ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Mar. 1987, p. 430–433
0066-4804/87/030430-04$02.00/0
Copyright © 1987, American Society for Microbiology
Vol. 31, No. 3

In Vitro and In Vivo Activity of Ciprofloxacin against Enterococci Isolated from Patients with Infective Endocarditis

MANUEL FERNANDEZ-GUERRERO,[1] MARK S. ROUSE,[2] NANCY K. HENRY,[2] JOSEPH E. GERACI,[2] AND WALTER R. WILSON[2]*

Fundacion Jimenez Diaz, Avda Reyes Catolicos, 2, Madrid, 3 Spain,[1] and Mayo Clinic and Foundation, Mayo Medical School, Rochester, Minnesota 55905[2]

Received 29 May 1986/Accepted 5 December 1986

In vitro activity of ciprofloxacin against 27 strains of enterococci was inoculum dependent. Using inocula of 10^5 to 10^6 or 10^7 to 10^8 CFU of enterococci per ml, the MICs for 50 and 90% of strains tested increased from 1 to ≥128 μg of ciprofloxacin per ml with the higher inoculum compared with the lower inoculum. The MBC for 50% of strains tested increased from 2 to >128 μg/ml and the MBC for 90% of strains tested increased from 8 to >128 μg of ciprofloxacin per ml with the lower and higher inocula, respectively. The combination of penicillin-gentamicin was more effective in vitro than the combination of ciprofloxacin-gentamicin against the low or high inoculum of enterococci. Using two strains of enterococci, we studied the efficacy of ciprofloxacin in the treatment of enterococcal experimental endocarditis in rabbits. Ciprofloxacin used alone or combined with gentamicin was significantly less effective ($P < 0.01$) than procaine penicillin alone or procaine penicillin combined with gentamicin for the treatment of enterococcal experimental endocarditis. The combination of ciprofloxacin-procaine penicillin was not a more effective therapy than procaine penicillin alone.

Enterococci are the third leading cause of infective endocarditis, accounting for 10 to 15% of the cases (14). Current antimicrobial therapy for patients with enterococcal endocarditis consists of penicillin or vancomycin combined with an aminoglycoside administered for 4 to 6 weeks. In our experience with the management of patients with enterococcal endocarditis, the frequency of streptomycin-associated vestibular toxicity was 19%, and the frequency of gentamicin-associated nephrotoxicity was 60% (15). High-level streptomycin resistance occurs in approximately 20 to 80% of enterococcal isolates, and high-level resistance to gentamicin may be increasing in frequency (8, 9, 11; D. Schaberg, S. Dembinski, M. Kish, and G. Power, Program Abstr. 24th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 510, 1984). Enterococci that are resistant to high concentrations of gentamicin are not killed synergistically by combinations of penicillin and the currently available aminoglycosides. Plasmid-mediated β-lactamase production by enterococci has been reported (10). These factors suggest that a less-toxic, alternative therapy for patients with enterococcal endocarditis needs to be evaluated.

Ciprofloxacin, a carboxyquinolone, is active in vitro against a wide array of aerobic and anaerobic bacteria and is reportedly bactericidal against enterococci (1, 3, 5, 7, 12; A. Weber, R. Scribner, W. Rollerson, and M. I. Marks, Abstr. Annu. Meet. Am. Soc. Microbiol. 1984, A62, p. 11). Accordingly, ciprofloxacin might be useful as a single-drug therapy for patients with enterococcal endocarditis. The purpose of this study was to determine the bactericidal effect of ciprofloxacin against enterococci in vitro and its efficacy in the treatment of enterococcal experimental endocarditis in rabbits.

(This study was presented in part at the 24th Interscience Conference on Antimicrobial Agents and Chemotherapy, 8–10 October 1984 [24th ICAAC, abstr. no. 275, 1984].)

* Corresponding author.

MATERIALS AND METHODS

In vitro studies. A total of 27 strains of enterococci isolated from patients with infective endocarditis were studied; 23 of these strains were *Streptococcus faecalis*, 3 strains were *Streptococcus faecium*, and 1 strain was *Streptococcus durans*. The enterococci were stored in defibrinated sheep blood at −70°C. Before testing each strain was thawed and subcultured on Trypticase (BBL Microbiology Systems, Cockeysville, Md.) soy agar containing 5% sheep blood. A total of 19 strains (70%) were susceptible to streptomycin, with a MIC of ≤2,000 μg/ml; 8 strains (30%) were highly resistant to streptomycin (MIC, >2,000 μg/ml). All strains were susceptible to ≤16 μg of gentamicin per ml.

A macrodilution method was used for susceptibility testing (13). Inocula were prepared from overnight broth cultures to yield a low inoculum (10^5 to 10^6 CFU/ml) or a high inoculum (10^7 to 10^8 CFU/ml) and inoculated into serial twofold dilutions of ciprofloxacin in dextrose-free Todd-Hewitt broth (pH 7.4) containing 0.1% L-cysteine. Subcultures were made for comfirmation of purity and for quantitation of the inoculum sizes. Tubes containing the inocula in serially diluted concentrations of ciprofloxacin were incubated for 24 h at 35°C in room air. The MIC was defined as the lowest concentration of ciprofloxacin in broth without visible growth of enterococci. The MBC was determined by subculturing 10 μl of broth from the control tube, with the first tube containing growth, and from all tubes without visible growth and was defined as the lowest concentration of antibiotic that killed ≥99.9% of the original inoculum. Tests for in vitro synergy were performed in triplicate by using the time-kill method with antibiotics singly or in combination with a low and a high inoculum with the two strains used for animal experimental endocarditis (12). Synergy was defined as at least a 100-fold increase in killing of enterococci by a combination of antibiotics compared with that achieved by an antibiotic singly.

TABLE 1. In vitro activity of ciprofloxacin combined with penicillin or gentamicin compared with penicillin combined with gentamicin

Antibiotic (concn [μg/ml])	Log_{10} CFU of enterococci per ml for:											
	Strain 1						Strain 2					
	Low inoculum			High inoculum			Low inoculum			High inoculum		
	0 h	4 h	24 h	0 h	4 h	24 h	0 h	4 h	24 h	0 h	4 h	24 h
Control	5.8	6.4	7.8	7.7	8.2	9.0	6.1	6.9	8.0	7.9	8.4	8.9
Gentamicin (3)	5.8	6.7	8.1	7.7	8.5	8.9	6.1	6.3	7.9	7.9	8.1	9.0
Penicillin (20)	5.8	5.5	4.9	7.7	7.5	7.2	6.1	5.5	4.9	7.9	7.8	7.8
Ciprofloxacin (1)	5.8	5.6	5.9	7.7	8.0	8.3	6.1	5.9	5.9	7.9	8.0	8.2
Ciprofloxacin (1)–penicillin (20)	5.8	5.2	5.0	7.7	7.9	8.0	6.1	5.7	5.2	7.9	8.1	7.6
Ciprofloxacin (1)–gentamicin (3)	5.8	4.9	3.1	7.7	7.5	7.5	6.1	5.0	3.4	7.9	7.3	7.1
Penicillin (20)–gentamicin (3)	5.8	4.2	Sterile	7.7	5.2	3.9	6.1	4.1	2.8	7.9	5.7	4.9

Animal studies. Experimental aortic valve endocarditis was established in 132 New Zealand white rabbits (weight, >2 kg) by modifications of the methods described by Garrison and Freedman (4). Briefly, animals were anesthetized with a mixture of ketamine-xylazene injected intramuscularly. An incision was made in the neck, and the right carotid artery was exposed. The artery was ligated distally, and a sterile polyethelene catheter (PE 90; Clay Adams) was inserted into the artery through a small incision and advanced proximally across the aortic valve into the left ventricle. A pressure-sensitive monitoring device was attached to the distal end of the catheter to ensure that the catheter tip crossed the aortic valve and entered the left ventricle. The end of the catheter was sealed and tied to the carotid artery, and the wound was closed over the catheter with surgical clips.

Two strains of *S. faecalis* were selected for experimental endocarditis from among the 27 strains studied. For strain 1 the MIC/MBC ratio with the lower or the higher inoculum was 0.5/1 and 128/>128 μg of ciprofloxacin per ml, respectively. For strain 2 the MIC/MBC with the lower inoculum was 1/4 μg of ciprofloxacin per ml, and with the higher inoculum the MIC/MBC was >128/>128 μg of ciprofloxacin per ml. Enterococci were inoculated into Todd-Hewitt broth, incubated overnight, and diluted 1:10. A 1-ml broth sample containing 10^7 to 10^8 CFU of enterococci per ml was injected into a peripheral ear vein 24 h after the insertion of the catheter. The presence of endocarditis was confirmed by a blood culture yielding enterococci obtained before the initiation of antimicrobial therapy.

Antimicrobial therapy was started 24 h after intravenous injection of enterococci and was administered for 3 days. After day 3 of treatment and at least 12 h after administration of the last dose of antimicrobial agent(s), animals were sacrificed by intravenous injection of sodium pentobarbital. The chest was opened, the heart was excised and opened, and the aortic valve vegetations were removed aseptically. The vegetations were weighed and homogenized with a sterile mortar and pestle. The entire vegetation was cultured, and the number of CFU of enterococci per gram of vegetation was quantitated by using a pour-plate method with agar containing bile and esculin as indicators. The results were expressed as the log_{10} CFU of enterococci per gram of valve vegetation.

Animals were placed into the following treatment groups: (i) control (10 animals that received no antimicrobial therapy), (ii) low-dose ciprofloxacin (20 animals that received ciprofloxacin [30 mg/kg] intramuscularly [i.m.] three times

TABLE 2. Results of treatment of enterococcal experimental endocarditis in rabbits

Antimicrobial agent (dose)	Strain 1		Strain 2	
	No. of animals	Mean log_{10} CFU/g of vegetation ± SD	No. of animals	Mean log_{10} CFU/g of vegetation ± SD
Control	5	10.4 ± 0.1	5	10.0 ± 0.1
Ciprofloxacin (30 mg/kg, i.m., TID)	10	8.4 ± 0.8[a,b]	10	8.8 ± 0.8[a,b]
Ciprofloxacin (50 mg/kg, i.m., TID)	12	8.8 ± 0.4[a,b]	12	8.5 ± 0.7[a,b]
Procaine penicillin (1.2 × 10^6 U, i.m., TID)	10	5.2 ± 1.0[b,c,d]	10	6.2 ± 1.1[b,c,d]
Procaine penicillin (1.2 × 10^6 U, i.m., TID) plus ciprofloxacin (30 mg/kg, i.m., TID)	5	6.3 ± 0.9[c,d]	5	6.2 ± 0.8[c,d]
Ciprofloxacin (30 mg/kg i.m., TID) plus gentamicin (1.05 mg/kg, i.m., TID)	12	5.5 ± 1.5[c]	12	7.9 ± 0.4[b,d]
Procaine penicillin (1.2 × 10^6 U, i.m., TID) plus gentamicin (1.05 mg/kg, i.m., TID)	12	3.9 ± 1.3[d]	12	4.2 ± 1.0[d]

[a] $P < 0.05$ for ciprofloxacin (30 or 50 mg/kg, i.m., TID) versus control.

[b] $P < 0.01$ for procaine penicillin (1.2 × 10^6 U, i.m., TID) versus ciprofloxacin (30 or 50 mg/kg, i.m., TID) or ciprofloxacin (30 mg/kg i.m.)-gentamicin (1.05 mg/kg, i.m., TID; strain 2).

[c] P is not significant for procaine penicillin (1.2 × 10^6 U, i.m., TID) versus procaine penicillin (1.2 × 10^6 U, i.m., TID)-ciprofloxacin (30 mg/kg, i.m., TID) or ciprofloxacin (30 mg/kg, i.m., TID)-gentamicin (1.05 mg/kg, i.m., TID; strain 1).

[d] $P < 0.01$ for procaine penicillin (1.2 × 10^6 U, i.m., TID)-gentamicin (1.05 mg/kg, i.m., TID) versus procaine penicillin (1.2 × 10^6 U, i.m., TID) or ciprofloxacin (30 mg/kg, i.m., TID)-gentamicin (1.05 mg/kg, i.m., TID) or procaine penicillin (1.2 × 10^6 U, i.m., TID)-ciprofloxacin (30 mg/kg, i.m., TID; 1.05).

daily [TID]), (iii) high-dose ciprofloxacin (24 animals that received ciprofloxacin [50 mg/kg] TID), (iv) penicillin alone (20 animals that received procaine penicillin [1.2×10^6 U] i.m. TID), (v) penicillin plus ciprofloxacin (10 animals that received procaine penicillin [1.2×10^6 U] i.m. TID plus ciprofloxacin [30 mg/kg] i.m. TID), (vi) ciprofloxacin plus gentamicin (24 animals that received ciprofloxacin [30 mg/kg] i.m. TID plus gentamicin [1.05 mg/kg] i.m. TID), (vii) penicillin plus gentamicin (24 animals that received procaine penicillin [1.2×10^6 U] i.m. TID plus gentamicin [1.05 mg/kg] i.m. TID).

Because of the large number of treatment groups, it was not possible to have an equal number of rabbits treated with each therapeutic regimen during each day of experiments. An effort was made to distribute the animals equally among the respective therapeutic regimens throughout the study period. The dosages of antimicrobial agents were selected because administration resulted in concentrations in serum in rabbits that were similar to those used to treat infections in humans.

On day 2 of therapy, 0.5 h after the administration of the fourth dosage of antibiotic, blood samples were obtained through a peripheral ear vein from all rabbits included in the study for measurement of antimicrobial concentrations in serum. Concentrations of penicillin in serum were determined by bioassay, concentrations of gentamicin in serum were measured by fluorescence polarization (6), and concentrations of ciprofloxacin in serum were meaured by bioassay by using *Klebsiella pneumoniae* ATCC 10031 (12). The mean concentration of gentamicin in serum at 0.5 h was 1.97 ± 0.3 μg/ml. In animals treated with 30 or 50 mg of ciprofloxacin per kg, the mean concentrations in serum at 0.5 h were 2.8 ± 0.4 and 5.9 ± 0.5 μg/ml, respectively. The mean concentration of penicillin in serum at 0.5 h was 15.6 ± 4.3 μg/ml.

Analysis of results. Differences in mean $\log_{10}$ CFU of enterococci per gram of vegetation were analyzed statistically by using the Kruskall-Wallis test and the Wilcoxon two-sample test, and the results were corrected for multiple comparisons (2).

RESULTS

By using an inoculum size of 10^5 to 10^6 CFU of enterococci per ml, the MIC of ciprofloxacin for 50% (MIC_{50}) and 90% (MIC_{90}) of strains tested was 1 μg/ml (range, 0.5 to 4 μg/ml); the MBC_{50} and MBC_{90} were 2 and 8 μg/ml, respectively (range, 1 to 16 μg/ml). With the larger inoculum size (10^7 to 10^8 CFU/ml), the MIC_{50} and MIC_{90} were 128 and >128 μg/ml, respectively (range, 16 to >128 μg/ml); the MBC_{50} and MBC_{90} were >128 μg/ml (range, 32 to >128 μg/ml).

The in vitro effect of the inoculum size of the two strains of enterococci used in the animal experiments on the effects of synergy testing is shown in Table 1. With the smaller inoculum the killing effect of ciprofloxacin in vitro was similar to that of penicillin, and both strains were killed synergistically by a combination of ciprofloxacin-gentamicin. The combination of ciprofloxacin-penicillin was no more effective in vitro than either antimicrobial agent alone.

With the larger inoculum, the activity of penicillin or ciprofloxacin in vitro was similar, but the magnitude of killing by either drug was less than that achieved against the smaller inoculum. While the combination of ciprofloxacin-gentamicin did not act synergistically in vitro against either strain of enterococci, the combination of penicillin-gentamicin killed both strains synergistically.

The results of treatment of enterococcal experimental endocarditis are shown in Table 2. Ciprofloxacin alone at either dosage or in combination with gentamicin (strain 2 only) was significantly less effective ($P < 0.01$) than that of procaine penicillin alone or the combination of procaine penicillin-gentamicin.

DISCUSSION

The in vitro bactericidal effect of ciprofloxacin in our study was highly inoculum dependent. Bauernfeind and Petermuller (1) found that the MICs of enterococci increased fourfold when tested with an inoculum of 5×10^6 CFU/ml compared with those obtained with an inoculum of 5×10^3 CFU/ml. In our study with an inoculum size similar to the larger inoculum used by Bauernfeind and Petermuller (1), ciprofloxacin was bactericidal at a concentration that was four times that of the MIC_{90}. When the inoculum size was increased to 10^7 to 10^8 CFU/ml, however, the MBC was ≥32 μg of ciprofloxacin per ml for all 27 strains tested. With the exception of enterococci, the inoculum size exerts a minimal effect on the in vitro activity of ciprofloxacin against other gram-positive or gram-negative microorganisms (3). The in vitro activity of ciprofloxacin against enterococci may also be strain dependent. The magnitude of killing in vitro by a combination of ciprofloxacin-gentamicin was greater against strain 1 than against strain 2.

It is possible that an in vivo inoculum effect occurred in our study of enterococcal experimental endocarditis. The number of enterococci in cardiac valve vegetations in untreated animals was approximately 10^{10} microorganisms per g of valve vegetation. This high concentration of enterococci may explain in part the relatively poor in vivo activity of ciprofloxacin alone or combined with gentamicin against enterococcal experimental endocarditis. The possibility is supported by the results of in vitro tests which demonstrated synergy with the combination of ciprofloxacin-gentamicin against a low inoculum of enterococci but no synergistic effect of this combination against a high inoculum of enterococci. The penetration of ciprofloxacin into experimental cardiac valve vegetations also may have influenced the in vivo activity of ciprofloxacin in our study. Additional data are necessary to clarify the role of these factors.

The results of our study suggest that the use of ciprofloxacin alone or combined with gentamicin or penicillin may be inferior to the use of penicillin combined with gentamicin for the treatment of enterococcal endocarditis. At this time we suggest that ciprofloxacin not be used for the treatment of enterococcal infections in humans. Because ciprofloxacin has variable activity against enterococci, however, additional studies of the efficacy of ciprofloxacin treatment of experimental infections with enterococci are justified to clarify the role of ciprofloxacin in the treatment of less severe infections caused by this microorganism.

LITERATURE CITED

1. **Bauernfeind, A., and C. Petermuller.** 1983. In vitro activity of ciprofloxacin, norfloxacin and naladixic acid. Eur. J. Clin. Microbiol. 2:111–115.
2. **Dixon, W. J., and F. J. Massa, Jr.** 1969. Introduction to statistical analysis, 3rd ed., p. 167–181. McGraw-Hill Book Co., New York.
3. **Forsgren, A.** 1985. Comparative in vitro activity of three new quinolone antibiotics against recent clinical isolates. Scand. J. Infect. Dis. 17:91–94.
4. **Garrison, P., and L. Freedman.** 1970. Experimental endo-

carditis. Staphylococcal endocarditis in rabbits resulting from placement of polyethylene catheter in right side of heart. Yale J. Biol. Med. **42**:394–410.

5. **Goossens, H., P. De Mol, H. Coignau, J. Levy, O. Grados, G. Ghysels, H. Innocent, and J. P. Butzler.** 1985. Comparative in vitro activity of azthreonam, ciprofloxacin, norfloxacin, ofloxacin, HR 810 (a new cephalosporin), RU 28965 (a new macrolide), and other agents against enteropathogens. Antimicrob. Agents Chemother. **27**:388–392.
6. **Jolley, M. E., S. D. Stroupe, K. S. Schwenzer, C. J. Wang, M. Lu-Steffes, H. D. Hill, S. R. Popelka, J. T. Holen, and D. M. Kelso.** 1981. Fluorescence polarization immunoassay. III. An automated system for therapeutic drug determination. Clin. Chem. **27**:1575–1579.
7. **King, A., K. Shannon, and I. Phillips.** 1985. The in vitro activities of enoxacin and ofloxacin compared with that of ciprofloxacin. J. Antimicrob. Chemother. **15**:551–558.
8. **Mederski-Samoraj, B. D., and B. E. Murray.** 1983. High-level resistance to gentamicin in clinical isolates of enterococci. J. Infect. Dis. **147**:751–757.
9. **Moellering, R. C. Jr., C. Wennersten, and A. N. Weinberg.** 1971. Studies on antibiotic synergism against enterococci. I. Bacteriologic studies. J. Lab. Clin. Med. **77**:821–828.
10. **Murray, B. E., and B. D. Moderski-Samoraj.** 1983. Transferable beta-lactamase. J. Clin. Invest. **72**:1168–1171.
11. **Sandiford, H. D., J. B. DeMain, and W. M. Kirby.** 1970. Antibiotic synergism of enterococci: relation to inhibitory concentrations. Arch. Intern. Med. **126**:255–259.
12. **Van Caekenberghe, D. L., and S. R. Pattyn.** 1984. In vitro activity of ciprofloxacin compared with those of other new fluorinated piperazinyl-substituted quinoline derivatives. Antimicrob. Agents Chemother. **25**:518–521.
13. **Washington, J. A., II (ed.).** 1985. Laboratory procedures in clinical microbiology, p. 691–735. Springer-Verlag. New York.
14. **Wilson, W. R., and J. E. Geraci.** 1985. Treatment of streptococcal endocarditis. Am. J. Med. **78**:128–137.
15. **Wilson, W. R., C. J. Wilkowske, A. J. Wright, M. A. Sande, and J. E. Geraci.** 1984. Treatment of streptomycin-susceptible and streptomycin-resistant enterococcal endocarditis. Ann. Intern. Med. **100**:816–823.