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Efficacy of Fleroxacin in Experimental Methicillin-Resistant *Staphylococcus aureus* Endocarditis

GLENN W. KAATZ,[1]* SUSAN M. SEO,[1] STEVEN L. BARRIERE,[2] LISA M. ALBRECHT,[3,4]† AND MICHAEL J. RYBAK[3,4]

Department of Internal Medicine, Division of Infectious Diseases, Wayne State University School of Medicine,[1] College of Pharmacy and Allied Health Professions, Wayne State University,[3] and Department of Pharmacy Services, Detroit Receiving Hospital/University Health Center,[4] Detroit, Michigan 48201, and Departments of Pharmaceutical Services and Pharmacology and Division of Infectious Diseases, Department of Medicine, UCLA Center for the Health Sciences, Los Angeles, California 90024[2]

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The efficacy of fleroxacin versus that of vancomycin was assessed by using the rabbit model of methicillin-resistant *Staphylococcus aureus* endocarditis. Animals were treated with fleroxacin (30 mg/kg of body weight every 8 h) or vancomycin (17.5 mg/kg every 6 h) for 4 days. These antimicrobial agents were equally effective in clearing bacteremia, reducing bacterial counts in vegetations and tissues, and curing endocarditis. However, resistance to fleroxacin at fivefold the MIC arose in the test strain of *S. aureus* in 8% of animals that received the drug. We conclude that fleroxacin is as efficacious as vancomycin in this model of a serious systemic *S. aureus* infection, but modest resistance to fleroxacin may develop during therapy.

The fluoroquinolones are potent antimicrobial agents having excellent activity against a broad range of bacteria, including methicillin-resistant *Staphylococcus aureus* (15). Fleroxacin, a new trifluorinated quinolone, has significant activity against both methicillin-susceptible and -resistant strains of *S. aureus* in vitro (2), and it has favorable pharmacokinetics, suggesting that once- or twice-daily dosing will be possible in humans (14).

Recently, reports of vancomycin resistance in previously susceptible gram-positive bacteria have appeared (8, 10). Because of this occurrence, it is sensible to examine other therapeutic options for infections caused by organisms for which vancomycin is the mainstay of therapy. Therefore, we examined the efficacy of fleroxacin versus that of vancomycin by using the rabbit model of methicillin-resistant *S. aureus* endocarditis. This model affords a severe test of antimicrobial efficacy in a serious systemic infection.

MATERIALS AND METHODS

Organism. The methicillin-resistant strain of *S. aureus* used (MRSA 494) was a bloodstream isolate from a patient with endocarditis.

In vitro studies. The MICs and MBCs of methicillin, vancomycin, and fleroxacin for MRSA 494 were determined by a microdilution method using cation-supplemented Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.) (4).

The frequency at which MRSA 494 developed spontaneous mutational resistance to 2-, 5-, and 10-fold its fleroxacin MIC was determined by exposing exponential-growth-phase organisms (~10^{10} CFU) to the appropriate concentration of fleroxacin incorporated into Mueller-Hinton agar (Difco). Colonies were counted 48 h later.

Serum inhibitory and bactericidal titers (SITs and SBTs) were determined by a microdilution method modified by using cation-supplemented Mueller-Hinton broth as the diluent, because the protein binding of fleroxacin (30%) or vancomycin (50%) is not significant (12).

Animal studies. All studies were done using male New Zealand White rabbits (2 to 3 kg). Single- and multiple-dose pharmacokinetic studies with fleroxacin were performed with healthy animals to determine a dose and dosing interval which produced levels in serum approximating those achievable in humans. Five animals received single intravenous (i.v.) bolus doses of 35 mg/kg of body weight, followed by frequent collection of serum samples for the next 12 h for the determination of fleroxacin content. For multiple-dose studies, five animals received 17.5 mg/kg i.v. every 12 h, for a total of 9 doses. Following dose 9, serum was collected on a schedule similar to that for single-dose studies and was assayed for fleroxacin content. Serum fleroxacin concentration-time curves were plotted, and the elimination half-life was estimated from the slope of the plotted line. Other pharmacokinetic parameters (plasma clearance and steady-state volume of distribution) were determined by model-independent methods (5). Similar studies have previously been performed with vancomycin (1).

Left-sided endocarditis was established as described previously by using an i.v. bacterial inoculum of 10^6 CFU (6). After 18 h, all animals had 1 ml of blood withdrawn for culture. Serial dilution and plating techniques were used to determine 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 autopsy.

Rabbits were randomized to receive 4 days of fleroxacin (30 mg/kg i.v. every 8 h), vancomycin (17.5 mg/kg i.v. every 6 h), or no treatment (controls). The dose administered was adjusted for weight on a daily basis. Controls were sacrificed at the time therapy was begun in animals receiving antimicrobial agents. Sacrifice was followed by the determination of bacterial counts in vegetations and tissues (see below).

* Corresponding author.
† Present address: College of Pharmacy, University of Illinois at Chicago, Chicago, IL 60680.

TABLE 1. Vegetation and tissue bacterial counts

Treatment	n	Mean ± SD $\log_{10}$ CFU/g (no. of culture-negative specimens) for[a]:		
		Vegetation	Kidney	Spleen
Control (no therapy)	10	8.78 ± 0.91 (0)	5.50 ± 1.70 (0)	5.32 ± 0.89 (0)
Vancomycin	16	2.48 ± 1.19 (14)	1.31 ± 0.54 (15)	1.64 ± 0.76 (14)
Fleroxacin	15	2.68 ± 0.51 (10)	1.36 ± 0.68 (14)	1.32 ± 0.10 (15)

[a] For all comparisons, significant reductions were noted for animals receiving either drug ($P < 0.0001$ versus control). There were no differences between drug-treated animals. The same was true for the frequency at which both drugs produced culture-negative vegetations or tissues ($P < 0.001$ versus control).

Serum samples for the measurement of peak (obtained at the beginning of the elimination phase) and trough (just before a scheduled dose) antibiotic content and peak SITs and SBTs were obtained from all animals at the time of dose 1 on day 2. Blood for culture was obtained before dose 1 on day 3.

Following 4 days of therapy, randomly selected animals were sacrificed 8 to 10 h (for vancomycin) or 10 to 12 h (for fleroxacin) after the final dose and were autopsied in an aseptic manner. Terminal blood cultures were obtained. Vegetations and 500-mg (mean weight) sections of left kidney and spleen, including areas with infarction or abscess formation or both, if visible, were removed for culture. These specimens were weighed, suspended in 0.85% NaCl (final volume, 1 ml), and homogenized. Quantitative bacterial counts, determined by serial dilution and plating techniques, were expressed as $\log_{10}$ CFU per gram (sensitivity limit, 10 CFU per vegetation or tissue section; for numerical and statistical purposes, culture-negative specimens were considered to contain 10 CFU). Other animals were monitored for 7 days after completion of therapy, with blood cultures being obtained on days 3 and 7 posttherapy. These animals then were sacrificed and autopsied as described above. Animals with sterile vegetations and tissues were considered cured of their infections.

Antibiotic content of serum. Fleroxacin concentrations in serum were determined by bioassay by using an agar well diffusion method (9); *Escherichia coli* 1346 was used as the indicator organism. Vancomycin concentrations were determined by fluorescence polarization immunoassay (TDx; Abbott Diagnostics, Irving, Tex.) (11). Pooled normal rabbit serum was used to prepare standards and dilute unknowns as needed.

Resistance to fleroxacin. Isolates of strain MRSA 494 recovered from blood, vegetations, or tissues of animals receiving fleroxacin were screened for the emergence of resistance to the drug by plating them onto tryptic soy agar (Difco) containing 5- and 10-fold the MIC for the organism. These plates were examined for growth 48 h later.

Statistical analysis. Comparisons of blood and vegetation-tissue bacterial densities were made by one-way analysis of variance. Comparisons of frequencies of blood and vegetation-tissue sterilization and of cure of endocarditis were made by the Fisher exact test. The Kruskal-Wallis H test was used for comparisons of SITs and SBTs, and the unpaired *t* test was used for comparisons of pharmacokinetic parameters. A *P* value of <0.05 was considered significant.

RESULTS

In vitro studies. The MICs and MBCs of methicillin, vancomycin, and fleroxacin for MRSA 494 were 21.8 and 59.5, 0.4 and 0.4, and 0.6 and 1.6 μg/ml, respectively. The frequency of spontaneous mutational resistance to twofold the fleroxacin MIC for strain MRSA 494 was 1.65×10^{-8}; a frequency of $<1 \times 10^{-10}$ was found at 5- and 10-fold the MIC.

Animal studies. The pharmacokinetic parameters (mean ± standard deviation) of fleroxacin following a single dose of 35 mg/kg were as follows: elimination half-life, 3.12 ± 1.17 h; steady-state volume of distribution, 3.63 ± 0.88 liters; plasma clearance, 1.74 ± 0.27 liters/h. Following multiple doses of 17.5 mg/kg, results were as follows: half-life, 3.32 ± 1.48 h; steady-state volume of distribution, 5.02 ± 1.49 liters; clearance, 2.34 ± 0.27 liters/h. No differences were observed between the regimens for half-life or steady-state volume of distribution, but clearance following multiple doses was significantly greater than that following a single dose ($P = 0.007$).

No differences were found in the intensity of pretreatment bacteremia (mean ± standard deviation $\log_{10}$ CFU per milliliter) for animals receiving vancomycin (3.14 ± 0.51) or fleroxacin (2.78 ± 0.78) and sacrificed after 4 days of therapy or for controls sacrificed 18 h after bacterial challenge (3.09 ± 0.81).

Peak and trough concentrations of vancomycin in serum were 29.70 ± 10.58 and 4.64 ± 3.38 μg/ml, respectively (mean ± standard deviation). For fleroxacin, the corresponding values were 14.92 ± 4.37 and 1.33 ± 0.84 μg/ml. Geometric mean peak SITs and SBTs for animals receiving vancomycin were 1:30 and 1:15, respectively, and for those receiving fleroxacin, they were 1:22 and 1:8. The SITs for the two groups were not statistically different, but the SBTs were significantly higher for animals receiving vancomycin ($P < 0.01$).

There were no differences in the frequency of blood culture sterilization after 2 or 4 days of therapy. For animals that received vancomycin, 15 of 16 had sterile cultures after 2 and 4 days, while all 15 animals that received fleroxacin had sterile cultures on both days.

Quantitative bacterial counts found in vegetations and tissues are shown in Table 1. The two drugs were equally effective in reducing these counts, and they produced equal proportions of culture-negative vegetations and tissues.

Cure of MRSA 494 endocarditis. Animals monitored for 7 days posttherapy had similar degrees of pretreatment bacteremia (vancomycin, 2.95 ± 0.95 [$n = 9$]; fleroxacin, 2.73 ± 0.73 [$n = 10$]). Mean drug levels in serum, SITs, and SBTs were similar to those found in animals sacrificed following 4 days of therapy. There were no differences in the frequency of blood culture sterilization during or posttherapy.

Eight of nine vancomycin-treated and eight of ten fleroxacin-treated animals had sterile vegetations and tissues 7 days posttherapy and were considered cured of endocarditis. This difference was not significant.

Resistance to fleroxacin. Isolates of MRSA 494 able to grow on agar containing fivefold the fleroxacin MIC for the strain were recovered from 2 of 25 animals that received the drug. The resistant organisms made up ≤3% of the total

number of CFUs recovered from any cultured site. No resistance at 10-fold the MIC was found.

DISCUSSION

Fluoroquinolones such as ciprofloxacin, enoxacin, and pefloxacin have been shown to compare favorably with vancomycin in animals with experimental methicillin-resistant *S. aureus* infections (3, 6, 13). We have shown the same to be true for fleroxacin, a new trifluorinated quinolone. The drug cleared bacteremia, reduced bacterial counts in vegetations and tissues, and cured endocarditis with an efficacy equal to that of vancomycin. However, resistance to the drug at fivefold the MIC for the test strain was seen in a small proportion of organisms recovered from two animals. Serum fleroxacin concentrations in these animals were somewhat low, with peaks and troughs being nearly 50% below mean levels. It is possible that these low concentrations favored the development of resistance. Maintenance of higher levels in serum, nearer the mean peak and trough we achieved, may help to prevent this problem.

A difference was observed in plasma clearance of fleroxacin in well animals when the 17.5-mg/kg dose was compared with the 35-mg/kg dose. This was probably due to saturation of elimination pathways by the higher dose, resulting in diminished clearance. This hypothesis is supported by our finding of no differences in pharmacokinetic parameters other than clearance for the two dosing regimens. Such phenomena become important if they are not compensated for by adjustments in dosing interval or dose size, because the outcome of experimental infection could be affected.

Animals receiving vancomycin had significantly higher peak SBTs than those found in fleroxacin-treated animals. This difference did not result in an improved microbiologic outcome for those receiving vancomycin. We have found this to be true for ciprofloxacin in our previous studies with this animal model (6, 7).

In conclusion, we have found fleroxacin to be equivalent to vancomycin in this model of a serious methicillin-resistant *S. aureus* infection. This drug may serve as an alternative to vancomycin in humans with similar infections, but modest resistance to the drug may develop during therapy.

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