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Comparative Efficacy of Trovafloxacin in Experimental Endocarditis Caused by Ciprofloxacin-Sensitive, Methicillin-Resistant *Staphylococcus aureus*

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The new fluoroquinolone trovafloxacin was tested against a ciprofloxacin-sensitive, methicillin-resistant *Staphylococcus aureus* strain in the rabbit model of endocarditis. Trovafloxacin was more effective than vancomycin (CFU/g of vegetation, 2.65 ± 1.87 versus 4.54 ± 2.80 [mean ± standard deviation]; $P < 0.05$) or ampicillin-sulbactam plus rifampin (4.9 ± 1.1 CFU/g). The addition of ampicillin-sulbactam to trovafloxacin tended to reduce titers further.

Staphylococcus aureus is a common cause of infective endocarditis (26, 31). To achieve high cure rates for staphylococcal endocarditis, prolonged therapy is required with antibiotics that achieve bactericidal activity at the site of infection, the cardiac vegetation (1). Staphylococci have shown a remarkable capacity over the years to develop resistance against commonly used antibiotics. Early after the introduction of penicillin into clinical practice, they acquired a penicillinase which now makes the majority of staphylococci resistant to penicillin G (11). In the 1960s, the development of penicillinase-stable penicillins, such as methicillin or nafcillin, was immediately followed by the discovery of staphylococci that are resistant to these drugs (16). These methicillin-resistant *S. aureus* (MRSA) strains are now important pathogens of nosocomial and, increasingly, community-acquired infections and cause endocarditis in hospitalized patients as well as in injection drug users (8). Vancomycin is currently the only clinically tested antibiotic for therapy for MRSA endocarditis (4). However, the drug is less rapidly bactericidal than penicillins to sensitive staphylococci, thus prolonging the time to sterilization of the infected site, and the outcome for serious infections is not always favorable (15, 20, 29). Moreover, reduced sensitivity of MRSA strains to vancomycin has recently been recognized in a few staphylococci isolated in Japan and the United States (14, 21). This occurrence had been expected based on laboratory observations, and the possibility exists that, similar to the increasing problem of vancomycin-resistant enterococci, the loss of activity of vancomycin against MRSA strains will become a clinically relevant problem (22). Thus, the need to develop alternative therapies for the treatment of serious infections with MRSA strains, such as endocarditis, is urgent.

Quinolones have good bactericidal activity against many sensitive pathogens and are generally well tolerated (27). The activity of older quinolones, such as ciprofloxacin, against gram-positive pathogens has not been consistent, and some studies have documented a lack of activity of ciprofloxacin against staphylococci in experimental endocarditis (9, 17). Recently, several new quinolones have entered the stage of clinical development, and one of the hallmarks of this new group of drugs is markedly improved activity against gram-positive pathogens, including staphylococci and pneumococci (25). Trovafloxacin is a representative of these new quinolones with extended activity against gram-positive pathogens (12, 18, 19). The purpose of the present study was to compare the in vivo efficacy of trovafloxacin in experimental endocarditis caused by a quinolone-sensitive strain of MRSA with those of vancomycin and the combination of ampicillin-sulbactam plus rifampin. The latter regimen has been proposed as a possible alternative to vancomycin in the treatment of MRSA endocarditis, based on promising results in the rabbit model of endocarditis (6).

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In vitro studies. MRSA strain 76 (13, 23) was used for all *S. aureus* endocarditis experiments. This strain is quinolone and vancomycin sensitive (Table 1). Trovafloxacin (in the form of alatrofloxacin, the parenterally applicable prodrug of trovafloxacin) was provided by Pfizer Inc. (Groton, Conn.). Ampicillin-sulbactam, vancomycin, and rifampin were obtained from commercial sources.

MICs were determined in Todd-Hewitt broth by the standard tube macrodilution method with an inoculum of 3.75×10^5 CFU/ml for the MRSA strain. The MIC was defined as the lowest concentration inhibiting visible growth after 24 h of incubation at 37°C in room air with 5% CO_2. The MICs for the MRSA strain are presented in Table 1. The low trovafloxacin MIC reflected the quinolone-sensitive nature of this MRSA strain. It is important to note, however, that many MRSA strains are resistant to quinolones.

Endocarditis model in rabbits. The animal studies were approved by the Committee on Animal Research of the University of California, San Francisco. The rabbit model of aortic valve endocarditis established by Perlman and Freedman was used (24). Endocarditis was induced in anesthetized rabbits by placing a permanent transaortal catheter through an incision in the carotid artery. Twenty-four hours after insertion of the catheter, rabbits were infected with an intravenous inoculum of the MRSA strain suspended in 1 ml of saline ($\log_{10}$ 6.5 to 7.7 CFU/ml). The accuracy of the inoculum was confirmed by quantitative cultures.

Twenty-four hours after infection, rabbits with MRSA en-

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TABLE 1. MICs (μg/ml) of drugs tested

Drug	MIC for *S. aureus* 76
Trovafloxacin	0.06
Vancomycin	2
Nafcillin	>128
Ampicillin	128
Ampicillin-sulbactam	128
Rifampin	0.06
Ciprofloxacin	2

docarditis were treated with one of the following antibiotic regimens for 4 days: (i) trovafloxacin (30 mg/kg of body weight twice a day [b.i.d.]), $n = 20$; (ii) vancomycin (50 mg/kg b.i.d.), $n = 23$; (iii) ampicillin-sulbactam (100 mg and 50 mg/kg, respectively, three times a day [t.i.d.]) plus trovafloxacin (30 mg/kg b.i.d.), $n = 13$; (iv) ampicillin-sulbactam (100 mg and 50 mg/kg, respectively, t.i.d.) plus rifampin (5 mg/kg t.i.d.), $n = 10$. All antibiotics were dissolved in sterile water except for rifampin, which was reconstituted in sterile diluent according to the manufacturer's instructions. Stock antibiotic solutions were diluted in saline and injected intramuscularly (i.m.). Trovafloxacin was protected from light during the injection. Infected control animals received saline. Twelve hours after the last antibiotic dose, animals were euthanized, aortic valves were removed under sterile conditions, and vegetations were homogenized and quantitatively cultured. The limit of quantitation was 1 CFU/g of vegetation.

Drug concentrations in serum were determined by the agar well diffusion method performed in antibiotic medium 11 (Difco Laboratories) in duplicate wells. Standard curves for serum were generated from rabbit serum. *Bacillus subtilis* (ATCC 6633) was used as the indicator strain for trovafloxacin and *S. aureus* 209P was used as the indicator strain for vancomycin. The limits of detection were 0.12 μg for trovafloxacin and 1 μg/ml for vancomycin.

All results are expressed as means ± standard deviations. Comparisons between groups were performed by analysis of variance. Significance was determined by the Student-Newman-Keuls test.

In vivo results. The dose of antibiotics chosen in the present study produced serum concentrations in the rabbits that approximated those achieved in humans (30). For trovafloxacin, serum concentration was 1.04 ± 0.35 μg/ml 1 h after a 30 mg/kg i.m. injection (approximate peak), while the trough concentration 12 h after injection was 0.49 ± 0.19 μg/ml. For vancomycin, serum concentrations 1 h after injection of a dose of 50 mg/kg were 18.4 ± 6.6 μg/ml, whereas trough concentrations were 3.4 ± 1.6 μg/ml. Because of the i.m. administration, the concentrations of the drugs at the approximate peak were somewhat lower than what would be expected after intravenous injection. Pharmacokinetic data for the administration of ampicillin-sulbactam had been determined previously (6). The mean serum concentrations 1 h after the dose used here were 23 ± 3 μg/ml for ampicillin and 40 ± 14 μg/ml for sulbactam. Both drugs had serum half-lives of approximately 1/2 h in rabbits (6).

All antibiotic treatments reduced the vegetation titers significantly compared to those for the control group (Table 2). Among the treatment groups, the trovafloxacin plus ampicillin-sulbactam-treated group had the lowest bacterial counts, followed by the trovafloxacin group. Importantly, groups treated with trovafloxacin (alone or in combination with ampicillin-sulbactam) had significantly lower bacterial titers than animals treated with vancomycin ($P < 0.05$; Table 2). The difference between trovafloxacin plus ampicillin-sulbactam and trovafloxacin alone was not significant, although there was a clear trend favoring the combination treatment.

The results of these studies show that trovafloxacin is efficacious in the treatment of experimental endocarditis caused by an MRSA strain with preserved sensitivity to quinolones. The fact that the organism was ciprofloxacin sensitive is important for several reasons. Many strains of MRSA today are ciprofloxacin resistant, and resistance to one quinolone tends to confer resistance to other drugs of the same class (28). However, some of the newer quinolones, such as trovafloxacin, may have only moderately increased MICs for such quinolone-resistant strains (3). The question of whether trovafloxacin or other new quinolones preserve effectiveness against these ciprofloxacin-resistant strains in difficult-to-treat infections such as endocarditis will need to be addressed in additional studies before an assessment can be made about the potential of these drugs for the entire group of MRSA strains. Treatment with quinolones of staphylococcal endocarditis is further complicated by the fact that with some of the quinolones, resistant mutants of the infecting strain rapidly develop (2, 9, 17). We have not screened for this occurrence in the present study, but a previous study with trovafloxacin in a similar model of staphylococcal endocarditis failed to detect such resistant mutants (18). In vitro, the frequency of spontaneous mutations leading to resistance to trovafloxacin was approximately 2 orders of magnitude below 1 organism in 10^6 to 10^7 CFU, the number of organisms present in cardiac vegetations of infected rabbits (18).

In the present study, trovafloxacin appeared more effective than vancomycin, based on significantly lower vegetation titers at the end of therapy. This finding contrasts with that of a previously published study comparing these two drugs in which there was no significant difference between trovafloxacin and vancomycin against either a methicillin-sensitive *S. aureus* strain or an MRSA strain (18). While this result could be due to strain-to-strain variation, regardless of susceptibility to vancomycin, an important difference between the two studies was the

TABLE 2. Vegetation titers, sterile vegetations, and number of animals surviving the duration of treatment in experimental endocarditis

Treatment group (*n*)	Vegetation titers ($\log_{10}$ CFU/g)	No. of sterile vegetations/total (%)	No. of animals surviving treatment/total (%)
Controls (22)[a]	8.42 ± 0.79[b]	0/22 (0)	Not applicable
Trovafloxacin (20)	2.55 ± 2.50[c]	7/20 (35)	19 (95)
Vancomycin (23)	4.58 ± 3.21	5/23 (22)	19 (83)
Ampicillin-sulbactam plus rifampin (10)	4.91 ± 1.10[c]	0/10 (0)	10 (100)
Trovafloxacin plus ampicillin-sulbactam (13)	1.43 ± 1.29	4/13 (31)	12 (92)

[a] Control animals were sacrificed at the institution of therapy.
[b] $P < 0.05$ versus all treatment groups.
[c] The difference between the trovafloxacin-containing regimens was not significant, but both regimens were lower ($P < 0.05$) than the vancomycin or ampicillin-sulbactam plus rifampin regimens.

sensitivity of the infecting organisms to vancomycin. The vancomycin MIC for *S. aureus* 76, used in the present study, was 2 μg/ml compared to 0.4 and 0.5, respectively, for the two strains used in the study by Kaatz et al. (18). Levels of vancomycin in serum were similar in the two studies, particularly with regard to the trough concentrations, which may contribute to efficacy in this model (7). Both peak and trough serum concentrations exceeded the MIC by a ratio that was lower in the present study than the ratio in the study of Kaatz et al. (18), providing a possible explanation for the inferior efficacy of the drug in the present study. Furthermore, time-kill curves in vitro showed that vancomycin at 5 μg/ml was less rapidly bactericidal than trovafloxacin at 1 μg/ml (data not shown). Although the present studies were not designed to address determinants of efficacy of vancomycin, these data support the hypothesis that the ratio by which vancomycin concentrations exceed the MIC for the infecting organism influences its efficacy.

The use of ampicillin-sulbactam produced some noteworthy results. The drug combination alone was not used in the present study, since previous studies with the same infecting strain had documented its ineffectiveness, as predicted by the high MIC (6). Others have found that the MIC for some strains of MRSA can be equal to that of the combination of an aminopenicillin with a β-lactamase inhibitor and that these drugs can be effective in vivo (5, 10). Against our strain of MRSA, the combination of ampicillin-sulbactam with rifampin produced activity similar to that of vancomycin, the standard therapy for MRSA. Previous studies, as well as the MICs indicate that rifampin was the active drug in this combination, and it is possible that such a combination may be an effective alternative to vancomycin, for example, in the treatment of infections caused by MRSA strains with reduced sensitivity to vancomycin (14). Equally interesting, the addition of ampicillin-sulbactam to trovafloxacin appeared to improve bactericidal activity. While mean vegetation titers did not reach a statistically significant difference, 8 of 20 animals treated with trovafloxacin alone had vegetation titers at the end of treatment in excess of $\log_{10}$ 3 CFU/g of vegetation, in contrast to 0 of 13 animals treated with the combination ($P < 0.01$ by Fisher's exact test post hoc).

Our results indicate that trovafloxacin has good bactericidal activity in experimental endocarditis caused by a ciprofloxacin-sensitive MRSA strain. Our data further suggest that the addition of an aminopenicillin plus β-lactamase inhibitor may increase the bactericidal rate of the quinolone. Finally, the efficacy of vancomycin may be reduced when serum concentrations, particularly trough concentrations, fail to exceed the MIC severalfold, as previously shown for teicoplanin (7). An important question not addressed by the present study is the efficacy of trovafloxacin in the therapy of ciprofloxacin-resistant MRSA endocarditis.

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