ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Dec. 1990, p. 2312–2317
0066-4804/90/122312-06$02.00/0

Vol. 34, No. 12

Treatment of Experimental Foreign Body Infection Caused by Methicillin-Resistant *Staphylococcus aureus*

JEAN-CHRISTOPHE LUCET,† MATHIAS HERRMANN, PETER ROHNER, RAYMOND AUCKENTHALER, FRANCIS A. WALDVOGEL, AND DANIEL P. LEW*

Division of Infectious Diseases, Department of Medicine, Geneva University Hospital, 1211 Geneva 4, Switzerland

Received 4 June 1990/Accepted 26 September 1990

A novel model of experimental foreign body infection was developed in rats: four perforated Teflon tissue cages per animal were implanted subcutaneously and 3 to 4 weeks later were infected with 0.5×10^5 to 2×10^5 CFU of methicillin-resistant *Staphylococcus aureus*. After 2 weeks, the number of CFU in the cage fluid was determined [day 1 mean, (7.25 ± 0.79) $\log_{10}$ CFU/ml], and treatment with vancomycin (50 mg/kg twice a day [BID]), fleroxacin (50 mg/kg BID), or rifampin (25 mg/kg BID), alone and in combination, was initiated for a duration of 6 days. Concentrations of antibiotics in cage fluids were in the range of those encountered in clinical conditions. Eighteen hours after the last injection (day 7), the number of CFU in the cage fluid was determined and the difference between day 1 and day 7 values was calculated. Rifampin, alone and in combination with fleroxacin or vancomycin, was the most effective regimen in reducing the bacterial counts in the tissue cage fluids [(1.87 ± 1.44, 2.18 ± 1.02, and 2.55 ± 1.09 $\log_{10}$) CFU/ml, $P < 0.001$, respectively]. After treatment, cage fluids and cages were analyzed for resistant bacteria. Resistance to rifampin occurred in 15 of 19 cages in animals treated with rifampin alone and in 4 of 25 in animals treated with rifampin plus vancomycin. We detected no development of resistance to rifampin in animals treated with rifampin plus fleroxacin or to fleroxacin in animals treated with this antimicrobial agent. In conclusion, regimens including rifampin alone or in combination with vancomycin or fleroxacin were an effective treatment of foreign body infection due to methicillin-resistant *S. aureus* in reducing bacterial counts, but rifampin monotherapy was compromised by significant emergence of resistance. The combined therapy of fleroxacin with rifampin prevents development of resistance to rifampin.

Infection is one of the most serious complications in the use of prosthetic devices and a major cause of morbidity and implant failure, and it usually requires the removal of infected material (3, 8). *Staphylococcus* species are the most frequently encountered microorganisms in this setting, with an increasing incidence of methicillin-resistant *Staphylococcus aureus* (MRSA) (6). Vancomycin is the reference antibiotic to treat MRSA infections, but its poor tissue diffusion and its moderate bactericidal rate often require an associated antimicrobial therapy, usually rifampin, in deep-seated infections (7); however, resistance to rifampin has emerged under this association (1, 21). The new generation of fluoroquinolones offers an alternative strategy for antibiotic treatment of MRSA infections on foreign materials: (i) these compounds show a good activity on staphylococci, can be administered orally, and are characterized by a low rate of adverse effects; (ii) in spite of a regular in vitro antagonism, the combined therapy of fluoroquinolones and rifampin has been recently proposed to treat serious *S. aureus* infections (10); and (iii) the emergence of resistance of MRSA to quinolones in monotherapy is not rare (16, 26, 27, 32), and their association with rifampin might prevent emergence of resistance.

In the present investigation, we developed a novel animal model for treatment of experimental chronic foreign body infection caused by MRSA. This model allowed us to establish a persistent and chronic infection dependent on the presence of foreign material and to evaluate the efficacy of prolonged antibiotic treatment. In particular, we studied (i) the activity of fleroxacin, a new fluoroquinolone with a long half-life and good tissue penetration, (ii) the therapeutic effect of fleroxacin, vancomycin, and rifampin in association, and (iii) the emergence of bacteria resistant to fleroxacin or rifampin alone or in combination.

MATERIALS AND METHODS

Organism. The strain of *S. aureus* used, MRGR3, was a methicillin-resistant bloodstream isolate from a patient with an indwelling-device infection.

In vitro studies. Fleroxacin (Hoffmann-La Roche & Co. Ltd., Basel, Switzerland) was provided as an injectable preparation containing 4 mg of drug per ml. Vancomycin hydrochloride (Laboratory Lilly, GmbH, Giessen, Federal Republic of Germany) was prepared at a concentration of 50 mg/ml and stored at 4°C for a maximum of 96 h. Rifampin (Laboratory Ciba-Geigy, Basel, Switzerland) was prepared at a concentration of 50 mg/ml, stored at −20°C, and used immediately after thawing.

The MICs and MBCs of methicillin, fleroxacin, vancomycin, and rifampin for strain MRGR3 in the exponential growth phase were determined in quadruplicate in Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.) by a macrodilution method with an inoculum of 0.9×10^6 to 3×10^6 CFU/ml. The MIC was defined as the lowest concentration of antibiotics which inhibited visible growth after 18 to 24 h of incubation at 37°C. The MBC was defined as the lowest concentration which killed 99.9% of the original inoculum (28).

Killing kinetic studies were determined in Mueller-Hinton broth with 1.2×10^6 CFU of exponential-growth-phase organisms per ml as an inoculum. Antibiotic concentrations

* Corresponding author.
† Present address: Intensive Care Unit, Hôpital Bichat-Claude Bernard, Paris, France.

were selected according to those found in tissue cage fluid, namely, 5 μg of vancomycin or fleroxacin per ml. In contrast, we tested rifampin at only 0.5 μg/ml in order to prevent a carry-over effect of this antibiotic. Test tubes were incubated at 37°C in a shaking water bath, and quantitative subcultures were performed after 0, 1, 2, 4, 6, and 24 h of incubation. To determine the carryover of antibiotics, 100 μl of undiluted or 10-fold serially diluted portions was plated on Mueller-Hinton agar plates with a Spiral Plater (Spiral System Inc., Cincinnati, Ohio). The plates were then incubated at 35°C for 48 h before the colonies were counted with a colony counter (Spiral System Inc.). This system allowed us (i) to determine suitable sample dilutions in order to prevent carry-over and (ii) to quantify reliably CFU counts even in the presence of inhibitory antibiotic concentrations due to dispersion of the sample in the periphery of the plate (E. Yourassowsky, M. P. Van der Linden, F. Crokaert, and Y. Glupczynski, Letter, J. Antimicrob. Chemother. **21**:138–140, 1988). Under these experimental conditions, the 10-fold-diluted samples exhibited no carry-over of antibiotics. Therefore, the minimal detection level was 1.3 $\log_{10}$ CFU/ml.

The frequency at which the strain developed spontaneous mutational resistance to 3-, 6-, and 12-fold MICs of fleroxacin or 10-, 100-, and 1,000-fold MICs of rifampin was determined by exposing 10^{10} to 10^{11} CFU in the stationary or exponential growth phase to the appropriate concentration of antibiotics incorporated into Mueller-Hinton agar; colonies were counted 48 h later.

Animal studies. Male Wistar rats weighing 220 to 240 g were used. Three to four multiperforated polytetrafluoroethylene (Teflon) tissue cages (internal and external diameters of 10 and 12 mm, respectively; length, 32 mm; volume, 2.5 cm^3) were implanted subcutaneously under aseptic conditions as previously described (41). Each cage contained triplicate polymethyl methacrylate cover slips (7 by 7 mm) which could be easily removed from the explanted tissue cage for analysis. Tissue cage fluids could be aseptically aspirated and analyzed for CFU.

A pilot study was performed to evaluate the natural course of infection. The subcutaneous injection of 10^8 CFU of MRGR3 could not produce any infection in the absence of tissue cages (12 animals). In contrast, 10^5 CFU injected into cages 3 to 4 weeks after implantation was found to be sufficient to produce a persistent infection of >95% of the cages, whereas inocula with fewer CFU did not constantly initiate infection. Tissue cage fluids were subsequently withdrawn twice a week for 2 months and assessed for bacterial counts. Results are shown in Fig. 1. Bacterial counts increased during weeks 1 to 3 after infection and then remained constant during weeks 3 to 7. Thereafter, chronic infection persisted in 10 cages, and only 1 of the 10 cages was extruded on week 12.

The experimental study was designed as follows: 4 weeks after implantation, cage fluid samples were analyzed to exclude bacterial contamination, and tissue cages were subsequently inoculated with 0.1 ml of saline containing 0.4 $\times$ 10^6 to 2 $\times$ 10^6 CFU of MRGR3 in stationary phase per ml. To confirm experimental infection, each tissue cage fluid was aspirated after 14 days and checked for the presence of bacterial infection by quantitative culture. Plates were incubated for 48 h, and results were expressed as $\log_{10}$ CFU per milliliter (sensitivity limit, 1.3 $\log_{10}$ CFU/ml). Tissue cages containing fewer than 10^5 CFU/ml in their fluid phase were excluded from the therapeutic protocols. Rats were then randomized to receive (by intraperitoneal route twice a day for 6 days) vancomycin (50 mg/kg), fleroxacin (50 mg/kg), rifampin (25 mg/kg), or the following combinations in the same concentrations: vancomycin plus rifampin, vancomycin plus fleroxacin, or rifampin plus fleroxacin. Animals without antibiotic treatment were examined in parallel (controls). Rats were sacrificed on day 7 of therapy, 18 h after the last injection of antibiotics. This lag time was selected to minimize any carry-over of rifampin. After aspiration of tissue cage fluid, tissue cages were removed aseptically. Quantitative cultures of 10-fold serially diluted tissue cage fluid were performed for 48 h at 37°C. Since the sensitivity limit was 1.3 $\log_{10}$ CFU/ml, the culture-negative fluids were referred to as containing 1.3 $\log_{10}$ CFU/ml. To determine the effect of antibiotic therapy on adherent bacteria, one cover slip was removed from each cage and rinsed three times in phosphate-buffered saline. Bacteria were removed from cover slips by sonication (120 W, 540 kC for 60 s; Bransonic 220; Dynatech, Kloten, Switzerland) in 1 ml of phosphate-buffered saline and incubation in trypsin (6 U/ml) (Bovin Trypsin; Serva, Westbury, N.Y.) for 20 min. Previous studies have shown that this method is harmless for the viability of staphylococci and allows the detachment of the majority of adherent microorganisms from cover slips (19). Quantitative bacterial counts were then determined with a minimal detection limit of 20 CFU/ml of phosphate-buffered saline.

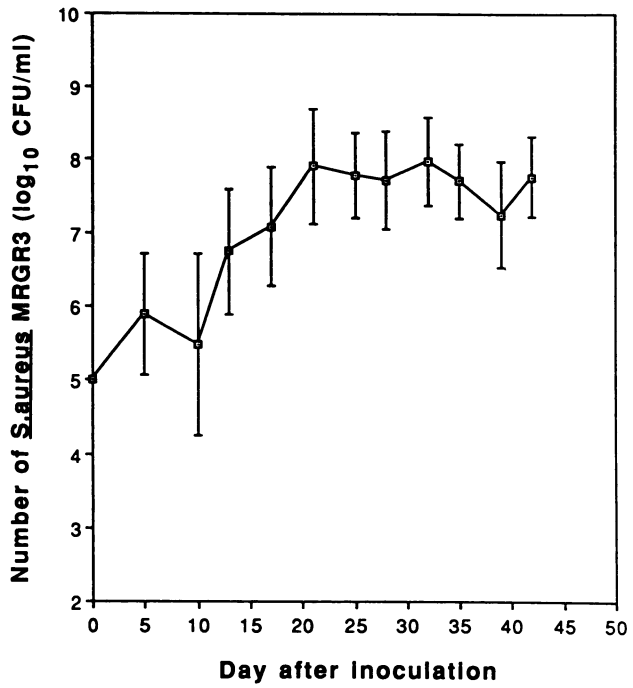

FIG. 1. In vivo growth of strain MRGR3 in subcutaneous tissue cages without antibiotics. At 4 weeks after implantation, MRGR3 (10^5 CFU) was inoculated into tissue cages (day 0), and subsequently the number CFU in aspirated cage fluid was determined. Results are means ± standard deviations from 10 cages tested.

Pharmacokinetics of antimicrobial agents. Cage fluids were obtained from all animals by pooling tissue cage fluids of each animal for measurement of trough (before administration) and peak (4 h after injection) antibiotic levels after 3 days of treatment. Vancomycin concentrations were measured by fluorescence polarization, kindly performed by A. Deom (Laboratoire Central de Chimie Clinique, Hôpital Cantonal Universitaire, Geneva, Switzerland). Concentra-

tions of fleroxacin in cage fluids were measured by high-performance liquid chromatography (E. Weidekamm, Hoffmann-La Roche). Concentrations of rifampin in cage fluids were estimated by a bioassay with *Micrococcus luteus* NCTC 8340 as the test strain. Rifampin levels in animals treated by vancomycin plus rifampin could not be determined, since the test strain is vancomycin susceptible.

Resistance to antimicrobial agents. Excised tissue cages and the two remaining polymethyl methacrylate cover slips were rinsed, and bacteria were removed as described above. Isolates of MRGR3 recovered from tissue cages or cage fluids were screened for the emergence of resistance to rifampin or fleroxacin during therapy: 100 μl of either the fluid of sonicated tissue cages or the 10-fold-diluted cage fluid aspirates was plated onto Mueller-Hinton agar containing either 3- and 10-fold MICs of fleroxacin or 10- and 100-fold MICs of rifampin. Plates were examined for growth 48 h later. The lower detection limits of resistant bacteria adherent to implanted foreign material and in tissue cage fluid were 10^{-1} and 10^{-2}, respectively. Thus, even low numbers of resistant bacteria could be detected by this method. The number of resistant bacteria was determined semiquantitatively. The serotypes of the original MRGR3 strain and of all resistant strains were determined to check that the microorganisms studied originated from the same parental strain.

Statistics. Results were expressed as $\log_{10}$ CFU per milliliter in arithmetic means ± standard deviations. Comparisons of bacterial counts were made by one-way analysis of variance, and multiple comparisons have subsequently been performed by the Dunnett *t* test. Comparisons of antimicrobial concentrations were made by an unpaired *t* test. Relative frequencies were compared by a chi-square test with the Yates correction.

RESULTS

In vitro studies. The MICs and MBCs of methicillin, vancomycin, fleroxacin, and rifampin for strain MRGR3 were >64 and >64, 1 and 2, 0.62 and 1.06, and 0.01 and 0.02 μg/ml, respectively. Timed-kill studies showed that rifampin antagonized the killing effect of fleroxacin for the test strain by reducing its bactericidal activity by 2.6 $\log_{10}$ CFU/ml at 6 h but not at 24 h. Rifampin antagonized the killing effect of vancomycin with a reduction of bactericidal activity of 1.2 and 3.6 $\log_{10}$ CFU/ml at 6 and 24 h, respectively. The association of vancomycin plus fleroxacin did not affect bactericidal activity (data not shown).

MRGR3 developed spontaneous mutational resistance to 3-, 6-, and 12-fold MICs of fleroxacin very rarely, i.e., at a frequency $<10^{-10}$ in the exponential or stationary growth phase. The frequency of mutational resistance to rifampin was 1.4×10^{-6}, 4.5×10^{-7}, and 2.0×10^{-7} in the exponential growth phase and 5.2×10^{-7}, 4.4×10^{-7}, and 1.7×10^{-7} in the stationary growth phase when exposed to 10-, 100-, and 1,000-fold MICs of rifampin, respectively.

Pharmacokinetics of antimicrobial drugs. Concentrations of antimicrobial agents in tissue cage fluids are shown in Table 1. No significant difference was noted in animals treated with antibiotics alone or in combination, with the exception of fleroxacin levels in animals treated with fleroxacin in association with vancomycin compared with its association with rifampin ($P < 0.05$). Vancomycin levels varied over a wide range, i.e., from 0.3 to 5.9 μg/ml at the trough levels and from 3.4 to 42 μg/ml at the peak levels. The lowest trough cage fluid levels of fleroxacin and rifampin measured were always above their MICs, i.e., 0.6 and 1.7 μg/ml, respectively. The levels of rifampin determined in six cage fluids at the time of sacrifice, 18 h after the last dose of antibiotic, were 0.69 ± 0.29 μg/ml.

TABLE 1. Antimicrobial concentrations in the tissue cage fluid[a]

Treatment	No. of animals	Concn (mean μg ± SD)	
		Trough	Peak
Vancomycin			
Alone	4	1.87 ± 1.18	11.53 ± 4.94
+Fleroxacin	5	3.46 ± 1.5	13.51 ± 5.44
+Rifampin	9	2.16 ± 1.2	18.8 ± 10.5
Rifampin			
Alone	6	3.31 ± 1.77	6.41 ± 3.01
+Fleroxacin	7	5.80 ± 3.2	8.73 ± 2.86
Fleroxacin			
Alone	4	2.47 ± 1.62	8.35 ± 2.74
+Rifampin	7	4.05 ± 2.83	14.77 ± 3.97
+Vancomycin	5	2.20 ± 1.02	8.01 ± 2.39

[a] After 3 days of antibiotic therapy. Antibiotic concentrations were measured after pooling fluid for the four cages in each animal.

Animals studies. Of 163 cages, 11 (6.7%) with quantitative bacterial counts in the tissue cage fluid below 10^5 CFU/ml before treatment were excluded from the study. Bacterial counts for the remaining 152 tissue cage fluids before treatment were 7.25 ± 0.79 $\log_{10}$ CFU/ml [range, (7.11 ± 0.87) $\log_{10}$ CFU/ml for animals receiving vancomycin to (7.4 ± 0.67) $\log_{10}$ CFU/ml for animals receiving fleroxacin].

The quantitative bacterial counts in cage fluids are shown in Table 2. In order to rule out the variations of bacterial counts between different cages on day 1, results were expressed as the difference between values from day 1, before treatment, and day 7, 18 h after the last injection, for each cage. A reduction of 0.17 $\log_{10}$ CFU/ml was observed for control animals (no significant difference between day 1 and day 7).

Compared with controls, all regimens except fleroxacin could significantly decrease the viable counts in tissue cage fluids from day 1 to day 7 and also reduce the number of microorganisms adherent to the tissue cage at day 7. Monotherapy with rifampin was more efficient than either vancomycin or fleroxacin alone on fluid-phase microorganisms ($P < 0.05$ and $P < 0.01$, respectively). The efficacies of vancomycin and fleroxacin were not significantly different when these drugs were used in monotherapy. The most effective treatment regimens were found to be the combinations including rifampin, resulting in a significant ($P < 0.05$ versus controls) reduction of >2 $\log_{10}$ CFU/ml from the initial number of microorganisms. Vancomycin plus rifampin was more active than fleroxacin plus rifampin, but this difference was not significant. The combination of vancomycin or fleroxacin with rifampin was more effective than each monotherapy without rifampin ($P < 0.001$). Despite the good efficacy of some antibiotic regimens, sterilization of the tissue cage fluids was not obtained, except for one cage treated with vancomycin plus rifampin. With respect to the number of bacteria adherent to the cages after 7 days of treatment, rifampin alone or in combination with vancomycin or fleroxacin was also significantly more active than monotherapy with vancomycin or fleroxacin ($P < 0.001$).

MRGR3 isolates resistant to rifampin were recovered from 15 of 19 cages in animals treated with rifampin monotherapy: in 8 of 15 cages, resistant organisms were found both in the

TABLE 2. Reduction in CFU and resistance in tissue cage fluid and number of bacteria and emergence of resistant mutants adherent to foreign material.

Treatment	No. of cages	Results[a] in:			
		Tissue cage fluid		Foreign body	
		Reduction in CFU[b] (mean $\log_{10}$ CFU/ml ± SD)	Resistance to rifampin[c]	Adherent bacteria[d] (mean $\log_{10}$ CFU ± SD)	Resistance to rifampin[c]
Control	28	0.17 ± 0.65	ND	5.35 ± 1.21	ND
Vancomycin	16	1.16** ± 0.59	ND	3.97*** ± 1.08	ND
Rifampin	19	1.87*** ± 1.44	8/19	3.34*** ± 1.30	15/19
Fleroxacin	16	0.78 ± 0.69	ND	4.61 ± 1.29	ND
Vancomycin + rifampin	33	2.55*** ± 1.09	0/25	2.53*** ± 1.28	4/25
Vancomycin + fleroxacin	18	1.50*** ± 0.92	ND	3.16*** ± 1.20	ND
Rifampin + fleroxacin	23	2.18*** ± 1.02	0/23	3.28*** ± 1.40	0/23

[a] ND, Not done. **, $P < 0.01$ versus control; ***, $P < 0.001$ versus control.
[b] Reduction of bacterial counts in tissue cage fluids between day 1 and day 7.
[c] Number of cages containing resistant bacteria/number of cages examined.
[d] Number of bacteria adherent to one cover slip at day 7.

cage fluid and adherent to the solid phase, whereas in the remaining 7 cages, resistant bacteria were found only in the adherent population. The MIC of rifampin was higher than 100 μg/ml for all of these strains. Of 15 cages, 3 contained large numbers ($>10^3$ CFU/ml) of resistant bacteria, whereas the other 12 cages contained amounts of resistant bacteria which did not significantly contribute to the number of CFU in the cage fluid. When the three cages with the largest number of resistant bacteria were excluded from statistical analysis, rifampin treatment alone was equally efficacious as combination treatment including rifampin [reduction of viable counts, (2.19 ± 1.30) $\log_{10}$ CFU/ml]. Of 33 cages in animals treated with vancomycin plus rifampin, 8 showed contamination with gram-negative organisms, and these cages were excluded from further evaluation for the emergence of resistance. In 4 of 25 (16%) remaining cages, a small number (<100 CFU/ml) of rifampin-resistant staphylococci adherent to the solid phase were found in two animals. No evidence of resistance was found in the fluids aspirated from these cages. Analysis of the vancomycin concentrations in the cage fluids of these two animals showed 0.3 and 3.4 μg/ml at the trough level and 3.4 and 17.4 μg/ml at the peak level. In contrast, no resistance to rifampin occurred in animals treated with rifampin plus fleroxacin. Resistance to fleroxacin was not detected under any of the conditions in which this antibiotic was used.

DISCUSSION

Many factors seem to interfere with antibiotic therapy of foreign body infections, in particular, those due to MRSA. Most of them are not completely elucidated, but they might involve either surface modifications of adherent bacteria (17) or the alteration of local immune defense mechanisms around the prosthetic material (40). Additional factors, such as the low number of antibiotics active on MRSA, their frequent ineffectiveness on quiescent bacteria within neutrophils (33), or the variable diffusion of antibiotics in deep infection sites, may further reduce the efficacy of antibiotic therapy (7).

Thus, there exists a need for an animal model assessing in vivo the efficacy of antibiotics in treating foreign body infections. A previously developed model from our group using guinea pigs is not suitable for curative treatment studies because of serious side effects of antibiotics and virulent infection on foreign bodies in these animals (41). Thus, this report describes a new experimental model in rats, reproducing several clinical characteristics of infection on foreign devices, in particular, persistence of infection for more than 2 months in untreated animals. This model allowed us to assess local levels of antibiotics and bacterial counts before and after treatment. It was also convenient to study the emergence of bacteria resistant to antibiotics.

The susceptibility pattern of our strain was typical: the MICs and MBCs of the three antibiotics tested were in the range of usually encountered values and confirmed the good in vitro antistaphylococcal activity of fleroxacin. Bactericidal activities of the antibiotics were evaluated by the kill curve method (23) with high inoculum and antibiotic concentrations achieved in humans. As previously reported, we found an antagonism between vancomycin and rifampin (5, 18) and between rifampin and fluoroquinolones (12, 18, 30). Since a carry-over effect could have positively biased the groups treated with rifampin, we selected a low concentration of rifampin. This low dosage, the sampling of material 18 h after the end of therapy, the 10-fold dilution before counting, and the incubation for 48 h allowed us to control the carry-over phenomenon.

Antibiotic levels in the tissue cage fluids were close to those reported in other studies of bone or interstitial tissues (2, 36). Trough levels were above the MICs of rifampin and fleroxacin and confirmed the good tissue diffusion of these antibiotics. At peak levels, fleroxacin concentrations were significantly higher when associated with rifampin; it is conceivable that rifampin modifies the pharmacokinetics of fleroxacin in rats. Vancomycin levels showed great variability; however, the mean levels were close to those obtained in humans at infected sites, which also showed quite variable local levels of vancomycin (15).

Foreign body infections due to MRSA raise critical therapeutic problems: they require a long-term therapy with well-diffusing antimicrobial agents in deep-seated infections. Vancomycin is the reference antimicrobial agent for treating infections due to MRSA (7, 35), but unfavorable diffusion characteristics may hamper its use as a single agent for therapy of prosthetic device infections (13). Our data demonstrate that vancomycin alone is an efficacious agent, even when the treatment is as short as 6 days. Fluoroquinolones are promising antistaphylococcal agents and allow long-term oral therapy with good tolerance. In contrast to promising results obtained in humans (9, 16, 39), the reduction of viable

counts with fleroxacin in our study was lower than results with vancomycin (difference not significant). It is possible that a longer treatment may have resulted in an increased efficacy of fleroxacin. No resistance to fleroxacin was observed. Resistance of *S. aureus* to fluoroquinolones generally occurs at a low frequency, $<10^{-9}$ (29), but is frequent in clinical conditions (9, 16, 32), usually with a moderate increase of MIC. By using plates containing a threefold MIC of fleroxacin for our strain, we should have been able to detect any resistant bacteria. The relatively low number of bacteria in the cage, the low percentage of in vitro-resistant mutants of strain MRGR3 ($<10^{-10}$), and fleroxacin levels in the tissue cage fluids constantly above the MIC may explain the absence of resistance development in our in vivo model.

In our therapeutic protocol, we have shown that rifampin alone or in combination was the best treatment for the reduction of bacterial counts either in the tissue cage fluid or on the solid phase. This optimal activity of rifampin is probably due to its low MIC for *S. aureus* MRGR3, its excellent diffusion (2) and intracellular penetration (24), and its possible activity in inactivated polymorphonuclear cells on contact with a foreign material (37). The emergence of resistant bacteria is unfortunately a constant event when rifampin is used as monotherapy (25, 31) and occurred in our study in 15 of 19 cages. This percentage seems unexpectedly high with an initial inoculum with low CFU concentrations and after a short-term antibiotic treatment: in 7 of 15 cases, a low number of resistant bacteria were found adherent only on the foreign implant, suggesting a possible role of the material favoring the mutation phenomenon.

Therefore, a combination of another antibiotic besides rifampin is necessary. The results observed in experimental infections, endocarditis (4), or osteomyelitis (31) suggest the combination of rifampin with vancomycin in the treatment of infections due to MRSA in humans (13, 22, 35, 38). The combination of vancomycin with rifampin improved our therapeutic results by limiting the emergence of rifampin-resistant strains. Emergence of rifampin resistance is a rare observation in clinical MRSA infections treated with vancomycin plus rifampin (1, 11, 14, 34). In these nine reported cases, it seems that the emergence of rifampin-resistant *S. aureus* strains is multifactorial: deep sites of infection, insufficient local levels of vancomycin, and frequent in vitro antagonism. In our studies, the high variability of vancomycin levels among animals and the in vitro antagonism could also explain the development of rifampin resistance.

Despite their usual in vitro antagonism, recent studies have stimulated interest in the combination of rifampin plus fluoroquinolones in the treatment of patients with osteomyelitis or osteoarthritis (9) and in the treatment of experimental (20) or human (10) endocarditis. Our results confirm the excellent effectiveness of the combination in infections involving foreign materials, regarding both the reduction of the bacterial inoculum and the prevention of resistance. These results should be compared with clinical observations, which have not yet described a strain resistant to either of these two antibiotics when they were associated. Good tissue diffusion, long half-life, and ability for intracellular penetration probably afford these two antibiotics mutual protection (18). Another advantage of this combination is the good tolerance and the possibility of prolonged oral administration.

Despite our promising results with combination chemotherapy, a sterilization of the implanted material could not be obtained. Thus, further experiments including a more prolonged duration of treatment and other combinations are warranted in order to try to achieve cure of foreign body infections caused by MRSA.

ACKNOWLEDGMENTS

This work was supported by grant 3.829.087 from the Swiss National Science Foundation and by a grant from Hoffmann-La Roche & Co. Ltd., Basel, Switzerland.

We thank I. Ratti for excellent technical assistance and P. Vaudaux and C. Chuard for helpful comments and suggestions.

LITERATURE CITED

1. **Acar, J. F., F. W. Goldstein, and J. Duval.** 1983. Use of rifampin for the treatment of serious staphylococcal and gram-negative bacillary infections. Rev. Infect. Dis **5**(Suppl. 3):S502–S506.
2. **Acocella, G.** 1983. Pharmacokinetics and metabolism of rifampin in humans. Rev. Infect. Dis. **5**(Suppl. 3):S428–S432.
3. **Archer, G. L.** 1984. *Staphylococcus epidermidis*: the organism, its disease and treatment, p. 25–48. *In* J. S. Remington and M. N. Schwartz (ed.), Current clinical topics in infectious disease, vol. 5. McGraw-Hill Book Co., New-York.
4. **Bayer, A. S., and K. Lam.** 1985. Efficacy of vancomycin plus rifampicin in experimental aortic-valve endocarditis due to methicillin-resistant *Staphylococcus aureus*: in vitro-in vivo correlations. J. Infect. Dis. **151**:157–165.
5. **Bayer, A. S., and J. O. Morrison.** 1984. Disparity between timed-kill and checkerboard methods for determination of in vitro bactericidal interaction of vancomycin plus rifampin versus methicillin-susceptible and -resistant *Staphylococcus aureus*. Antimicrob. Agents Chemother. **26**:220–223.
6. **Calderwood, S. B., L. A. Swinski, A. W. Karchmer, C. M. Waternaux, and M. J. Buckley.** 1985. Risk factors for the development of prosthetic valve endocarditis. Circulation **72**: 31–37.
7. **Chambers, H. F.** 1988. Methicillin-resistant staphylococci. Clin. Microbiol. Rev. **1**:173–186.
8. **Dankert, J., A. H. Hogt, and J. Feijen.** 1986. Biomedical polymers: bacterial adhesion, colonization and infection. Crit. Rev. Biocompat. **2**:219–301.
9. **Desplaces, N., and J. F. Acar.** 1988. New quinolones in the treatment of bone and joint infections. Rev. Infect. Dis. **10**(Suppl. 1):S179–S183.
10. **Dworkin, R. J., B. L. Lee, M. A. Sande, and H. F. Chambers.** 1989. Treatment of right-sided *Staphylococcus aureus* endocarditis in intravenous drugs users with ciprofloxacin and rifampicin. Lancet **ii**:1071–1073.
11. **Eng, R. H. K., S. M. Smith, M. Tillem, and C. Cherubin.** 1985. Development of rifampicin resistance during the therapy of methicillin-resistant *Staphylococcus aureus* infection. Arch. Intern. Med. **145**:146–148.
12. **Fass, R. J., and V. L. Helsel.** 1987. In vitro antistaphylococcal activity of pefloxacin alone and in combination with other antistaphylococcal drugs. Antimicrob. Agents Chemother. **31**: 1457–1460.
13. **Faville, R. J., D. E. Zaske, and E. L. Kaplan.** 1978. *Staphylococcus aureus* endocarditis: combined therapy with vancomycin and rifampicin. J. Am. Med. Assoc. **240**:1963–1965.
14. **Garaud, J. J., B. Regnier, K. Lassoued, B. Pangon, and C. Leport.** 1985. Traitement des staphylococcies graves. Echec de la rifampicine en association. Quatre observations. Presse Med. **14**:1013–1016.
15. **Graziani, A. L., L. A. Lawson, G. A. Gibson, M. A. Steinberg, and R. R. MacGregor.** 1988. Vancomycin concentrations in infected and noninfected bone. Antimicrob. Agents Chemother. **32**:1320–1322.
16. **Greenberg, R. N., D. J. Kennedy, P. M. Reilly, K. L. Luppen, W. J. Weinandt, M. R. Bollinger, F. Aguirre, F. Kodesch, and A. M. K. Saeed.** 1987. Treatment of bone, joint, and soft-tissue infections with oral ciprofloxacin. Antimicrob. Agents Chemother. **31**:151–155.
17. **Gristina, A. G., and J. W. Costerton.** 1985. Bacterial adherence to biomaterials and tissue: the significance of its role in clinical sepsis. J. Bone J. Surg. Am. Vol. **67-A**:264–273.

18. **Hackbarth, C. J., H. F. Chambers, and M. A. Sande.** 1986. Serum bactericidal activity of rifampin in combination with other antimicrobial agents against *Staphylococcus aureus*. Antimicrob. Agents Chemother. **29**:611–613.
19. **Herrmann, M., M. E. E. Jaconi, C. Dahlgren, F. A. Waldvogel, O. Stendahl, and D. P. Lew.** 1990. Neutrophil bactericidal activity against *Staphylococcus aureus* adherent on biological surfaces. J. Clin. Invest. **86**:942–951.
20. **Kaatz, G. W., S. M. Seo, S. L. Barriere, L. M. Albrecht, and M. J. Ribak.** 1989. Ciprofloxacin and rifampin, alone and in combination, for therapy of experimental *Staphylococcus aureus* endocarditis. Antimicrob. Agents Chemother. **33**:1184–1187.
21. **Kapusnik, J. E., F. Parenti, and M. A. Sande.** 1984. The use of rifampin in staphylococcal infections. A review. J. Antimicrob. Chemother. **13**(Suppl. C):61–66.
22. **Karchmer, A. W., G. L. Archer, and W. E. Dismukes.** 1983. Rifampin treatment of prosthetic valve endocarditis due to *Staphylococcus epidermidis*. Rev. Infect. Dis. **5**(Suppl. 3): S543–S548.
23. **Krogstad, D. J., and R. C. Moellering.** 1986. Antimicrobial combinations, p. 537–595. *In* V. Lorian (ed.), Antibiotics in laboratory medicine. The Williams & Wilkins Co., Baltimore.
24. **Mandell, G. L.** 1983. The antimicrobial activity of rifampicin-emphasis on the relation to phagocytes. Rev. Infect. Dis. **5**(Suppl. 3):S463–S467.
25. **Mandell, G. L., and D. R. Moorman.** 1980. Treatment of experimental staphylococcal infections: effect of rifampin alone and in combination on the development of rifampin resistance. Antimicrob. Agents Chemother. **17**:658–662.
26. **Maple, P. A. C., J. M. T. Hamilton-Miller, and W. Brumfitt.** 1989. World-wide antibiotic resistance in methicillin-resistant *Staphylococcus aureus*. Lancet **i**:537–540.
27. **Maple, P. A. C., J. M. T. Hamilton-Miller, and W. Brumfitt.** 1989. Ciprofloxacin resistance in methicillin and gentamycin resistant *Staphylococcus aureus*. Eur. J. Clin. Microbiol. **8**:622–624.
28. **National Committee for Clinical Laboratory Standards.** 1987. Methods for determining bactericidal activity of antimicrobial agents. M26-P. National Committee for Clinical Laboratory Standards, Villanova, Pa.
29. **Neu, H. C.** 1988. Bacterial resistance to fluoroquinolones. Rev. Infect. Dis. **10**(Suppl. 1):S57–S63.
30. **Neu, H. C.** 1989. Synergy of fluoroquinolones with other antimicrobial agents. J. Infect. Dis. **11**(Suppl. 5):1025–1035.
31. **Norden, C. W., and M. Shaffer.** 1983. Treatment of experimental chronic osteomyelitis due to *Staphylococcus aureus* with vancomycin and rifampin. J. Infect. Dis. **147**:352–357.
32. **Piercy, E. A., D. Barbaro, J. P. Luby, and P. A. Mackowiak.** 1989. Ciprofloxacin for methicillin-resistant *Staphylococcus aureus* infections. Antimicrob. Agents Chemother. **33**:128–130.
33. **Rogers, D. E., and R. Tompsett.** 1952. The survival of staphylococci within human leukocytes. J. Exp. Med. **95**:209–212.
34. **Simon, G. L., R. H. Smith, and M. A. Sande.** 1983. Emergence of rifampin-resistant strains of *Staphylococcus aureus* during combination therapy with vancomycin and rifampin. Rev. Infect. Dis. **5**(Suppl. 3):S507–S508.
35. **Sorell, T. C., D. R. Packham, S. Shanker, M. Foldes, and R. Munro.** 1982. Vancomycin therapy for methicillin-resistant *Staphylococcus aureus*. Ann. Intern. Med. **97**:344–350.
36. **Sörgel, F., R. Metz, K. Naber, R. Seelman, and P. Muth.** 1988. Pharmacokinetics and body fluid penetration of fleroxacin in healthy subjects. J. Antimicrob. Chemother. **22**(Suppl. D):155–168.
37. **Tshefu, K., W. Zimmerli, and F. A. Waldvogel.** 1983. Short term rifampin administration in the prevention/eradication of foreign body infection. Rev. Infect. Dis. **5**(Suppl. 3):S474–S480.
38. **Van der Auwera, P., F. Meunier-Carpentier, and J. Klastersky.** 1983. Clinical study of combination therapy with oxacillin and rifampicin for staphylococcal infections. Rev. Infect. Dis. **5**(Suppl. 3):S515–S522.
39. **Wolff, M., B. Regnier, B. Pangon, E. Rouveix, M. J. Laisné, F. Vachon, C. Brun-Buisson, M. Rapin, R. Leclerq, C. J. Soussy, and J. Duval.** 1985. Utilisation des nouvelles quinolones chez 29 malades atteints de septicémies ou d'endocardites, p. 133–150. *In* J. J. Pocidalo, F. Vachon, and B. Regnier (ed.), Les nouvelles quinolones. Arnette, Paris.
40. **Zimmerli, W., P. D. Lew, and F. A. Waldvogel.** 1984. Pathogenesis of foreign body infections. Evidence of a local granulocyte defect. J. Clin. Invest. **73**:1191–1200.
41. **Zimmerli, W., F. Waldvogel, P. Vaudaux, and U. E. Nydegger.** 1982. Pathogenesis of foreign body infection: description and characteristics of an animal model. J. Infect. Dis. **146**:487–497.