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Efficacy of Trovafloxacin against Experimental *Staphylococcus aureus* Endocarditis

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Trovafloxacin is a new fluoronaphthyridone chemically and functionally related to members of the fluoroquinolone class of antimicrobial agents. The in vivo efficacy of the drug was compared with that of vancomycin by using the rabbit model of left-sided endocarditis. Rabbits infected with either a nafcillin-susceptible or -resistant test strain were treated with trovafloxacin (13.3 mg/kg of body weight every 12 h) or vancomycin (25 mg/kg of body weight every 8 h) for 4 days. In comparison with untreated controls, both antimicrobial agents effectively cleared bacteremia and significantly reduced bacterial counts in vegetations and tissues of animals infected with either test strain. No resistance to trovafloxacin emerged in test strains during therapy. We conclude that in this model trovafloxacin is as efficacious as vancomycin is and may serve as a viable alternative to vancomycin for use in humans with similar infections.

Trovafloxacin (CP-99,219-27) is a fluoronaphthyridone antimicrobial agent related to fluoroquinolones such as ciprofloxacin and norfloxacin (2). It has potent activity against a broad range of bacterial species, including *Staphylococcus aureus* (4, 5, 15, 17, 22). The antibacterial activity of trovafloxacin versus *S. aureus* surpasses that of ciprofloxacin, and trovafloxacin may maintain clinically relevant activity against ciprofloxacin-resistant strains (1).

The in vivo activity of trovafloxacin against selected pathogens has been assessed by using animal models, and the drug has shown therapeutic efficacy comparable to that of standard forms of therapy (3, 6, 13, 21). However, the efficacy of trovafloxacin in treating serious *S. aureus* infections has not been determined. In order to address this issue, we compared the therapeutic activities of trovafloxacin and vancomycin by using the rabbit model of left-sided *S. aureus* endocarditis. This model affords a severe test of antimicrobial activity in a serious systemic infection.

MATERIALS AND METHODS

Antimicrobial agents. Trovafloxacin (lot number, 32438-280-1M) and the L-Ala–L-Ala prodrug of trovafloxacin, alatrofloxacin (CP-116,517-27; lot number, 34037-098-04), were obtained from Pfizer Inc., Groton, Conn. Vancomycin was purchased from commercial sources. Alatrofloxacin is used for parenteral administration; this compound is rapidly hydrolyzed in serum to form trovafloxacin (23). However, as the molecular weight of alatrofloxacin is greater than that of trovafloxacin (654.6 versus 512), 1 mg of the prodrug is equivalent to 0.782 mg of active trovafloxacin. For the sake of clarity, we will refer to both compounds as trovafloxacin and take into account the conversion factor when appropriate.

Organisms. The nafcillin-susceptible and -resistant strains of *S. aureus* used (SA-1199 and MRSA-494, respectively) were bloodstream isolates from patients with endocarditis.

In vitro studies. The MICs and MBCs of vancomycin and trovafloxacin for the test strains were determined in Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.), which was cation adjusted with calcium and magnesium according to the guidelines of the National Committee for Clinical Laboratory Standards (14). The susceptibilities of both strains to nafcillin have been established previously (12). Agar dilution MICs of trovafloxacin were determined with Mueller-Hinton agar (14).

The frequencies at which test strains developed spontaneous mutational resistance to trovafloxacin at fivefold the agar dilution MIC for the strains were determined by exposing exponential-growth-phase organisms (10^{10} CFU) to the appropriate concentration of antimicrobial agent incorporated into Mueller-Hinton agar. Colonies were counted 48 h later.

Animal studies. All studies were done with male New Zealand White rabbits (weight, 2 to 3 kg). Left-sided endocarditis was established as described previously by using an intravenous (i.v.) bacterial inoculum of 10^6 CFU (7). In this model, a catheter is placed across the aortic valve and remains in place for the duration of the study. Sixteen hours after bacterial challenge, all animals had 1 ml of blood withdrawn for culture. Serial dilution and plating techniques were used to determine the number of CFU per milliliter of blood. Inclusion in the study required that this blood culture be positive and that the catheter be positioned properly across the aortic valve at the time of autopsy. Following randomization of animals, a 4-day treatment course with either trovafloxacin (13.3 mg/kg [equivalent to 17 mg/kg of alatrofloxacin] of body weight i.v. every 12 h) or vancomycin (25 mg/kg of body weight i.v. every 8 h) was initiated. Both drugs were administered by i.v. bolus injection, and the dose administered was adjusted for weight on a daily basis. Rabbits randomized to no treatment (controls) were sacrificed at the time that therapy was begun in animals receiving antimicrobial agents; this was followed by the determination of bacterial counts in vegetations and tissues (see below).

Serum samples for the measurement of peak (obtained 1 h postdose) and trough (obtained just before a scheduled dose) antibiotic contents were collected from all animals at the time of the first dose on day 2. Repeat blood cultures were obtained prior to the first dose on day 3.

Following 4 days of therapy, all animals were sacrificed 10 to 12 h (for vancomycin) or 14 to 16 h (for trovafloxacin) following the final dose and were autopsied in an aseptic manner. Terminal blood cultures and serum samples for the measurement of antibiotic content were obtained, followed by the removal of vegetations and 500-mg (mean weight) sections of left kidney and spleen for culture. These specimens were weighed, suspended in 0.9% NaCl (final volume, 1 ml), and homogenized. Quantitative bacterial counts, determined by serial dilution and plating techniques, were expressed as the $\log_{10}$ of the number of CFU per gram (sensitivity limit, 10 CFU per vegetation or tissue section; culture-negative specimens were considered to contain 10 CFU for numerical and statistical purposes). The effect of antibiotic carryover was minimized by using a dilution factor of at least 200-fold for cultured material. Based upon mean vegetation weights and terminal drug levels (see below), this degree of dilution was sufficient to eliminate antibiotic carryover even if vegetation drug concentrations were more than 100-fold those found in serum.

Antibiotic content of serum. Trovafloxacin concentrations in serum were determined by a bioassay using an agar well diffusion method (18); *Klebsiella*

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TABLE 1. Antimicrobial agent concentrations in serum[a]

Infecting strain and treatment	Concn (μg/ml) in serum	
	Peak	Trough
SA-1199		
Vancomycin	43.6 ± 11.7	3.0 ± 1.6
Trovafloxacin	3.84 ± 1.37	<0.11 ± 0.08[b]
MRSA-494		
Vancomycin	37.8 ± 6.2	2.3 ± 1.2
Trovafloxacin	4.39 ± 1.48	<0.20 ± 0.18[b]

[a] Dosages employed: trovafloxacin, 13.3 mg/kg i.v. every 12 h; vancomycin, 25 mg/kg i.v. every 8 h. Peak and trough serum concentrations (means ± standard deviations) were determined for samples obtained 1 h after and just prior to dose administration, respectively.

[b] Concentrations in some specimens were below the assay limit of 0.06 μg/ml. In such instances the assay limit was used for numerical purposes.

pneumoniae 10031 was used as the indicator organism. Vancomycin concentrations were determined by fluorescence polarization immunoassay (TDx; Abbott Diagnostics, Irving, Tex.) (16). Pooled normal rabbit serum was used to prepare standards and dilute unknowns as needed. The limits of detection for these methods were 0.06 μg/ml for trovafloxacin and 0.5 μg/ml for vancomycin.

Resistance to trovafloxacin. All isolates recovered from blood, vegetations, or tissues of animals receiving trovafloxacin were screened for the development of raised MICs of the drug during therapy. This was done by plating undiluted blood and homogenized vegetation and tissue specimens onto Mueller-Hinton agar containing 0.25 μg trovafloxacin per ml (fivefold the agar dilution MIC for both strains; see below). Plates were examined for growth 48 h later.

Statistical analysis. Comparisons of blood, vegetation, and tissue bacterial counts were made by using the Kruskal-Wallis one-way analysis of variance on ranks followed by Dunn's test for multiple comparisons. Comparisons of the frequencies of sterilization of blood, vegetations, and renal and splenic tissues were made by use of the Fisher exact test. A P value of <0.05 was considered significant.

RESULTS

In vitro studies. The broth microdilution MICs and MBCs of nafcillin, vancomycin, and trovafloxacin for SA-1199 were 0.4 and 0.4, 0.5 and 0.5, and 0.08 and 0.08 μg/ml, respectively. Corresponding results for MRSA-494 were 25 and 100, 0.4 and 0.4, and 0.08 and 0.31 μg/ml, respectively. The MIC of trovafloxacin as determined by agar dilution was 0.05 μg/ml for both strains.

The frequency of spontaneous mutational resistance for SA-1199 to trovafloxacin at fivefold its agar dilution MIC was 1.2×10^{-9}. The corresponding result for MRSA-494 was 3.5×10^{-9}.

Animal studies. No differences were found in the intensities of pretreatment bacteremia ($\log_{10}$ of the number of CFU per milliliter [mean ± standard deviation]) for animals infected with SA-1199 and randomized to receive vancomycin (4.05 ± 0.76) or trovafloxacin (3.49 ± 0.85) or controls sacrificed 16 h after bacterial challenge (3.93 ± 1.06). The same was true for those infected with MRSA-494 and treated with vancomycin (3.13 ± 0.69) or trovafloxacin (2.88 ± 0.57) and for controls (3.16 ± 0.75).

The peak and trough concentrations in serum achieved with vancomycin and trovafloxacin are shown in Table 1. The peak concentrations of vancomycin were modestly higher than the usual range (20 to 30 μg/ml) targeted for most therapeutic applications. The peak concentrations of trovafloxacin were very similar to those achieved in humans following an oral or i.v. dose of 300 mg (19, 20, 23). Trough concentrations of trovafloxacin were below detectable limits for some animals infected with either test strain (SA-1199, $n = 5$; MRSA-494, $n = 1$). In those instances, the assay limit (0.06 μg/ml) was used for numerical purposes. Terminal antimicrobial agent concentrations were, in all cases, low enough that antibiotic carryover would not have affected the results observed. The actual concentrations were very similar to the trough concentrations reported in Table 1.

There were no differences in the frequencies of blood culture sterilization during therapy with either antimicrobial agent in animals infected with either test strain. For vancomycin-treated animals with SA-1199 endocarditis, 100% of animals had sterile blood cultures after 2 and 4 days of therapy. For those treated with trovafloxacin, 88% had sterile cultures at both of these times. For animals infected with MRSA-494 and treated with vancomycin, blood cultures were sterile in 93 and 100% of animals at 2 and 4 days, respectively. For those treated with trovafloxacin, 100% of animals had sterile cultures at both times.

Quantitative bacterial counts in vegetations and tissues are given in Table 2. Compared to those in control animals, there were highly significant reductions in bacterial counts at each of these sites in rabbits infected with either test strain and treated with either drug ($P < 0.001$ for all comparisons). There were no significant differences in bacterial counts between trovafloxacin- or vancomycin-treated animals, and both drugs sterilized all assayed sites at similar rates. It was noted, however, that both drugs were more effective at sterilizing the vegetations of animals infected with MRSA-494 than those of animals infected with SA-1199 ($P < 0.001$). Sterilization of renal and splenic tissues was achieved at comparable rates regardless of the infecting organism.

Resistance to trovafloxacin. No resistance to trovafloxacin emerged in either test strain during therapy.

DISCUSSION

Many studies have shown that fluoroquinolones compare favorably with standard therapy in animals with experimental *S. aureus* infections (7–12). On the basis of the results presented here, the same appears to be true for trovafloxacin. Compared to vancomycin as standard therapy, trovafloxacin was just as efficacious in clearing bacteremias and reducing vegetation and tissue bacterial counts regardless of the test organism used. In addition, both drugs were equally effective in sterilizing these sites. As we have shown previously in studies similar to the present one, both drugs were more effective in reducing bacterial counts (and sterilizing vegetations) of rabbits infected with MRSA-494 than of those infected with SA-1199. This likely was the result of the somewhat smaller vegetations that MRSA-494 produces (mean weight, 56 versus 76 mg for SA-1199 in this study), a fact which conceivably could result in improved drug penetration and improved bacterial killing in animals infected with this organism.

We found that the emergence of resistance to trovafloxacin at fivefold the MIC was not detected in vivo with either test

TABLE 2. Vegetation and tissue bacterial counts

Infecting strain and treatment	No. of rabbits	Bacterial counts ($\log_{10}$ CFU/g) in[a]:		
		Vegetation	Kidney	Spleen
SA-1199				
Vancomycin	15	5.72 ± 2.33 (1)	1.72 ± 1.26 (12)	1.48 ± 0.57 (12)
Trovafloxacin	17	4.51 ± 2.38 (5)	1.67 ± 1.04 (14)	1.72 ± 1.23 (14)
None (control)	11	9.71 ± 0.71 (0)	5.60 ± 1.53 (0)	6.34 ± 0.76 (0)
MRSA-494				
Vancomycin	15	2.01 ± 0.30 (14)	1.30 ± 0.09 (15)	1.31 ± 0.08 (14)
Trovafloxacin	17	2.35 ± 0.32 (17)	1.30 ± 0.11 (17)	1.30 ± 0.07 (17)
None (control)	10	8.43 ± 1.16 (0)	5.65 ± 1.65 (0)	5.57 ± 0.80 (0)

[a] Values are means ± standard deviations. Values in parentheses are numbers of culture-negative (sterile) specimens.

strain. However, resistance to trovafloxacin at this multiple of the MIC did occur at a low frequency in vitro for both strains. The simplest explanation for this apparent discrepancy lies in the number of bacteria contained in rabbit vegetations compared to the frequency of spontaneous mutational resistance for each test strain. In the present study, the vegetation bacterial population ranged from $10^{6.0}$ to $10^{9.3}$ CFU; in many vegetations, bacterial density likely was below that necessary to allow resistant subpopulations to emerge. Perhaps conditions more favorable to the development of resistance might be found in larger vegetations containing more CFU. However, based on our spontaneous mutation frequency data the emergence of resistance to trovafloxacin at fivefold the MIC appears unlikely to occur if the inoculum at the infected site contains less than approximately $10^{8.5}$ CFU.

Our inability to detect the emergence of trovafloxacin resistance in vivo is in contrast to what we have found previously using ciprofloxacin, fleroxacin, and clinafloxacin in the same animal model. In these studies we found the spontaneous mutation frequencies at fivefold the MIC of the appropriate fluoroquinolone to be similar to or even lower than that found for trovafloxacin (8, 9, 11, 12). In those studies, resistance was found to emerge mainly in animals infected with SA-1199; however, in one instance it also was found in MRSA-494-infected animals treated with fleroxacin (9). In our study with clinafloxacin (12), resistance was seen to develop in one animal infected with a highly ciprofloxacin-resistant derivative of SA-1199 but not in animals infected with SA-1199 itself.

Resistance in these earlier studies may have been the result of the lower intrinsic antistaphylococcal activities of ciprofloxacin and fleroxacin compared with that of trovafloxacin and the significantly elevated MIC of clinafloxacin observed for the ciprofloxacin-resistant SA-1199 derivative that developed resistance to that drug (40-fold less susceptible to clinafloxacin than the parent strain) (12). It is probable that the improved potency of newer fluoroquinolones such as clinafloxacin and trovafloxacin, with a resultant improvement in the serum concentration-to-MIC ratio, will help to reduce the incidence of in vivo resistance. Whether drugs such as these will have efficacy in the therapy of infections caused by strains resistant to the earlier fluoroquinolones remains to be seen. Based on our previous in vivo work with clinafloxacin and in vitro work done with trovafloxacin, this appears to be a possibility (1, 12).

The model used in this study does permit a severe test of antimicrobial activity in a serious systemic infection. The presence of a foreign body (the catheter) increases the difficulty of the treatment situation. An additional and perhaps even more severe test of the antimicrobial activity of trovafloxacin would be to allow treated animals to survive for a period of time following the completion of therapy and then determine bacterial counts in vegetations and tissues. We have employed this technique previously when evaluating other fluoroquinolones; the data generated allows a true rate of cure to be established (7, 9). Additional work is needed to establish this rate for trovafloxacin.

In conclusion, we have found trovafloxacin to be equivalent to vancomycin in a model of a serious *S. aureus* infection regardless of the susceptibility of the test strain to nafcillin. Trovafloxacin may serve as a viable alternative to vancomycin for use in humans with similar infections. Controlled clinical trials are required to address this potential indication.

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