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Comparison of Sparfloxacin, Temafloxacin, and Ciprofloxacin for Prophylaxis and Treatment of Experimental Foreign-Body Infection by Methicillin-Resistant *Staphylococcus aureus*

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The prophylactic and therapeutic activities of three broad-spectrum fluoroquinolones were evaluated in two different experimental models of foreign-body infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) susceptible to quinolones. In a guinea pig model of prophylaxis, subcutaneously implanted tissue cages were infected at a >90% rate by 10^2 CFU of MRSA in control animals. A single dose of 50 mg of ciprofloxacin per kg of body weight administered intraperitoneally 3 h before bacterial challenge was less effective than an equivalent regimen of either sparfloxacin or temafloxacin in decreasing the rate of experimental infection in tissue cages challenged with increasing inocula of MRSA. In a rat model evaluating the therapy of chronic tissue cage infection caused by MRSA, the efficacy of a 7-day high-dose (50-mg/kg twice-daily) regimen of sparfloxacin, temafloxacin, or ciprofloxacin was compared to that of vancomycin (50 mg/kg twice daily). Active levels of sparfloxacin, temafloxacin, or ciprofloxacin were continuously present in tissue cage fluid during therapy, exceeding their MBCs for MRSA by 6- to 20-fold. Either temafloxacin, sparfloxacin, or vancomycin was significantly ($P < 0.01$) more active than ciprofloxacin in decreasing the viable counts of MRSA in tissue cage fluids. The different activities of ciprofloxacin compared with those of the other two quinolones against chronic tissue cage infections caused by MRSA did not involve the selective emergence of quinolone-resistant mutants. Temafloxacin and ciprofloxacin, which showed the most prominent differences in their in vivo activities, however, exhibited similar bactericidal properties and pharmacokinetic parameters in the rat model. In conclusion, both temafloxacin and sparfloxacin were significantly more active than ciprofloxacin for the prophylaxis or treatment of experimental foreign-body infections caused by a quinolone-susceptible strain of MRSA.

Biomaterial implants are markedly susceptible to bacterial infections. Antimicrobial therapy of prosthetic device infections caused by *Staphylococcus aureus* is notoriously difficult, and microbial eradication frequently requires the removal of the infected material. An additional serious problem for prophylaxis and therapy of foreign-body infections is the increasing incidence of strains of methicillin-resistant *S. aureus* (MRSA) that frequently express a large number of additional determinants for resistance to several other antimicrobial agents (6). Standard antibiotic regimens, such as vancomycin alone (22) or in combination with rifampin (9, 20, 30, 33), may remain the only available therapy for implant-related staphylococcal infections. Furthermore, combined therapy does not always prevent the emergence of rifampin-resistant mutants (17, 18, 39).

The fluoroquinolones, a new generation of quinolones, display broad-spectrum antibacterial activity, including activity against gram-positive organisms, in particular, the staphylococci (16, 23). Several clinical and experimental studies have reported the therapeutic value of the fluoroquinolones ciprofloxacin, ofloxacin, or pefloxacin, alone or in combination with other agents (9, 15, 22, 26, 30), against serious *S. aureus* infections. However, the development of resistance has been a problem, especially with MRSA (1, 4, 24, 34, 35, 39).

The major objective of our experimental study was to evaluate the efficacies of three broad-spectrum fluoroquinolones in two related models of foreign-body infections caused by a quinolone-susceptible strain of MRSA. The first of these models was suitable for assessing the prophylactic activity of each fluoroquinolone on MRSA challenge, whereas the second one evaluated its activity for the treatment of chronic subcutaneous implant-related infections caused by MRSA. In this comparative study, the most frequently used quinolone agent, ciprofloxacin, was tested in parallel with temafloxacin (14, 40) and sparfloxacin (12, 25, 40), which exhibit increased activities against aerobic gram-positive cocci. Despite the early withdrawal of one of these agents (temafloxacin) after completion of this experimental study, both fluoroquinolones yielded interesting results for both the prophylaxis and treatment of experimental tissue cage infections caused by MRSA which were markedly superior to those for equivalent regimens of ciprofloxacin.

(The results of the study described here were presented in part at the 33rd Interscience Conference on Antimicrobial Agents and Chemotherapy [8].)

MATERIALS AND METHODS

Bacterial strains. MRSA MRGR3 (9, 30), which was used in both animal models, was isolated from a patient with catheter-related sepsis and was selected for its virulence properties in the animal models of tissue cage infections. Strain MRGR3 is heterogeneously resistant to methicillin and has additional determinants for resistance to penicillin, gentamicin, chloramphenicol, erythromycin,

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tetracycline, and polymyxin B. However, strain MRGR3 is susceptible to fleroxacin (9, 30), ciprofloxacin, temafloxacin, and sparfloxacin.

Antimicrobial agents. For the in vitro studies, the following laboratory standards with known potencies were supplied by the indicated manufacturers: sparfloxacin by Rhône-Poulenc (Paris, France), temafloxacin by Abbott Laboratories (Chicago, Ill.), ciprofloxacin by Bayer (Zürich, Switzerland), and vancomycin hydrochloride by Laboratory Lilly (Giessen, Germany). For animal studies, ciprofloxacin (Bayer) and vancomycin (Lilly) were dissolved in solvents as recommended by their manufacturers. Sparfloxacin (Rhône-Poulenc) was provided as a stock solution of 100 mg/ml which was diluted in saline to 10 mg/ml just before use. Temafloxacin (Abbott) was provided as a powder for injection and was freshly solubilized in saline at a concentration of 5 mg/ml just before use.

In vitro studies. The MICs of each agent for MRSA MRGR3 were determined by a macrodilution method by using Mueller-Hinton broth (Difco, Detroit, Mich.) and a standard inoculum of 10^6 CFU/ml (31). To screen for the possible carryover effects of each antibiotic during the MBC determinations, 100-μl portions were taken from all tubes with no visible growth. These were subcultured, either undiluted or serially diluted 10-fold in saline, on Mueller-Hinton agar for 36 h at 37°C. The MBC was defined as the lowest concentration that killed 99.9% of the original inoculum.

Time-kill studies were performed with exponential-phase bacteria by using standard assay conditions (32). Glass tubes containing 10 ml of Mueller-Hinton broth with either 4 μg of vancomycin per ml, 0.25 μg of sparfloxacin per ml, 1 μg of temafloxacin per ml, or 1 μg of ciprofloxacin per ml were incubated with 10^6 CFU of *S. aureus* per ml in a shaking water bath at 37°C. The number of viable organisms was determined by subculturing 50 μl of 10-fold serially diluted portions on Mueller-Hinton agar (Difco) after 0, 2, 4, 6, and 24 h of incubation. Bacteria were plated with a spiral plater (Spiral System, Cincinnati, Ohio), and the colonies were counted with a laser colony counter (Spiral) after 24 h of incubation at 37°C. The detection limit was 2 $\log_{10}$ CFU/ml with all antibiotics tested. No significant carryover of antibiotics was observed by using these experimental conditions (10, 30).

Prophylaxis of tissue cage infections. Four polytetrafluoroethylene (Teflon) multiperforated tissue cages each containing three polymethylmethacrylate (PMMA) coverslips (7 by 7 mm) were implanted subcutaneously in guinea pigs under aseptic conditions as described previously in detail (5, 43). At 3 weeks after implantation, tissue cage fluids, which are sterile inflammatory exudates allowing easy access to antimicrobial agents, were aseptically aspirated and were checked for the absence of accidental bacterial contamination. Then, tissue cages were inoculated with 0.1 ml of saline containing 10^2, 10^3, or 10^4 CFU of MRSA MRGR3, as routinely checked by plating on Mueller-Hinton agar. Experimental infection was confirmed by quantitatively culturing aspirated tissue cage fluid (5, 43).

To study the prevention of experimental infection by the antimicrobial agents, a single dose of either sparfloxacin (50 mg/kg of body weight), temafloxacin (50 mg/kg), ciprofloxacin (50 mg/kg), or vancomycin (30 mg/kg) was administered intraperitoneally 3 h before inoculation of live staphylococci into the tissue cages. This lag time was necessary to obtain peak levels of each antimicrobial agent in the tissue cage fluid at the time of bacterial inoculation. At 24 h, 48 h, and 7 days after the injection of 10^2, 10^3, and 10^4 CFU of MRSA MRGR3, quantitative cultures were performed by plating 100 μl of tissue cage fluid, either undiluted or serially diluted 10-fold on Mueller-Hinton agar. Because of the small volume (100 μl) of tissue cage fluid which could be aspirated during repeated punctures, samples yielding no single organism were scored as containing <10 CFU/ml (5). At day 7 the cages were removed and the coverslips were cultured in Mueller-Hinton broth at 37°C for 7 days (37). A brief sonication (Branson 2200; Branson Ultrasonics, Danbury, Conn.) was performed for 1 min at 60 W to disrupt the biofilm and phagocytic cells in order to optimize the yield of viable bacteria (10, 37). The detection limit was 1 CFU per coverslip (37).

The numbers of tissue cage fluid samples and coverslips protected by each antimicrobial agent from infection by an identical number of inoculated organisms were compared by Fisher's two-tailed (2-by-2) exact probability test with the Bonferoni correction for multiple comparisons. Using such correction, P values of $<0.05/6 = 0.008$ were required to achieve statistical significance.

Treatment of chronic tissue cage infections. Four tissue cages each containing three PMMA coverslips (7 by 7 mm) were implanted subcutaneously into rats as described previously (30). At 3 weeks after implantation, tissue cage fluid was aspirated and was checked for sterility. To establish a chronic local MRSA infection, tissue cages were inoculated with 0.1 ml of saline containing 0.2×10^6 to 2×10^6 CFU of stationary-phase organisms as described previously (9, 30, 36). Three weeks later, all tissue cages containing more than 10^5 CFU/ml of fluid were included in the therapeutic protocols (30, 36, 37).

Rats infected with MRSA MRGR3 were randomized to receive (by the intraperitoneal route for 7 days) either sparfloxacin (50 mg/kg twice a day), temafloxacin (50 mg/kg twice a day), ciprofloxacin (50 mg/kg twice a day), or vancomycin (50 mg/kg twice a day) or were left untreated. At 12 h after the last injection of antibiotic, quantitative cultures of 10-fold serially diluted tissue cage fluid were performed on Mueller-Hinton agar. Possible bacterial clumps were disrupted by sonication (60 W, 1 min) before plating. Quantitative bacterial counts were determined with a detection limit of 100 CFU/ml and are expressed as $\log_{10}$ CFU per milliliter. For each cage, the differences between CFU counts from day 1 and day 8 were determined and expressed as delta $\log_{10}$ CFU per milliliter. For each treatment group, results were expressed as means ± standard errors of the means. Comparison of bacterial counts in the different groups was performed by one-way analysis of variance and Newman-Keuls multiple comparisons procedure. Data were considered significant when P was <0.05 by using two-tailed significance levels.

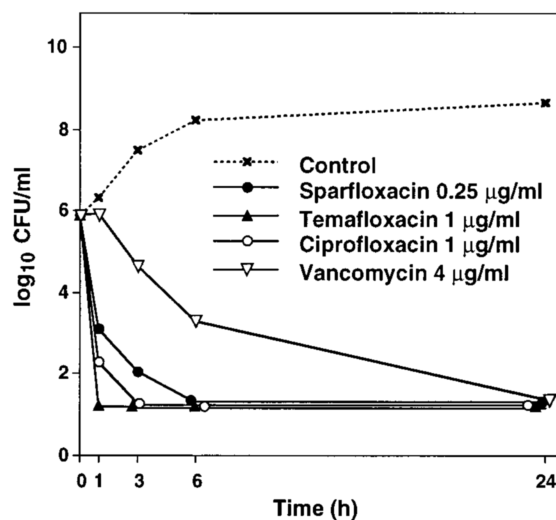

FIG. 1. Rate of in vitro elimination of exponential-phase cultures of MRSA MRGR3 grown in Mueller-Hinton broth by either sparfloxacin, temafloxacin, ciprofloxacin, or vancomycin.

Resistance to antimicrobial agents. The bacteria recovered from tissue cage fluids or coverslips on day 8 were screened for the emergence of resistance to sparfloxacin, temafloxacin, or ciprofloxacin: 100-μl samples of 10-fold-diluted tissue cage fluid or sonicated coverslips were plated onto Mueller-Hinton agar containing a fourfold MIC of each fluoroquinolone for MRSA. Plates were incubated for 48 h at 37°C. The detection limits were 2 and 1 $\log_{10}$ CFU/ml for tissue cage fluids and coverslips, respectively.

Pharmacokinetics of antimicrobial agents. The pharmacokinetic properties of vancomycin in tissue cage fluids of guinea pigs (5) or rats (30, 36) have been estimated previously. The concentrations of sparfloxacin, temafloxacin, and ciprofloxacin in serum and tissue cage fluid were estimated by a bioassay with *Escherichia coli* 1346 as the test strain (5). In guinea pigs, the concentrations of sparfloxacin, temafloxacin, or ciprofloxacin in tissue cage fluid of guinea pigs were measured at various time intervals (1, 2, 4, 6, 8, 12, and 24 h) after intraperitoneal administration of a single dose of 50 mg of antimicrobial agent per kg. In rats treated twice a day with a 50-mg/kg regimen of sparfloxacin, temafloxacin, or ciprofloxacin, the pharmacokinetics of each antimicrobial agent in both serum and tissue cage fluids were determined at similar time intervals on day 4 (to allow equilibrium concentrations for each agent) of therapy.

In the guinea pig model, the areas under the concentration-time curve (AUC) of each fluoroquinolone above its MBCs for MRSA MRGR3 were estimated from 0 to 24 h (AUC_{0-24}) after administration of each antimicrobial agent by the linear trapezoidal rule. A similar approach was used to determine the AUC of each fluoroquinolone above its MBC for MRSA from 0 to 12 h above the MBC (AUC between each dose) in the rat model.

RESULTS

In vitro studies: MICs and MBCs. The MICs and MBCs of sparfloxacin, temafloxacin, ciprofloxacin, and vancomycin for MRSA MRGR3 were 0.06 and 0.125, 0.25 and 0.25, 0.25 and 0.25, and 1 and 2 μg/ml, respectively.

Time-kill studies showed a more rapid elimination of exponential-phase cultures of strain MRGR3 by four times the MICs of each fluoroquinolone than by four times the MIC of vancomycin during the initial 6-h period (Fig. 1). In contrast, all three fluoroquinolones and vancomycin eliminated MRSA by at least 3 $\log_{10}$ CFU/ml at 24 h (Fig. 1). Additional time-kill studies demonstrated that markedly higher concentrations of each fluoroquinolone, equivalent to 24 times their MICs for strain MRGR3 and averaging those found in rat tissue cage fluid during therapy, led to elimination rates of the test strain

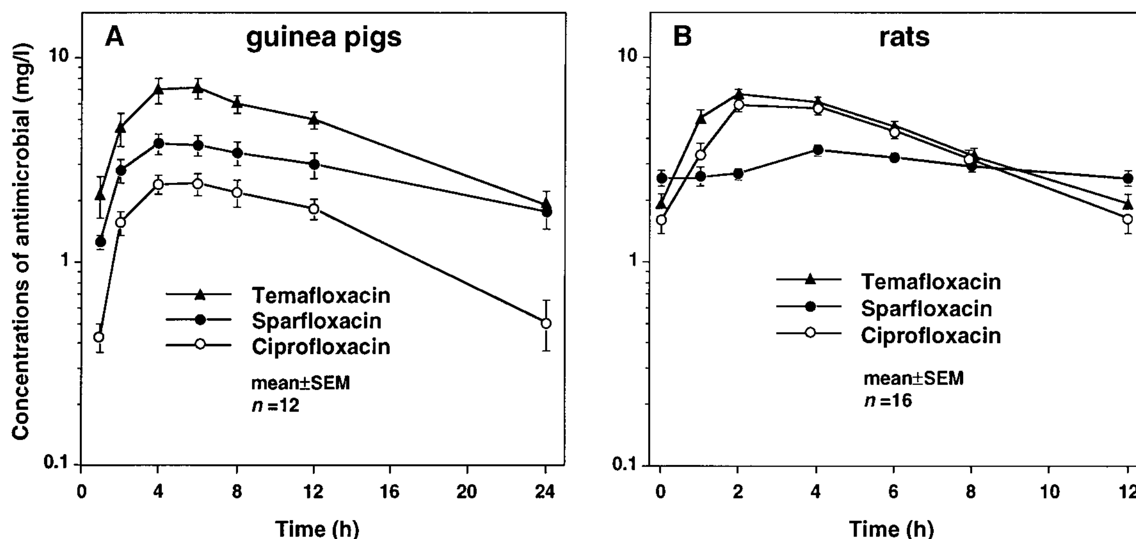

FIG. 2. Levels of temafloxacin, sparfloxacin, or ciprofloxacin in tissue cage fluid of guinea pigs that received a single 50-mg/kg dose of antimicrobial agents (A) or rats on day 4 of twice-a-day therapy with 50 mg of each fluoroquinolone per kg (B).

similar those recorded with 4 times the MICs of each agent (data not shown).

Prophylaxis of tissue cage infections. In untreated animals, 11 of 12 tissue cages challenged with 10^2 CFU of MRSA MRGR3 developed infection, with bacterial counts exceeding 10^3 CFU/ml of fluid at 24 h or later. With higher bacterial counts, the infection rate was 100%. These rates of tissue cage infection were similar to those recorded in previous studies (5, 37, 43).

The mean concentrations of sparfloxacin, temafloxacin, or ciprofloxacin in the tissue cage fluid of guinea pigs at various time intervals after administration are shown in Fig. 2A. The average levels of sparfloxacin, temafloxacin, and ciprofloxacin in tissue cage fluids ($n = 12$) were 2.8, 4.6, and 1.6 μg/ml at 2 h, 3.8, 7.1, and 2.4 μg/ml at 6 h, 3.0, 5.0, and 1.8 μg/ml at 12 h, and 1.8, 1.9, and 0.5 μg/ml at 24 h, respectively. Thus, a single prophylactic dose of each fluoroquinolone produced bactericidal levels (two to six times the MBC) in tissue cage fluid for the next 24-h period. In comparison, the mean concentrations of vancomycin in tissue cage fluid determined in a previous study (5) were 7.1, 12.2, and 2.0 μg/ml at 3, 6, and 24 h, respectively. These comparative pharmacokinetic data for each fluoroquinolone and vancomycin indicated optimal lag times between drug administration and bacterial challenge of 3 h for each antimicrobial agent.

Table 1 shows that both sparfloxacin and temafloxacin reduced colony counts below the detection limit of 10 CFU/ml of tissue cage fluid within 48 h in all but one cage challenged with either 10^2 ($n = 8$), 10^3 ($n = 8$), or 10^4 ($n = 8$) CFU of MRSA MRGR3. Almost identical protection rates were recorded in each treatment group at 7 days (Table 1). Furthermore, coverslips from culture-negative tissue cage fluids cultured at 7 days were all found to be sterile (limit of detection, 1 CFU/ml). In contrast to sparfloxacin and temafloxacin, ciprofloxacin was markedly less effective for preventing tissue cage infections, since only 63% of tissue cages challenged with 10^3 ($n = 8$) CFU and <13% (0 of 8) of the cages challenged with 10^4 CFU ($P < 0.0002$) were culture negative at 48 h or 7 days (Table 1). In comparison, vancomycin protected from infection 100 and 75% of tissue cages challenged with 10^2 ($n = 8$) or 10^3 ($n = 8$) CFU, respectively. However, the prophylactic effect of vancomycin was not significant ($P > 0.2$) against higher bacterial inocula since only 38% of tissue cages challenged with 10^4 CFU ($n = 8$) were protected from MRSA infection (Table 1).

To analyze additional pharmacokinetic parameters that might explain the significantly lower prophylactic activity of ciprofloxacin compared with those of sparfloxacin and temafloxacin, we estimated the areas under the tissue cage fluid concentration-time curves (AUC) of all three fluoroquinolones above their respective MBCs for MRSA MRGR3. The AUC_{0-24} of ciprofloxacin over its MBC for MRSA was 30.7 mg · h/liter, being indeed lower than those of sparfloxacin and

TABLE 1. Comparison of sparfloxacin, temafloxacin, ciprofloxacin, and vancomycin in the prophylactic treatment of tissue cage infection caused by *S. aureus* MRGR3

Antibiotic	Dose (mg/kg)	No. of CFU of *S. aureus* inoculated	No. of negative tissue cage[a]/no. analyzed (%) after:	
			48 h	7 days[b]
None (control)		10^2	1/12 (8)	1/12 (8)
Sparfloxacin	50	10^2	7/8 (88)	8/8 (100)
	50	10^3	8/8 (100)	8/8 (100)
	50	10^4	8/8 (100)	8/8 (100)
Temafloxacin	50	10^2	8/8 (100)	8/8 (100)
	50	10^3	8/8 (100)	7/8 (88)
	50	10^4	8/8 (100)	8/8 (100)
Ciprofloxacin	50	10^2	8/8 (100)	7/8 (88)
	50	10^3	5/8 (63)	5/8 (63)
	50	10^4	0/8[c] (<13)	0/8[c] (<13)
Vancomycin	30	10^2	8/8 (100)	8/8 (100)
	30	10^3	6/8 (75)	6/8 (75)
	30	10^4	3/8 (38)	3/8 (38)

[a] Culture-negative samples (100 μl) of tissue cage fluid were scored as <10 CFU/ml, which was the lower limit of detection.
[b] Identical results were obtained for coverslips and tissue cage fluids at 7 days.
[c] $P < 0.002$ for sparfloxacin and temafloxacin versus ciprofloxacin.

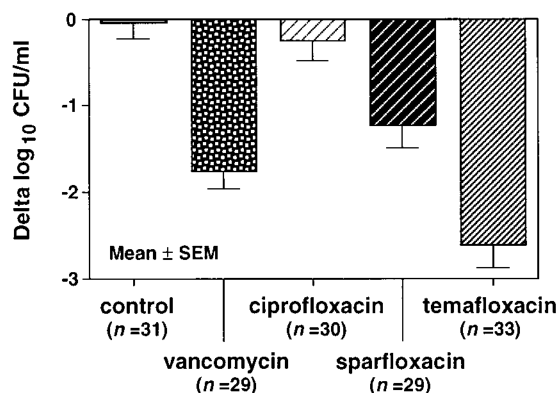

FIG. 3. Decrease in viable counts of MRSA MRGR3 in tissue cage fluids of rats treated with the different regimens for 7 days.

temafloxacin, which were 62.5 and 100.0 mg · h/liter, respectively.

Treatment of chronic tissue cage infections. The average peak and trough concentrations of sparfloxacin, temafloxacin, and ciprofloxacin assayed in rat serum at day 4 of therapy were 9.3, 19.1, and 15.4 μg/ml at 0.5 h and 1.6, 0.4, and 0.1 μg/ml at 12 h, respectively (data not shown). In tissue cage fluid at day 4 of therapy, the peak and residual levels of sparfloxacin, temafloxacin, and ciprofloxacin were 3.5, 6.7, and 5.9 μg/ml at 2 h and 2.6, 1.9, and 1.7 μg/ml at 12 h, respectively (Fig. 2B). Since these residual levels of sparfloxacin, temafloxacin, and ciprofloxacin were 20-, 8-, and 6-fold higher than their MBCs for MRSA MRGR3, respectively, all three fluoroquinolones were present in tissue cage fluid at concentrations constantly exceeding their MBCs for the test strain. At day 4 of therapy, the tissue cage fluid AUC_{0-12} of each fluoroquinolone above its MBC for MRSA was 34.0 mg · h/liter for sparfloxacin, 48.1 mg · h/liter for temafloxacin, and 43.4 mg · h/liter for ciprofloxacin.

Average peak and trough levels of vancomycin in tissue cage fluid were 12 and 2 μg/ml at 4 and 12 h, respectively, as described previously (30).

Of 161 tissue cages infected with MRSA MRGR3, 9 were excluded because of inadequate bacterial counts at the onset of therapy or because they were spontaneously expelled from animals during treatment. At the onset of therapy, bacterial counts for the remaining 152 tissue cages were 6.68 ± 0.17 $\log_{10}$ CFU/ml for controls (n = 31), 6.58 ± 0.13 $\log_{10}$ CFU/ml for animals receiving vancomycin (n = 29), 6.67 ± 0.14 $\log_{10}$ CFU/ml for animals receiving ciprofloxacin (n = 30), 6.58 ± 0.17 $\log_{10}$ CFU/ml for animals receiving sparfloxacin (n = 29), and 6.71 ± 0.16 $\log_{10}$ CFU/ml for animals receiving temafloxacin (n = 33). At the end of the 7-day treatment period, bacterial counts in the tissue cages of control animals showed a slight and nonsignificant decrease of 0.04 ± 0.18 $\log_{10}$ CFU (n = 31). The ciprofloxacin regimen was ineffective (Fig. 3), since the viable counts of MRSA showed a marginal and nonsignificant decrease of 0.25 ± 0.22 $\log_{10}$ CFU in tissue cage fluids (n = 30). In contrast, both the sparfloxacin and temafloxacin regimens (Fig. 3) led to significant reductions in bacterial counts in tissue cage fluids of 1.22 ± 0.26 $\log_{10}$ CFU/ml (n = 29) and 2.58 ± 0.25 $\log_{10}$ CFU/ml (n = 33), respectively, in comparison with the reductions in tissue cage fluids from controls and ciprofloxacin-treated animals ($P < 0.01$). Finally, the vancomycin regimen (Fig. 3) also led to a significant reduction in bacterial counts in tissue cage fluids (n = 29) of 1.77 ± 0.20 $\log_{10}$ CFU/ml in comparison with the reduction in tissue cage fluids from controls and ciprofloxacin-treated animals ($P <$ 0.01).

Emergence of resistant organisms during therapy. The potential emergence of quinolone-resistant mutants during therapy of chronic tissue cage infections by MRSA was studied. No MRGR3 isolates resistant to any fluoroquinolone were recovered from tissue cage fluids or coverslips treated for 7 days with either sparfloxacin, temafloxacin, or ciprofloxacin. Therefore, the markedly different efficacy of each fluoroquinolone for the treatment of chronic tissue cage infections caused by MRSA was not associated with any significant development of resistance to any antimicrobial agent.

DISCUSSION

In recent years, several clinical and experimental studies have evaluated the in vivo activities of various fluoroquinolones for the treatment of severe staphylococcal infections such as endocarditis (42), osteomyelitis and septic arthritis (29), and skin and soft tissue infections (21). The most interesting characteristics of the broad-spectrum 4-quinolones are their favorable pharmacokinetic properties, good tissue penetration, and the possibility that they can be administered orally (27). These properties of fluoroquinolones raised a high degree of interest for their use in the treatment of implant-related infections, as confirmed by initial experimental (9, 30) or clinical (29) studies. However, the rapid emergence of quinolone resistance, especially when fluoroquinolones were used as single agents against *S. aureus* infections, not only led to a significant percentage of clinical failures (29) but also compromised their further use against deep-seated staphylococcal infections (34, 35). Since quinolone resistance is predominant among MRSA, the prevalence of these multiply resistant organisms in many hospitals severely limits the oral use of any fluoroquinolone as a replacement for vancomycin (1, 34, 35). Despite a number of experimental (9, 15, 26, 30) and clinical (29) studies reporting the improved efficacy of a fluoroquinolone combined with rifampin for treating deep-seated staphylococcal infections, such a combination did not prove to be effective (34, 35, 39) for preventing the emergence of resistance in staphylococci.

Despite these limitations in the use of fluoroquinolones for prophylaxis or treatment of *S. aureus* infections, the results from experimental tissue cage infections by MRSA may provide useful information on the in vivo efficacies of different fluoroquinolone agents. In recent years, a number of theoretical and experimental approaches have been proposed for use in evaluating the relevance of various pharmacokinetic parameters of antimicrobial agents on their therapeutic efficacies (13, 14, 27, 38, 40). The most extensively studied parameters in these studies were the serum concentration-time profiles in vivo, the protein binding, the area and time above the MIC, the duration of the postantibiotic effect, the volume of distribution, and tissue penetration.

Useful information derived from studies with the different tissue cage models of localized infections caused by *S. aureus* or *Staphylococcus epidermidis* (41) is the possibility of directly assessing the levels of antimicrobial agents at the infected sites, thus yielding the tissue cage concentration-time profiles of each antimicrobial agent used for prophylaxis or therapy. In the prophylactic model, single-dose regimens of antimicrobial agents were adjusted to yield bactericidal levels of each fluoroquinolone in tissue cage fluid for a minimum period of 24 h. Despite reaching adequate levels in tissue cage fluids, ciprofloxacin proved markedly less effective against MRSA challenge than sparfloxacin or temafloxacin administered at equivalent single-dose regimens. The different prophylactic activities

of the three fluoroquinolones in the guinea pig model were unexpected in view of their similar bactericidal activities against MRSA in standard in vitro assays. The only significant difference in the in vivo properties of the different fluoroquinolones was the relatively lower tissue cage fluid AUC of ciprofloxacin over its MBC for MRSA compared with those of temafloxacin and sparfloxacin. Although such differences in the pharmacokinetics of each fluoroquinolone may contribute to their different prophylactic efficacies, they may not be sufficient to explain the whole phenomenon. It is possible that differences in antibiotic uptake within phagocytic cells, recruited locally, and rates of exchange between intra- and extracellular compartments may contribute to the different in vivo activity of each fluoroquinolone, as suggested in a previous report of a study comparing ofloxacin, ciprofloxacin, temafloxacin, and sparfloxacin in an animal model of lung infection (40).

In comparison with the results of the prophylactic model, the very different in vivo activities of ciprofloxacin and temafloxacin for the treatment of chronically infected rat tissue cages represent an even greater paradox. Indeed, all major pharmacokinetic parameters of both fluoroquinolones in tissue cage fluids were almost identical, including equivalent concentration time-profiles, AUCs, MICs, and MBCs for the challenge strain. Not only were the in vitro bactericidal activities of temafloxacin and ciprofloxacin nearly identical against MRSA and similar to those reported previously (for a review, see reference 23), but no selective emergence of resistant organisms to either fluoroquinolone which might explain their widely different in vivo activities in the rat model was detected.

A number of recent studies suggest that standard conditions of in vitro susceptibility testing of bactericidal antibiotics (32) are not representative of the in vivo situation. Bacteria recovered from foreign body infections, especially those colonizing artificial surfaces, are frequently reported as slowly growing organisms showing altered susceptibilities to bactericidal antibiotics (2, 7, 10, 11, 19, 32, 37, 41). However, data from our own laboratory and from others indicate that the decrease in antibiotic susceptibility of slowly growing bacteria, whether they are surface attached or in the fluid phase, is moderate for fluoroquinolones, more important for beta-lactams and glycopeptides, and extensive for aminoglycosides (for a review, see reference 11). Furthermore, several in vitro studies have shown that supplementation of the medium with serum or other variables such as pH, magnesium concentration, and inoculum size do not significantly influence the in vitro activities of any major fluoroquinolone used for therapy (for a review, see reference 16). Previous in vitro and in vivo observations from our laboratory indicate that tissue cage bacteria indeed show an altered metabolic state and diminished susceptibility to antibiotics (10). However, resistance of tissue cage bacteria to antibiotic killing is mostly expressed against cell wall antibiotics, in particular, teicoplanin (37). It is unlikely that the metabolic state of tissue cage bacteria is responsible for expression of a specific resistance to ciprofloxacin but not any other fluoroquinolone during therapy of chronic tissue cage infections by MRSA.

Finally, the low level of activity of ciprofloxacin in both animal models of chronic MRSA infections is even more surprising in view of the good in vivo results obtained with this antimicrobial agent against a variety of experimental and clinical staphylococcal infections except for the occurrence of resistant organisms (for reviews, see references 21, 28, 29, and 42). We found only one report mentioning the lack of activity of ciprofloxacin for the treatment of 2-week-old abscesses in rabbits (3) without the concomitant emergence of quinolone-resistant organisms (3).

In conclusion, the rational selection of prophylactic or therapeutic regimens of antibiotics against foreign body infections is still problematic. These data further emphasize the value of performing experiments in animal for the primary evaluation of new therapeutic agents. In particular, comparative studies of different fluoroquinolones with similar in vitro activities but different in vivo efficacies in this and other (40) experimental models may help to discover important parameters influencing their in vivo activities.

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