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Ciprofloxacin Therapy of Experimental Endocarditis Caused by Methicillin-Resistant *Staphylococcus epidermidis*

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Ciprofloxacin or rifampin was significantly ($P < 0.05$) more effective than vancomycin or the combination of vancomycin plus gentamicin for the treatment of *Staphylococcus epidermidis* experimental endocarditis. There were no significant differences in efficacy among any of the combinations of antimicrobial agents that included ciprofloxacin or rifampin. One animal treated with rifampin alone and one treated with the combination of vancomycin, rifampin, and gentamicin were found to be infected with rifampin-resistant strains of *S. epidermidis* during therapy. Resistant subpopulations of *S. epidermidis* were not detected during therapy with any other antimicrobial agent used alone or in combination. Ciprofloxacin alone or in combination with rifampin was effective therapy against *S. epidermidis* experimental endocarditis.

Staphylococcus epidermidis is the most common causative agent of prosthetic valve endocarditis (4, 9, 11, 15, 16). Strains of *S. epidermidis* recovered from patients with prosthetic valve endocarditis are usually resistant to beta-lactam antimicrobial agents, and the mortality rate for patients with this infection is very high, 58 to 74% (9). The current antimicrobial therapy recommended for patients with methicillin-resistant *S. epidermidis* prosthetic valve endocarditis consists of administration of vancomycin either alone or in combination with either rifampin or gentamicin or administration of all three agents simultaneously (9). The use of these antimicrobial agents is costly and may be associated with significant adverse or toxic effects.

Ciprofloxacin is effective in vitro against a wide variety of gram-positive and gram-negative microorganisms, including *S. epidermidis* (2, 6). Accordingly, ciprofloxacin might be useful therapy for patients with *S. epidermidis* infective endocarditis. The purpose of this study was to determine the efficacy of ciprofloxacin alone or in combination with rifampin for treatment of *S. epidermidis* experimental endocarditis and to compare the results with those resulting from therapy with other antimicrobial regimens.

MATERIALS AND METHODS

In vitro studies. Eighteen strains of *S. epidermidis* isolated from patients with infective endocarditis seen at the Mayo Clinic were studied. A macrodilution method was used for in vitro susceptibility testing (19). The MIC was defined as the lowest concentration of antimicrobial agent in broth without visible growth of staphylococci. The MBC was determined by subculturing 100 μl of nonsupplemented Mueller-Hinton 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.

Of these 18 strains, 1 strain was selected for in vitro studies and for use in an experimental endocarditis model. This strain was chosen because of its ability to consistently produce experimental endocarditis in rabbits.

Killing curves were performed with antibiotic singly or in combination by using an inoculum size of $>10^6$ CFU of staphylococci per ml (19). Tests were performed in triplicate, and the results were expressed as the mean value.

Animal studies. Experimental aortic valve endocarditis was established in a total of 119 New Zealand White rabbits (weight, >2 kg) by modifications of the methods described by Garrison and Freedman (8). 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 polyethylene catheter (PE 90; Intramedic; 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 insertion of the catheter, 1 ml of broth containing 10^7 to 10^8 CFU of *S. epidermidis* per ml was injected into a peripheral ear vein. The presence of endocarditis was confirmed by a blood culture that yielded staphylococci that were obtained 24 h after infection and before the initiation of antimicrobial therapy.

Antimicrobial therapy was initiated 24 h after intravenous (i.v.) injection of *S. epidermidis*. Animals were placed into treatment groups as follows: (i) For the control group, nine animals received no antimicrobial therapy. (ii) For the vancomycin group, 16 animals received therapy with vancomycin (25 mg/kg of body weight i.v. two times daily [b.i.d.]). (iii) For the ciprofloxacin group, 16 animals received therapy with ciprofloxacin (30 mg/kg of body weight i.m. three times daily [t.i.d.]). (iv) For the rifampin group, 15 animals received therapy with rifampin (10 mg/kg of body weight i.m. b.i.d.). (v) For the vancomycin-gentamicin group, 16 animals received therapy with vancomycin (25 mg/kg of body weight i.v. b.i.d.) and gentamicin (1.05 mg/kg of body weight i.m. b.i.d.). (vi) For the vancomycin-rifampin group, 15 animals received therapy with vancomycin (25 mg/kg of body weight i.v. b.i.d.) and rifampin (10 mg/kg of body weight i.m. b.i.d.). (vii) For the ciprofloxacin-rifampin group, 16 animals received therapy with ciprofloxacin (30 mg/kg of body weight

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TABLE 1. In vitro susceptibility of 18 strains of *S. epidermidis*

Antimicrobial agent	MIC (μg/ml)[a]			MBC (μg/ml)[b]		
	50%	90%	Range	50%	90%	Range
Ciprofloxacin	0.25	0.25	≤0.125–0.5	0.25	0.5	≤0.125–1.0
Rifampin	≤0.125	0.25	≤0.125–0.25	≤0.125	0.5	≤0.125–0.5
Vancomycin	≤0.125	1.0	≤0.125–2.0	0.25	4	0.25–>8
Methicillin	>8	>8	0.25–>8	>8	>8	2–>8
Gentamicin	≤0.125	0.5	≤0.125–1	0.25	4	0.25–4

[a] 50% and 90%, MIC for 50% and 90% of strains, respectively.
[b] 50% and 90%, MBC for 50% and 90% of strains, respectively.

i.m. t.i.d.) and rifampin (10 mg/kg of body weight i.m. b.i.d.). (viii) For the vancomycin-gentamicin-rifampin group, 16 animals received therapy with vancomycin (25 mg/kg of body weight i.v. b.i.d.), gentamicin (1.05 mg/kg of body weight i.m. b.i.d.), and rifampin (10 mg/kg of body weight i.m. b.i.d.).

Animals were treated for a total of 3 days. At 12 to 14 h after administration of the last dosage of antimicrobial agent(s), animals were sacrificed by i.v. injection of sodium pentobarbital. The chest was opened, the heart was removed and opened, and aortic valve vegetations were removed aseptically. The vegetations were weighed and homogenized, and the entire valve vegetation was cultured. The homogenized valve vegetation were diluted 1:1,000 during quantitative culture, which minimized the carry-over effect, if any, of the antimicrobial agent(s) on cultures of vegetations. The number of CFU of *S. epidermidis* per gram of valve vegetation was quantitated by a pour plate method with tryptic soy agar. Results were expressed as the $\log_{10}$ CFU of *S. epidermidis* per gram of valve vegetation. Sterile vegetations were considered to have 2 $\log_{10}$ CFU per gram of valve vegetation. Portions of homogenized valve vegetation were screened for subpopulations of *S. epidermidis* which developed in vitro resistance during treatment of ≥8 μg of vancomycin, rifampin, or gentamicin per ml or ≥4 μg of ciprofloxacin per ml.

The concentrations of antimicrobial agents in serum were determined in five uninfected animals after administration of the third dosage of antimicrobial agent. Blood samples were obtained through a peripheral ear vein at 0.5, 1, 2, 4, 8, 12, and 24 h after administration of the antimicrobial agent. Among the animals with endocarditis, on day 2 of therapy at 0.5 h after administration of the dosage of the antimicrobial agent(s), blood samples were obtained for measurement of the antimicrobial agent concentration in serum. Concentrations of vancomycin and gentamicin in serum were measured by fluorescence polarization, and those of ciprofloxacin and rifampin were measured by bioassay (20).

Analysis of results. Differences in mean $\log_{10}$ CFU per gram of valve vegetation were analyzed by the Student-Newman-Keuls tests for variables (5).

RESULTS

The MICs and MBCs for 90% of the 18 strains of *S. epidermidis* tested are shown in Table 1. The MICs and MBCs for the strain used for in vitro studies and experimental endocarditis were as follows: methicillin, >8 μg/ml; gentamicin, 0.25 and 1.0 μg/ml; vancomycin, 0.5 and 1.0 μg/ml; ciprofloxacin, 0.25 and 1.0 μg/ml; and rifampin, 0.25 and 0.5 μg/ml, respectively. Table 2 shows the results of time-kill curves. The magnitude of in vitro killing was greatest with the combination of ciprofloxacin and rifampin.

The concentrations of antimicrobial agents in the serum of uninfected animals are shown in Table 3. Among infected animals on day 2 of therapy, the mean concentrations in serum at 0.5 h were 62.5 ± 6.2 μg of vancomycin per ml, 3.0 ± 0.6 μg of gentamicin per ml, and 3.8 ± 0.9 μg of ciprofloxacin per ml and 4.2 ± 0.6 μg of rifampin per ml.

The results of treatment of *S. epidermidis* experimental endocarditis are shown in Table 4. Treatment with ciprofloxacin or rifampin was significantly ($P < 0.05$) more effective than treatment with vancomycin alone or the combination of vancomycin plus gentamicin. There were no significant differences in the effects of therapy among any of the combinations of antimicrobial agents that included rifampin compared with the effects of treatment with ciprofloxacin or

TABLE 2. In vitro activity of antimicrobial agents alone or in combination against *S. epidermidis*

Antimicrobial agent (concn [μg/ml])	$\log_{10}$ CFU of *S. epidermidis*/ml at[a]:		
	0 h	4 h	24 h
Control	6.8	7.4	8.9
Vancomycin (2)	6.8	6.2	4.7
Gentamicin (0.5)	6.8	6.5	7.1
Rifampin (0.5)	6.8	5.9	4.4
Ciprofloxacin (0.5)	6.8	6.2	4.6
Vancomycin (2) plus gentamicin (0.5)	6.8	5.1	2.6
Vancomycin (2) plus rifampin (0.5)	6.8	5.7	5.1
Ciprofloxacin (0.5) plus rifampin (0.5)	6.8	4.9	1.6
Vancomycin (2) plus rifampin (0.5) plus gentamicin (0.5)	6.8	5.2	1.9

[a] Data represent the mean values of three separate tests performed in triplicate. The standard deviations were ≤0.5.

TABLE 3. Concentrations of antimicrobial agents in rabbit shown from 0.5 to 24 h after administration

Antimicrobial agent (dose)	Concn in serum (μg/ml) at the following times (h)[a]:						
	0.5	1	2	4	8	12	24
Vancomycin (25 mg/kg i.v. b.i.d.)	57	36	19.3	10.9	2.5	1.2	0.8
Gentamicin (1.05 mg/kg i.m. b.i.d.)	4.4	2.6	1.9	0.4	<0.1	<0.1	<0.1
Rifampin (10 mg/kg i.m. b.i.d.)	3.8	4.5	5.9	4.3	3.2	1.7	0.6
Ciprofloxacin (30 mg/kg i.m. t.i.d.)	3.3	2.7	1.9	1.2	0.6	0.5	0.3

[a] Results are mean values from five uninfected animals measured after administration of a third dose of antimicrobial agent. The standard deviations were ≤2.5 μg/ml.

TABLE 4. Results of treatment of *S. epidermidis* experimental endocarditis in rabbits

Antimicrobial agent (dose)	No. of animals	No. of animals with sterile valve vegetations	Mean $\log_{10}$ CFU/g of valve vegetation ± SD
None	9		8.4 ± 0.4[a]
Vancomycin (25 mg/kg i.v. b.i.d.)	16	2	4.8 ± 2.1[a,b]
Vancomycin (25 mg/kg i.v. b.i.d.) plus gentamicin (1.05 mg/kg i.m. b.i.d.)	16	1	4.4 ± 1.8[a,b]
Ciprofloxacin (30 mg/kg i.m. t.i.d.)	16	0	3.2 ± 1.0[a,b,c]
Rifampin (10 mg/kg i.m. b.i.d.)	15	1	2.8 ± 1.5[a,b,c]
Vancomycin (25 mg/kg i.v. b.i.d.) plus rifampin (10 mg/kg i.m. b.i.d.)	15	8	2.2 ± 0.8[a,b,c]
Ciprofloxacin (30 mg/kg i.m. t.i.d.) plus rifampin (10 mg/kg i.m. b.i.d.)	16	6	2.1 ± 1.2[a,b,c]
Vancomycin (25 mg/kg i.v. b.i.d.) plus gentamicin (1.05 mg/kg i.m. b.i.d.) plus rifampin (10 mg/kg i.m. b.i.d.)	16	5	2.0 ± 0.5[a,b,c]

[a] $P < 0.01$ for no antimicrobial therapy versus treatment with any drug used alone or in combination.
[b] $P < 0.05$ for antimicrobial therapy with vancomycin or vancomycin-gentamicin versus therapy with any other drug used alone or in combination.
[c] P was not significant for therapy with ciprofloxacin or rifampin versus treatment with vancomycin-rifampin, ciprofloxacin-rifampin, or vancomycin-rifampin-gentamicin.

rifampin administered alone. The number of animals with sterile cardiac valve vegetations was greater ($P < 0.03$) among animals treated with combinations of antimicrobial agents that included rifampin than among animals treated with a single drug alone or with the combination of vancomycin and gentamicin.

In one animal treated with rifampin alone and in one animal treated with the combination of vancomycin, gentamicin, and rifampin, resistance to rifampin (MIC, >8 μg/ml) was developed during therapy. Subpopulations of *S. epidermidis* resistant to other antimicrobial agents were not detected among any of the other treatment groups. There was no cross-resistance of rifampin-resistant strains to the other antimicrobial agents used in our study.

DISCUSSION

In our study, ciprofloxacin or rifampin was the most effective single agent for the treatment of *S. epidermidis* experimental endocarditis, and either agent alone was more effective ($P < 0.05$) than vancomycin or the combination of gentamicin and vancomycin. Galetto et al. (7) and Kobasa and colleagues (13) have reported that the combination of vancomycin and gentamicin is more effective therapy for *S. epidermidis* experimental endocarditis than is vancomycin alone. In our study, the combination of vancomycin and gentamicin was no more effective therapy than was vancomycin alone.

The results of our study were also in agreement with previously published studies which described the in vitro activity of rifampin and the efficacy of rifampin therapy for *S. epidermidis* experimental endocarditis (1, 10, 13, 14, 17, 18). Combinations of ciprofloxacin and rifampin or vancomycin and rifampin were as effective as triple-drug therapy with vancomycin-rifampin-gentamicin. Additionally, significantly more ($P < 0.03$) animals had sterile vegetations following therapy with combinations of antimicrobial agents that included rifampin.

Rifampin-resistant strains of *S. epidermidis* were detected in only two animals (one treated with rifampin alone and one treated with the combination of rifampin, vancomycin, and gentamicin). The emergence of rifampin-resistant strains of *S. epidermidis* during treatment of experimental endocarditis in rabbits and during treatment of *S. epidermidis* prosthetic valve endocarditis in humans has been observed previously (1, 9, 11, 14, 18). In these reports, rifampin-resistant strains occurred more frequently when rifampin was administered alone rather than in combination with other antimicrobial agents. The results of these experimental and human studies suggest that rifampin should not be used alone for the treatment of *S. epidermidis* endocarditis.

Katz and associates (12) have reported that the concentrations of ciprofloxacin in the serum of rabbits with *S. aureus* experimental endocarditis were higher on day 2 of therapy than those noted in single-dose studies in uninfected animals. These investigators suggested that this finding was likely the result of accumulation of ciprofloxacin in serum in infected animals. This factor could influence the outcome of treatment of experimental endocarditis. In our study, mean concentrations of ciprofloxacin in serum were similar in uninfected animals at 0.5 h after administration of three dosages of ciprofloxacin (3.3 μg/ml) compared with those in infected animals on day 2 of therapy (3.8 μg/ml).

Serious nosocomial infections caused by *S. epidermidis* are increasing in frequency. Methicillin-resistant *S. epidermidis* infections associated with the use of intravascular or other prosthetic devices frequently require the administration of vancomycin, which is expensive and potentially toxic. Current therapy for *S. epidermidis* prosthetic valve endocarditis requires prolonged, costly hospitalization and administration of a combination of antimicrobial agents such as vancomycin and rifampin, with or without gentamicin, which may be associated with potentially serious adverse or toxic effects (9, 11). Ciprofloxacin and rifampin are both well absorbed from the gastrointestinal tract, and the administration of oral dosages of ciprofloxacin has resulted in concentrations in serum similar to those observed in our study following parenteral administration (3). If the combination of ciprofloxacin and rifampin was shown to be effective for human infections caused by *S. epidermidis*, such therapy might be an attractive alternative to treatment with vancomycin alone or in combination with other antimicrobial agents. Moreover, because ciprofloxacin and rifampin therapy may be administered orally, such therapy would be considerably more cost-effective than a comparable course with parenteral therapy. Results of our studies suggest that further studies are warranted to clarify the role of ciprofloxacin alone or in combination with rifampin for the treatment of methicillin-resistant *S. epidermidis* infections in humans. These studies need to define whether the combination of ciprofloxacin and rifampin reduces the likelihood of emergence of ciprofloxacin- or rifampin-resistant strains of *S. epidermidis* when either of these agents is administered alone.

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