ciprofloxacin and results with vancomycin.

Staphylococcus aureus is the second most common cause of infective endocarditis, accounting for 25 to 35% of cases, and is associated with frequent complications, including metastatic abscesses and rapid cardiac valve destruction resulting in heart failure (2, 5). Current antimicrobial therapy for patients with methicillin-susceptible *Staphylococcus aureus* (MSSA) endocarditis consists of administration of a beta-lactam with or without gentamicin or the use of vancomycin (1, 15). Imipenem was effective therapy for MSSA experimental endocarditis and for infective endocarditis in humans (8, 20). The frequency of endocarditis caused by strains of *S. aureus* resistant to methicillin (MRSA) is increasing (16). Vancomycin alone or in combination with rifampin or gentamicin is recommended for the therapy of patients with MRSA endocarditis (3, 16, 21).

Ciprofloxacin, a carboxyquinolone, was reportedly bactericidal in vitro against strains of MSSA and MRSA and was fourfold more active in vitro against *S. aureus* than was norfloxacin or enoxacin (6, 10, 11, 14, 17, 18). Accordingly, ciprofloxacin might be useful therapy for patients with staphylococcal endocarditis. The purpose of this study was to determine the efficacy of ciprofloxacin therapy of MSSA or MRSA experimental endocarditis and to compare the results of therapy with those resulting from treatment of animals with nafcillin, vancomycin, or imipenem, alone or combined with gentamicin.

MATERIALS AND METHODS

In vitro studies. Four strains of *S. aureus* isolated from patients with infective endocarditis were used; two were MSSA and two were MRSA. The microorganisms were stored in defibrinated sheep blood at −70°C. Before testing, each strain was thawed and subcultured in tryptic soy agar containing 5% sheep blood.

A macrodilution method was used for susceptibility testing (21). Inocula were prepared from broth cultures in the log phase of growth to yield an inoculum size of $\geq 5.5 \times 10^5$ CFU of staphylococci per ml and were inoculated into serial twofold dilutions of antimicrobial agent in Mueller-Hinton broth. Subcultures were made for confirmation of purity and quantitation of the inoculum size. Tubes containing the inoculum in serially diluted concentrations of antimicrobial agents were incubated for 18 to 24 h at 35°C in room air. The MIC was defined as the lowest concentration of antimicrobial agent in broth without visible growth of staphylococci. The MBC was determined by subculture of 100 μl of broth from the control tube, the first tube containing growth, and from all tubes without visible growth and was defined as the lowest concentration of antibiotic that killed ≥99.9% of the original inoculum.

Tests for in vitro synergy were performed by the time-kill method with nafcillin or gentamicin singly or in combination, using an inoculum size of $\geq 10^7$ CFU of staphylococci per ml (22). Synergy was defined as at least a 100-fold increase in killing of staphylococci by a combination of nafcillin and gentamicin compared with that achieved by either drug alone. Tests were performed in triplicate, and the results are expressed as mean values.

Animal studies. Experimental aortic valve endocarditis was established in 352 New Zealand White rabbits (weight, >2 kg) by modifications of the methods described by Garrison and Freedman (12). Briefly, animals were anesthetized with a mixture of ketamine and xylazene injected intramuscularly (i.m.). An incision was made in the neck, and the right carotid artery was exposed. The artery was ligated distally, and a sterile polyethelene catheter (PE 90; Clay-Adams) was inserted into the artery through a small incision and advanced proximally across the aortic valve into the left ventricle. A pressure-sensitive monitoring device was attached to the distal end of the catheter to ensure that the catheter tip crossed the aortic valve and entered the left ventricle. The end of the catheter was sealed and tied to the carotid artery, and the would was closed over the catheter with surgical clips. The catheter was left in place throughout the experiment.

Twenty-four hours after the insertion of the catheter, 1 ml of broth containing 10^6 to 10^7 CFU of *S. aureus* per ml was injected into a peripheral ear vein. The presence of endocar-

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TABLE 1. In vitro susceptibility of two strains of MSSA and two strains of MRSA used for experimental endocarditis

Antimicrobial agent	μg/ml							
	MSSA				MRSA			
	Strain 1		Strain 2		Strain 1		Strain 2	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Nafcillin	0.5	1	0.25	0.5	>16	>16	>16	>16
Imipenem	0.06	0.06	0.06	0.06	8	>16	>16	>16
Ciprofloxacin	0.25	0.25	0.25	0.5	0.13	0.5	0.13	1
Vancomycin	0.5	1	1	4	1	2	0.5	1
Gentamicin	0.25	1.0	0.25	2.0	0.25	1	0.25	1

ditis was confirmed by a blood culture yielding staphylococci obtained before initiation of antimicrobial therapy.

Antimicrobial therapy was started 24 h after intravenous injection of *S. aureus*. Animals were placed into treatment groups as follows.

For MSSA, (i) controls included 32 animals (16 with each strain) that received no antimicrobial therapy; (ii) the imipenem group consisted of 40 animals (20 with each strain) that received therapy with imipenem, 40 mg/kg of body weight i.m. three times daily (t.i.d.) for 2 (16 animals) or 3 (24 animals) days; (iii) the nafcillin group consisted of 48 animals (24 with each strain) that received therapy with nafcillin, 200 mg/kg of body weight i.m. t.i.d. for 2 (24 animals) or 3 (24 animals) days; (iv) the ciprofloxacin group was made up of 48 animals (24 with each strain) that received therapy with ciprofloxacin, 30 mg/kg of body weight i.m. t.i.d. for 2 (24 animals) or 3 (24 animals) days; and (v) the nafcillin-plus-gentamicin group was composed of 48 animals (24 with each strain) that received therapy with nafcillin, 200 mg/kg of body weight i.m. t.i.d., plus gentamicin, 1.5 mg/kg i.m. t.i.d., for 2 (24 animals) or 3 (24 animals) days.

For MRSA, (i) controls included 16 animals (8 with each strain) that received no antimicrobial therapy; (ii) the nafcillin group consisted of 24 animals (12 with each strain) that received therapy with nafcillin, 200 mg/kg of body weight i.m. t.i.d. for 3 (12 animals) or 5 (12 animals) days; (iii) the vancomycin group consisted of 48 animals (24 with each strain) that received therapy with vancomycin, 25 mg/kg of body weight intravenously twice daily for 3 (24 animals) or 5 (24 animals) days; and (iv) the ciprofloxacin group was made up of 48 animals (24 with each strain) that received therapy with ciprofloxacin, 30 mg/kg of body weight i.m. t.i.d. for 3 (24 animals) or 5 (24 animals) days.

After the final day of treatment and at least 12 h after administration of the last dosage of antimicrobial agent(s), animals were sacrificed by intravenous injection of sodium pentobarbital. The chest was opened, the heart was excised and opened, and the aortic valve vegetations were removed aseptically. The vegetations were individually weighed and homogenized, and the entire vegetation was cultured. The number of CFU of *S. aureus* per gram of valve vegetation was quantitated by using a pour plate method with tryptic soy agar. The results were expressed as $\log_{10}$ CFU of *S. aureus* per gram of valve vegetation. Sterile vegetations were considered to have 2 $\log_{10}$ CFU/g of valve vegetation. Staphylococci recovered from cardiac valve vegetations were screened for emergence of resistance during therapy to >4 μg of ciprofloxacin, >16 μg of imipenem, >4 μg of nafcillin, >4 μg of gentamicin, and >4 μg of vancomycin per ml.

Measurement of serum concentration of antimicrobial agents. Pharmacokinetic studies were performed in uninfected control animals (five rabbits per antibiotic). Blood samples were obtained for measurement of serum concentration 0.5, 1, 2, 3, 4, 5, 6, and 8 h after administration of gentamicin, nafcillin, imipenem, ciprofloxacin, and vancomycin. Additional blood samples were obtained 10 and 12 h after administration of vancomycin. On day 2 of therapy in rabbits with endocarditis, 0.5 h after the administration of antibiotic, blood samples were obtained through a peripheral ear vein for measurement of serum antimicrobial concentration. Serum concentrations of gentamicin were measured by fluorescence polarization, and those of nafcillin, imipenem, ciprofloxacin, and vancomycin were measured by bioassay (23).

Analysis of results. Differences in mean $\log_{10}$ CFU of *S. aureus* per gram of valve vegetation were analyzed statistically, using the Wilcoxon test and two-way analysis of variance (9).

RESULTS

The MICs and MBCs for the four strains of *S. aureus* determined by the macrodilution method are shown in Table 1. Figures 1 to 4 show the results of in vitro killing by antibiotics alone or in combination.

The mean 0.5-h concentrations of antimicrobial agents in serum in uninfected control rabbits were as follows: nafcillin, 72.8 ± 4.3 μg/ml; gentamicin, 3.2 ± 0.8 μg/ml; ciprofloxacin, 4.2 ± 0.7 μg/ml; imipenem, 42.3 ± 6.4 μg/ml; vancomycin, 57.0 ± 3.4 μg/ml. Eight hours after administration, the mean concentrations in serum in uninfected controls and in rabbits with endocarditis were <1.0 μg of imipenem, nafcillin, ciprofloxacin, and gentamicin per ml and 1.2 μg of vancomycin per ml. The mean concentration of vancomycin in serum 12 h after administration was <1 μg/ml. On day 2 of infection in animals with endocarditis, 0.5 h after administration, the mean concentrations of antimicrobial agents in serum were as follows: nafcillin, 70.6 ± 3.1 μg/ml; gentami-

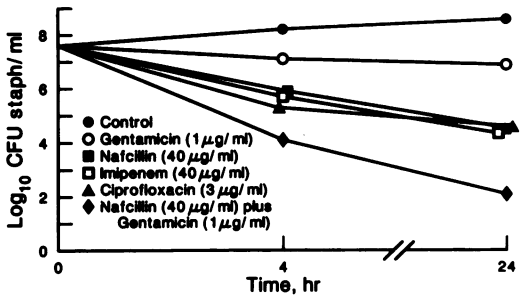

FIG. 1. Rate of killing in vitro of MSSA (strain 1) by ciprofloxacin, nafcillin, imipenem, gentamicin, or nafcillin plus gentamicin.

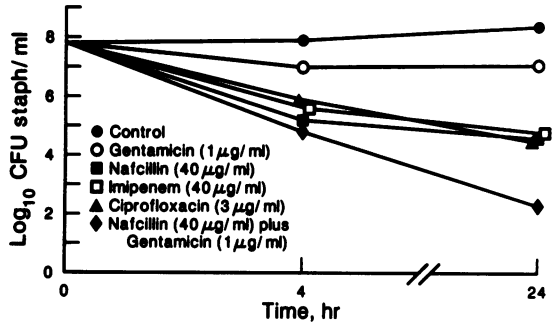

FIG. 2. Rate of killing in vitro of MSSA (strain 2) by ciprofloxacin, nafcillin, imipenem, gentamicin, or nafcillin plus gentamicin.

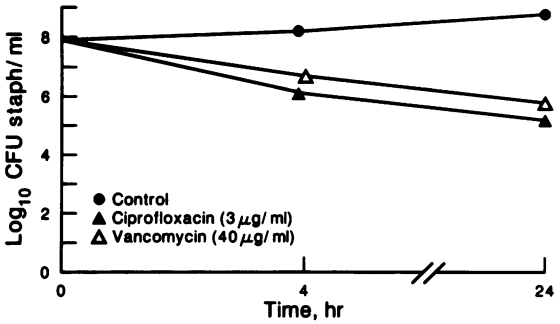

FIG. 4. Rate of killing in vitro of MRSA (strain 2) by ciprofloxacin or vancomycin.

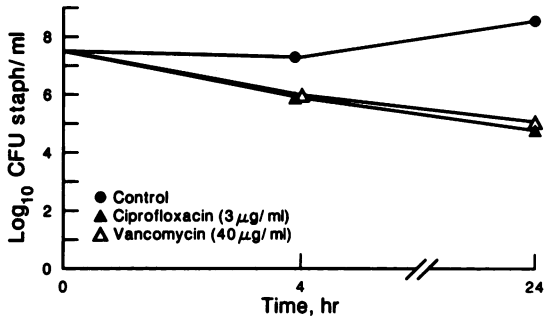

FIG. 3. Rate of killing in vitro of MRSA (strain 1) by ciprofloxacin or vancomycin.

cin, 3.6 ± 0.2 μg/ml; ciprofloxacin, 5.8 ± 0.5 μg/ml, imipenem, 39.7 ± 7.4 μg/ml; vancomycin, 60.2 ± 2.8 μg/ml.

The results of treatment of MSSA experimental endocarditis are shown in Table 2. Ciprofloxacin was more effective ($P < 0.01$) therapy than either imipenem or nafcillin. There was no significant difference between the results of treatment with ciprofloxacin and those with the combination of nafcillin and gentamicin. Imipenem was more effective ($P < 0.01$) after 3 days of therapy than after 2 days of treatment of endocarditis caused by either strain of MSSA. The numbers of rabbits excluded from analysis because of early death during antimicrobial therapy were two with imipenem, three with nafcillin, two with ciprofloxacin, and one with nafcillin plus gentamicin. The differences in mortality were not significant. The in vitro susceptibility of the staphylococci recovered from cardiac valve vegetations did not change when compared with pretreatment results.

Table 3 shows the results of treatment of MRSA experimental endocarditis. After 3 days of therapy, ciprofloxacin was more effective ($P < 0.01$) than vancomycin against both strains of MRSA. After 5 days of therapy, there was no significant difference in the results of treatment with ciprofloxacin compared with those of vancomycin. The numbers of rabbits excluded from analysis because of early death during therapy were nine with nafcillin (three in 3-day group and six in 5-day group), five with vancomycin (one in 3-day group and four in 5-day group), and four with ciprofloxacin (one in 3-day group and three in 5-day group). The mortality among animals treated with nafcillin was higher ($P < 0.05$) than in rabbits treated with vancomycin or ciprofloxacin. The mortality was not significantly different in animals treated with vancomycin compared with ciprofloxacin. The in vitro susceptibilities of the staphylococci recovered from cardiac valve vegetations did not change when compared with pretreatment results.

DISCUSSION

In our study, ciprofloxacin was the most effective single antimicrobial agent for the treatment of MSSA or MRSA experimental endocarditis. In addition to an increased magnitude of killing in vivo of *S. aureus*, ciprofloxacin therapy resulted in more rapid killing in vivo of MSSA than did therapy with imipenem or nafcillin. Ciprofloxacin therapy was also more rapidly bactericidal in vivo than was vancomycin therapy of MRSA endocarditis. Emergence of resistant subpopulations of staphylococci did not occur during

TABLE 2. Results of treatment of MSSA experimental endocarditis

Dosage of antimicrobial agent, i.m. t.i.d. (mg/kg)	Mean $\log_{10}$ CFU/g of valve vegetations ± SD[a]			
	Strain 1		Strain 2	
	2-day treatment	3-day treatment	2-day treatment	3-day treatment
None	9.8 ± 0.2[b]	10.3 ± 0.3[b]	10.1 ± 0.3[b]	9.9 ± 0.4[b]
Imipenem, 40	7.4 ± 1.5[b,c,e]	3.8 ± 1.6[b,d,e]	6.8 ± 1.6[b,c,e]	4.1 ± 0.9[b,e]
Nafcillin, 200	4.6 ± 1.9[c,e]	5.1 ± 1.4[d,e]	5.3 ± 1.1[c,e]	4.5 ± 1.8[e]
Ciprofloxacin, 30	3.2 ± 1.3[e,f]	2.4 ± 1.1[e,f]	2.3 ± 0.9[e,f]	2.7 ± 0.5[e,f]
Nafcillin, 200, + gentamicin, 1.5	2.9 ± 0.8[f]	2.8 ± 0.6[f]	2.7 ± 0.5[f]	2.1 ± 0.3[f]

[a] Each value represents the mean of results from 8 to 12 rabbits.
[b] $P < 0.01$, imipenem versus control.
[c] $P < 0.01$, nafcillin (2-day treatment) versus imipenem (2-day treatment).
[d] $P < 0.01$, imipenem (3-day treatment) versus nafcillin (3-day treatment), strain 1.
[e] $P < 0.01$, ciprofloxacin versus imipenem or nafcillin.
[f] P not significant, ciprofloxacin versus nafcillin plus gentamicin.

TABLE 3. Results of treatment of MRSA experimental endocarditis

Dosage of antimicrobial agent (mg/kg)	Mean $\log_{10}$ CFU/g of valve vegetations ± SD[a]			
	Strain 1		Strain 2	
	3-day treatment	5-day treatment	3-day treatment	5-day treatment
None	10.3 ± 0.2[b]	—[c]	10.1 ± 0.3[b]	—[c]
Nafcillin, 200, i.m. t.i.d.	9.6 ± 0.8[b]	10.1 ± 1.0[b]	10.3 ± 0.5[d]	9.7 ± 0.6[b]
Vancomycin, 25, i.v. b.i.d.[e]	3.5 ± 1.4[b,d]	2.5 ± 0.5[b,f]	3.8 ± 1.8[b,d]	2.5 ± 0.3[b,f]
Ciprofloxacin, 30, i.m. t.i.d.	2.4 ± 0.2[d]	2.9 ± 1.0[f]	2.3 ± 0.8[d]	2.4 ± 0.1[f]

[a] Each value represents the mean of results from 6 to 12 animals.
[b] $P < 0.001$, vancomycin versus control (3 days) or nafcillin.
[c] —, None of the animals survived 5 days without treatment.
[d] $P < 0.01$, ciprofloxacin (3-day treatment) versus vancomycin (3-day treatment).
[e] i.v., Intravenously; b.i.d., twice a day.
[f] P not significant, ciprofloxacin (5-day treatment) versus vancomycin (5-day treatment).

treatment with any of the antimicrobial agents used. This result is not surprising, however, because of the relatively short duration of therapy.

We and others (19) demonstrated that the combination of nafcillin and gentamicin was more effective therapy than nafcillin alone for the treatment of MSSA experimental endocarditis. In our study, combined therapy with nafcillin and gentamicin was no more effective than was ciprofloxacin alone. Carpenter et al. (4) reported that the efficacy of ciprofloxacin therapy was similar to that of nafcillin or vancomycin for the treatment of MSSA or MRSA experimental endocarditis, respectively. This study used only one strain of MSSA or MRSA and did not evaluate the results of therapy with a combination of antimicrobial agents or assess the rapidity of killing in vivo. Kaatz and colleagues (13) reported that ciprofloxacin was as effective as vancomycin therapy of MRSA experimental endocarditis. These authors observed that the concentrations of ciprofloxacin in serum on day 2 of therapy in animals with endocarditis were higher than those noted in single-dose studies in uninfected animals and suggested that this finding was likely the result of changes in pharmacokinetics of ciprofloxacin with multiple dosing in infected animals compared with a single dose in uninfected rabbits. In our study, the mean concentration of ciprofloxacin in serum 0.5 h after administration increased from 4.2 ± 0.7 μg/ml in uninfected animals to 5.8 ± 0.7 μg/ml in animals on day 2 of infection with endocarditis. However, it is difficult to compare the results of concentrations of ciprofloxacin in serum reported by Kaatz et al. (13) with those in our study because the time of collection of serum samples for determination of ciprofloxacin concentrations differed in the two studies.

Current therapy for serious staphylococcal infection in humans requires prolonged costly hospitalization and the use of antimicrobial agents alone or in combination which are associated with potentially serious adverse or toxic effects. Ciprofloxacin is well adsorbed from the gastrointestinal tract, and administration of oral dosages to humans resulted in concentrations in serum similar to those observed in our study in rabbits following parenteral administration (7). If therapy with ciprofloxacin were shown to be effective for human cases of *S. aureus* infection, ciprofloxacin might be an attractive alternative to therapy with a beta-lactam or vancomycin. Moreover, because ciprofloxacin can be administered orally, such therapy would be considerably more cost effective than a comparable course with parenteral therapy. The results of our study with *S. aureus* experimental endocarditis suggest that further studies are warranted to clarify the role of ciprofloxacin for the treatment of MSSA and MRSA infection in humans.

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