ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, June 1990, p. 1014–1016
0066-4804/90/061014-03$02.00/0

Vol. 34, No. 6

Comparative Efficacies of Ciprofloxacin, Pefloxacin, and Vancomycin in Combination with Rifampin in a Rat Model of Methicillin-Resistant *Staphylococcus aureus* Chronic Osteomyelitis

R. DWORKIN,[1] G. MODIN,[1] S. KUNZ,[2] R. RICH,[2] O. ZAK,[2] AND M. SANDE[1]*

University of California, San Francisco, and The Medical Service, San Francisco General Hospital, 1001 Potrero Avenue, San Francisco, California 94110,[1] and Infectious Diseases, Pharmaceutical Department Research Laboratories, Ciba-Geigy, Ltd., Basel, Switzerland[2]

Received 13 December 1989/Accepted 15 March 1990

The efficacies of the quinolones ciprofloxacin and pefloxacin alone and in combination with rifampin were compared with those of vancomycin alone and in combination with rifampin in a rat model of chronic osteomyelitis caused by methicillin-resistant *Staphylococcus aureus*. Neither the quinolones nor vancomycin alone was effective in reducing titers of organisms in bone after therapy, while rifampin alone was effective. All combination regimens with rifampin were more effective than the regimen with rifampin alone was, although these differences did not achieve statistical significance. Rifampin-resistant isolates were detected rarely. Quinolone-rifampin combination regimens may offer a nonparenteral option for the treatment of chronic osteomyelitis caused by methicillin-resistant *S. aureus*.

Staphylococcus aureus remains an important pathogen in patients with acute and chronic osteomyelitis, diseases which traditionally require prolonged parenteral antimicrobial therapy and hospitalization. Methicillin-resistant strains have emerged as a particular problem in this regard, with a limited number of agents being active against these pathogens.

Acute staphylococcal osteomyelitis in children has been successfully treated with predominantly oral regimens (2, 7). Such regimens are being used increasingly in adults with acute and chronic staphylococcal osteomyelitis (1). An effective oral regimen for chronic osteomyelitis caused by methicillin-resistant *S. aureus* (MRSA) has yet to be proven, however.

Rifampin is an antimicrobial agent with important and unique properties in the treatment of this disease. It is highly active against *S. aureus*, including methicillin-resistant strains. It is well absorbed orally and is generally well tolerated, even in prolonged courses of therapy. It is bactericidal and penetrates well into bone and polymorphonuclear leukocytes. Monotherapy with this agent has been avoided, however, because animal data and clinical reports in humans suggest that resistant organisms may develop readily (14). Combination therapy with other antistaphylococcal agents may help prevent the emergence of rifampin-resistant organisms (4).

A companion drug for rifampin has therefore been sought. The new quinolones ciprofloxacin and pefloxacin have excellent bioavailability, few side effects, good bone penetration, and good activity against both methicillin-susceptible *S. aureus* and MRSA strains. Their mechanism of action is inhibition of bacterial DNA gyrase, while rifampin inhibits bacterial RNA polymerase. The targeting of different steps in bacterial metabolism may lead to synergistic antimicrobial activity, although in vitro rifampin has shown synergy, antagonism, or indifference (5) when used with other agents.

The rat model of chronic osteomyelitis has been described previously by Zak et al. (O. Zak, F. Zak, and R. Rich, Program Abstr. 21st Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 530, 1981), who worked with *S. aureus* and *Pseudomonas aeruginosa*. This model seems to produce a refractory infection (11) that closely mimics the situation of chronic osteomyelitis in the presence of a foreign body. For this reason we chose it to determine the efficacies of the quinolones ciprofloxacin and pefloxacin, either alone or in combination with rifampin, compared with those of the traditional agent vancomycin alone or in combination with rifampin in the therapy of osteomyelitis caused by MRSA.

MATERIALS AND METHODS

Animals. Male SPF RA 22 Madorin rats (weight, 165 to 200 g) were used.

Bacteria. *S. aureus* A68 is methicillin resistant, and the MIC of cloxacillin for this organism is 64 μg/ml. Bacterial inocula were prepared by taking 0.05 ml of a suspension containing $10^{8.1}$ to $10^{9.3}$ organisms of strain A68 that were prepared from cultures stored in liquid nitrogen.

MICs. MICs were determined by serial twofold drug dilution in brain heart infusion broth (BBL Microbiology Systems, Cockeysville, Md.) that was supplemented with 5% NaCl by using a standard inoculum of 10^6 CFU/ml and incubated at 37°C for 48 h. The MIC was defined as the lowest concentration of antibiotic that inhibited visible growth in relation to the control.

Procedure. Rats were anesthetized with pentobarbital (Nembutal; Abbott AG, Basel, Switzerland) 30 mg/kg intraperitoneally, and the left tibia was surgically exposed. A hole (diameter, 1 mm) was bored with a dental drill into the medullary cavity of the proximal tibia, and 0.05 ml of 5% sodium morrhuate (Torigian Laboratories, Queens Village, N.Y.) was injected, followed by 0.05 ml of the bacterial inoculum. The hole was plugged with dental gypsum (Contura, Zurich, Switzerland), and the wound was closed. Therapy was begun 22 to 30 days (mean, 25.6 days) after the procedure. This produced a chronic infection, as evidenced by the regrowth of organisms to a high titer in all treatment groups when they were examined at 1 or 2 months after the completion of therapy.

* Corresponding author.

Pharmacokinetics. Pharmacokinetic investigations were performed for ciprofloxacin, pefloxacin, vancomycin, and rifampin. A single dose of the drug was administered to groups of four animals each, which were then sacrificed at intervals and from which blood and bone samples were taken for determination of the antibiotic concentration. Bone samples from tibiae were weighed, pulverized in a Mixer Mill (Retsch, Haan, Federal Republic of Germany), suspended in sterile saline, and centrifuged. The concentrations of drug in the bone supernatant and plasma were determined by the agar well diffusion bioassay technique by using antibiotic medium 1 (Oxoid Ltd., London, England). *Bacillus subtilis* ATCC 6633 was the test strain for ciprofloxacin and pefloxacin, *B. subtilis* in 8993 was the test strain for vancomycin, and *S. aureus* 10B (a standard isolate) was the test strain for rifampin. Standard curves were developed by producing known concentrations of antibiotic in water for the bone standard curve and in rat plasma for the plasma standard curve. The inhibition zones were determined by using a QUANTIMET 720P (Cambridge Instruments, Herts, England).

Treatment. Treatment regimens were designed to achieve levels of drug in serum that would closely mimic those obtained in humans. All drugs were administered subcutaneously. Drug administration regimens are abbreviated as follows: b.i.d., every 12 h; q.d., every 24 h. All doses are expressed in milligrams per kilogram.

There were eight treatment groups: ciprofloxacin 30 mg/kg b.i.d.; ciprofloxacin 30 mg/kg b.i.d. with rifampin 20 mg/kg q.d.; pefloxacin 60 mg/kg b.i.d.; pefloxacin 60 mg/kg b.i.d. with rifampin 20 mg/kg q.d.; vancomycin 60 mg/kg b.i.d.; vancomycin 60 mg/kg b.i.d. with rifampin 20 mg/kg q.d.; rifampin 20 mg/kg q.d.; and untreated controls.

Treatment was continued for 21 to 30 days (mean, 24.2 days). Groups of animals treated identically were sacrificed at 4 days, 1 month, or 2 months after the termination of therapy or, for controls, after a period of time equal to that in treated animals. The left tibias were isolated, frozen in liquid nitrogen, and crushed in an autopulverizer. The bone particles were weighed and suspended in sterile saline, and bacterial titers determined by plating serial dilutions on brain heart infusion agar. Titers were expressed as $\log_{10}$ CFU per gram of bone. In addition, the presence of organisms resistant to the antimicrobial agents to which they were exposed was determined by plating bone that was prepared as described above on brain heart infusion agar impregnated with 100 μg of antibiotic per ml of agar for each antibiotic which the animals received.

Statistical analysis. Titers of organisms in bone were compared statistically by a Kruskal-Wallis one-way analysis of variance followed by the Mann-Whitney test with the Bonferroni correction for multiple comparisons. A nonparametric test was chosen because 13% of the data was expressed as less than a certain lower limit of detection.

RESULTS

MIC determinations. MIC determinations were as follows: ciprofloxacin, 1 μg/ml; pefloxacin, 2 μg/ml; vancomycin, 4 μg/ml; and rifampin, 0.5 μg/ml.

Pharmacokinetics. Pharmacokinetic data for ciprofloxacin, pefloxacin, vancomycin, and rifampin are given in Table 1. Rifampin had the longest half-life (5.5 h). Ciprofloxacin and pefloxacin had similar half-lives (approximately 2 h) in plasma, and had half-lives in bone that were slightly longer than their half-lives in plasma. Rifampin had the best bone penetration (highest peak bone/plasma ratio), as well as the highest peak bone level/MIC ratio.

TABLE 1. Pharmacokinetic data for ciprofloxacin, pefloxacin, vancomycin, and rifampin

Drug (dose [mg/kg])	Site	Peak level (mean)[a]	Half-life (h)
Ciprofloxacin (30)	Plasma	3.4	2.4
	Bone	1.7	2.5
Pefloxacin (60)	Plasma	12.1	2.8
	Bone	8.3	3.5
Vancomycin (60)	Plasma	42.2	1.4
	Bone	9.0	2.2
Rifampin (20)	Plasma	12.6	5.5
	Bone	19.1	5.0

[a] Levels in plasma are in micrograms per milliliter, and levels in bone are in micrograms per gram.

Results of therapy. Sterilization of bone was not achieved by any treatment regimen in this highly refractory model of chronic osteomyelitis. This was evidenced by the regrowth of organisms to a high titer (10^5 to 10^6) in all treatment groups, with no differences among groups at the time of sacrifice 1 or 2 months later (data not shown).

Table 2 compares titers of organisms in bone at 4 days posttherapy in the seven treatment groups and one control group. The only single-agent regimen that was effective in lowering titers compared with those in controls was rifampin alone ($P < 0.01$). All of the combination regimens with rifampin were also effective in lowering titers, including the combinations of ciprofloxacin with rifampin ($P < 0.002$), pefloxacin with rifampin ($P < 0.01$), and vancomycin with rifampin ($P < 0.002$). None of the combination regimens were significantly more effective than rifampin alone.

Development of resistance. Rifampin-resistant organisms were detected after therapy in only one animal, which was on the vancomycin-rifampin regimen, at a titer of $10^{1.33}$ at the time of sacrifice 4 days posttherapy. Resistant organisms were not detectable in this group at 1 or 2 months posttherapy. Rifampin resistance did not develop in the group treated with rifampin alone.

TABLE 2. Titers of antibiotics in bone 4 days after completion of therapy in groups of rats with osteomyelitis caused by *S. aureus* A68 (methicillin resistant)

Group[a]	No.	Mean ± SD titer ($\log_{10}$ CFU/g of bone)[b]	Comparison with controls (*P* value)
Control	24	6.10 ± 0.43	
P60	6	6.06 ± 0.26	NS[c]
CIP30	6	6.04 ± 0.38	NS
V60	8	5.43 ± 1.05	NS
R20	6	4.74 ± 0.60	<0.01
P60-R20	6	2.88 ± 1.25	<0.01
CIP30-R20	16	3.07 ± 1.15	<0.002
V60-R20	18	2.76 ± 1.49	<0.002

[a] Abbreviations: P60, pefloxacin 60 mg b.i.d.; CIP30, ciprofloxacin 30 mg b.i.d.; V60, vancomycin 60 mg b.i.d.; R20, rifampin 20 mg q.d. All drugs were administered subcutaneously.

[b] When data were less than a certain lower limit of detection, the next lowest number to the same number of significant figures was used.

[c] NS, Not significant at the $P < 0.05$ level.

DISCUSSION

The rat model of chronic osteomyelitis used in this experiment is a demanding one for testing an antimicrobial agent regimen, as evidenced by the lack of sterilization of bone with any regimen. It should not be surprising that there is difficulty in sterilizing bone in the presence of a foreign body with only 3 to 4 weeks of therapy. Although in the rabbit model of osteomyelitis some 14- to 28-day regimens have achieved complete sterilization, the rat model appears to be more refractory (9–11). The reasons for the greater refractoriness of the rat model are unclear, but they may be related to the longer period of time during which infection is allowed to establish itself prior to the beginning of therapy in the rat model as compared with that in the rabbit model.

Henry et al. (6) compared the efficacies of ciprofloxacin, vancomycin, and rifampin alone with those of ciprofloxacin or vancomycin in combination with rifampin in a rat model of chronic osteomyelitis caused by an MRSA isolate. They found that rifampin alone was able to reduce titers but that neither of the other single agents was effective. Vancomycin-rifampin was not statistically significantly more effective than rifampin alone, but ciprofloxacin-rifampin was significantly more effective than rifampin alone, but ciprofloxacin-rifampin was significantly more effective than the other regimens. The dose of ciprofloxacin used was higher than that used in our study, 50 instead of 30 mg/kg b.i.d. Our data support these findings. All of the combination regimens were effective in lowering the organism titers, including ciprofloxacin, pefloxacin, and vancomycin when they were used with rifampin. Probably because of the large number of comparisons that were made and the small sample size in some of the groups, no statistically significant differences were found between the rifampin group and the combination groups, even though the mean titer in the rifampin-treated group was almost 100-fold higher than those in the combination antibiotic-treated groups.

In summary, these data suggest that the quinolones ciprofloxacin and pefloxacin in combination with rifampin are as effective as vancomycin-rifampin for treatment of experimental chronic osteomyelitis caused by MRSA.

Resistance. A somewhat surprising finding was the absence of widespread rifampin resistance in the rifampin-treated groups of rats, with the only resistant organisms being found in one animal in the vancomycin-rifampin group. Norden (9), in a rabbit model of osteomyelitis caused by MRSA, found that all isolates from rabbits that received rifampin alone developed rifampin resistance during therapy, as did two of five strains from rabbits that received vancomycin-rifampin. However, the technique used in those studies was more apt to pick up rare resistant organisms than ours was. In the rabbit model, the entire marrow cavity is flushed and the undiluted supernatant is plated, whereas in our experiments a more quantitative approach is taken by grinding whole bone, diluting it, and plating small portions. Henry et al. (6), who used a rat model similar to ours, had findings similar to ours, with 2 of 34 rats treated with rifampin alone and 1 of 33 rats treated with vancomycin-rifampin developing rifampin resistance.

The finding of detectable rifampin resistance only in rats treated with the vancomycin-rifampin regimen is of concern, since this phenomenon has also been observed clinically in patients on this regimen (3, 13). In contrast, rifampin resistance was not seen to develop with the quinolone-rifampin regimens. Whether this truly represents a significant advantage of the quinolone regimens or simply a statistical aberration needs to be explored further. Recent reports (8, 12) of the increasing resistance of staphylococci to quinolones is of concern. Theoretically, the use of combination therapy with rifampin should help prevent the development of such resistance on therapy. In vitro data (4) support this, but more extensive in vivo studies are necessary to confirm it.

LITERATURE CITED

1. **Black, J., T. L. Hunt, P. J. Godley, and E. Matthew.** 1987. Oral antimicrobial therapy for adults with osteomyelitis or septic arthritis. J. Infect. Dis. **155**:968–972.
2. **Cole, W. G., R. E. Dalziel, and S. Leitl.** 1982. Therapy of acute osteomyelitis in childhood. J. Bone Joint Surg. **64B**:218–223.
3. **Eng, R. H., S. M. Smith, M. Tillem, and C. Cherubin.** 1985. Rifampin resistance; development during the therapy of methicillin-resistant *Staphylococcus aureus* infection. Arch. Intern. Med. **145**:146–148.
4. **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.
5. **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.
6. **Henry, N. S., M. Rouse, A. Whitesell, M. McConnell, and W. R. Wilson.** 1987. Treatment of methicillin resistant *S. aureus* experimental osteomyelitis with ciprofloxacin or vancomycin alone or in combination with rifampin. Am. J. Med. **82**(Suppl. 4A):73–75.
7. **Kolyvas, E., G. Ahronheim, M. I. Marks, R. Gledhill, H. Owen, and L. Rosenthall.** 1980. Oral antibiotic therapy of skeletal infections in children. Pediatrics **65**:867–871.
8. **Kotilainen, P., J. Nikoskelainen, and P. Huovinen.** 1990. Emergence of ciprofloxacin-resistant coagulase-negative staphylococcal skin flora in immunocompromised patients receiving ciprofloxacin. J. Infect. Dis. **161**:41–44.
9. **Norden, C.** 1983. Experimental chronic staphylococcal osteomyelitis in rabbits: therapy with rifampin alone or in combination with other antimicrobial agents. Rev. Infect. Dis. **5**(Suppl. 3):S491–S494.
10. **Norden, C. W., and M. Shaffer** 1983. Treatment of experimental chronic osteomyelitis due to *S. aureus* with vancomycin and rifampin. J. Infect. Dis. **147**:352–357.
11. **Rissing, J. P., T. B. Buxton, R. S. Weinstein, and R. K. Shockley.** 1985. Model of experimental chronic osteomyelitis in rats. Infect. Immun. **47**:581–586.
12. **Schaefler, S.** 1989. Methicillin-resistant strains of *Staphylococcus aureus* resistant to quinolones. J. Clin. Microbiol. **27**: 335–336.
13. **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: a report of two cases. Rev. Infect. Dis. **5**(Suppl. 3):S507–S508.
14. **Wehrli, W.** 1983. Rifampin: mechanisms of action and resistance. Rev. Infect. Dis. **5**(Suppl. 3):S407–S411.