ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Oct. 1986, p. 528–531
0066-4804/86/100528-04$02.00/0

Vol. 30, No. 4

Efficacy of Ciprofloxacin in Experimental Aortic Valve Endocarditis Caused by a Multiply β-Lactam-Resistant Variant of *Pseudomonas aeruginosa* Stably Derepressed for β-Lactamase Production

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Received 5 February 1986/Accepted 8 May 1986

The emergence of multi-β-lactam resistance is a limiting factor in treating invasive *Pseudomonas* infections with newer cephalosporins. The in vivo efficacy of ciprofloxacin, a new carboxy-quinolone, was evaluated in experimental aortic valve endocarditis caused by a strain of *Pseudomonas aeruginosa* which is stably derepressed for β-lactamase production and is resistant to ceftazidime and multiple other β-lactam agents. A total of 51 catheterized rabbits with aortic catheters in place were infected with this strain and then received no therapy (controls), ceftazidime (75 mg/kg per day), or ciprofloxacin (80 mg/kg per day). Ciprofloxacin sterilized all blood cultures and significantly lowered vegetation densities of *P. aeruginosa* by day 2 of treatment versus controls ($P < 0.0005$) and animals receiving ceftazidime ($P < 0.0005$). This beneficial effect of ciprofloxacin was also noted on therapy days 6 and 11. Ciprofloxacin rendered most vegetations (85%) culture negative over the 11-day treatment period and achieved bacteriologic cure in 73% of animals ($P < 0.0005$ versus other therapy groups). Ciprofloxacin prevented bacteriologic relapse at 6 days posttherapy. No ciprofloxacin resistance was detected among *Pseudomonas* isolates from cardiac vegetations. Ciprofloxacin warrants further evaluation in vivo versus multi-drug-resistant gram-negative bacillary infections.

Recent experiences in the treatment of *Pseudomonas aeruginosa* endocarditis in humans and experimental animals have emphasized the difficulties in achieving cures with antibiotics alone, especially in left-sided valve involvement (13–15; K. Rajashekarasah, L. Bhatia, T. Price, J. Kowalski, D. McCulley, and C. Kallick, Program Abstr. 22nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 367, 1982). This has been related to either primary drug failures or development of resistance in vivo, resulting in recrudescent bacteremia and endocarditis (13–15).

One limiting factor in the therapy of endocarditis and other invasive pseudomonal infections has been in vivo development of antibiotic resistance that may cross class lines (e.g., β-lactams → aminoglycosides) (11, 19). We investigated the in vivo efficacy of ciprofloxacin, a new carboxy-quinolone with potent antipseudomonal activity, in experimentally induced *P. aeruginosa* endocarditis caused by a multiply β-lactam-resistant variant.

(This study was presented in part at the 26th Interscience Conference on Antimicrobial Agents and Chemotherapy, 29 September 1986, in New Orleans, La.)

MATERIALS AND METHODS

Organism. The *P. aeruginosa* strain (PA-48) used in this study is a ceftazidime-resistant mutant strain isolated from cardiac vegetations during the treatment of experimental endocarditis with ceftazidime monotherapy (3). The identification, serotyping, and rabbit serum resistance of the parental strain for PA-48 used to initially infect rabbits (PA-96) have been previously described (7). The resistant variant (PA-48) has been characterized in detail elsewhere (3; A. S. Bayer, D. C. Norman, D. Anderson, J. O. Morrison, and K. S. Kim, Program Abstr. 23rd Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 370, 1983). Briefly, PA-48 is a mutant of PA-96 that is stably derepressed for β-lactamase production which is inducible in the parental strain. The MICs/MBCs (micrograms per milliliter) for PA-48 at an inoculum of approximately 10^5 log-phase cells per ml in Mueller-Hinton broth are ceftazidime (128/128), cefoperazone (64/128), moxalactam (64/128), azlocillin (128/128), amikacin (2/2), and ciprofloxacin (0.195/0.195). The ciprofloxacin MIC and MBC remained at 0.195 μg/ml when testing was performed with PA-48 in stationary phase or at approximately 10^7 CFU/ml.

Induction and treatment of *Pseudomonas* endocarditis. A total of 51 female New Zealand White rabbits weighing 2 to 2.5 kg each were anesthetized with 50 mg of ketamine hydrochloride (Bristol Laboratories, Syracuse, N.Y.) given intramuscularly. The left ventricle was catheterized by the transcarotid→transaortic route with a polyethylene catheter as previously described (1). Each animal was then inoculated intravenously with approximately 10^8 CFU of PA-48 24 h after catheterization. Positive blood cultures for *P. aeruginosa* 24 h after inoculation were used as presumptive evidence of endocarditis (1). Macroscopic and bacteriologic data obtained at sacrifice provided ultimate confirmation of vegetative endocarditis. The catheter was left in place for the duration of the study; this created a form of endocarditis that is a severe test of antibiotic efficacy. The 51 rabbits were randomized to three therapy groups of 17 rabbits each: untreated controls; ceftazidime, 75 mg/kg per day (intramuscularly, in three divided doses); or ciprofloxacin, 80 mg/kg per day (intramuscularly, in two divided doses). The ceftazidime treatment group was included as an additional control in this study, to confirm in vivo stability of ceftazidime resistance during therapy with that agent. All therapy was begun 48 h after inoculation. The dose of ciprofloxacin was identical to that successfully used in other studies of exper-

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TABLE 1. Mean vegetation titers and vegetation sterilization in experimental *Pseudomonas* endocarditis

Treatment group	Day of antibiotic therapy									6 days after end of treatment[a]			Frequency of cure[b] (%)
	2			6			11						
	No. sac[c]	No. pos veg/total[d] (%)	Mean veg titers[e]	No. sac	No. pos veg/total	Mean veg titers	No. sac	No. pos veg/total	Mean veg titers	No. sac	No. pos veg/total	Mean veg titers	
Control	3	10/10 (100)	8.18 ± 0.43[f]	3	12/12	7.85 ± 0.67[f]	2	8/8	10.25 ± 0.26[f]				0/8[g]
Ciprofloxacin	4	1/12 (85)	2.25 ± 0.24[f]	3	4/11	3.56 ± 0.65[f]	3	0/11	2.0 ± 0.0[f]	2	0/8	2.0 ± 0.0[f]	8/12 (67)[g]
Ceftazidime	3	13/13 (100)	8.09 ± 0.42[f]	3	12/12	7.68 ± 0.73[f]	2	8/8	8.31 ± 0.43[f]	2	7/7	9.27 ± 0.37[f]	0/11[g]

[a] All untreated controls had died or been sacrificed by this day.
[b] Number of rabbits with all vegetations culture negative/total sampled.
[c] No. sac, Number of rabbits sacrificed.
[d] No. pos veg/total, Number of culture-positive vegetations/total sampled.
[e] Mean veg titers, Mean vegetation titers of *P. aeruginosa*, expressed as $\log_{10}$ CFU per gram ± standard error of the mean.
[f] $P < 0.0005$.
[g] $P < 0.005$.

imental *Pseudomonas* endocarditis in our laboratory (1a, 1b); also, this dosage regimen consistently produces supra-MBC serum levels for the infecting *Pseudomonas* strain at approximately 1 h postinjection. The ceftazidime dosage used in the present investigation was similar to that successfully used in treating experimental *Pseudomonas* endocarditis previously in our laboratory (2).

Sacrifices. Animals were sacrificed by rapid intravenous injection of 150 mg of sodium pentobarbital on day 2, 6, or 11 of therapy, or 6 days posttherapy to evaluate bacteriologic relapse. At sacrifice, aortic valve vegetations were individually excised, weighed, homogenized, and quantitatively cultured as previously described (2). No attempt was made to inactivate ceftazidime or ciprofloxacin in vegetation homogenates, as all sacrifices were performed at least 18 h after the final drug dose (16). Samples of each vegetation homogenate were also quantitatively cultured into Mueller-Hinton agar containing ceftazidime (50 μg/ml) or ciprofloxacin (8 μg/ml) depending on the therapy regimen. For ciprofloxacin-treated animals, this was to detect in vivo development of resistance to ciprofloxacin; for ceftazidime-treated animals, it was to confirm persistence of ceftazidime resistance in vivo for the duration of the study. The antibiotic concentrations chosen to detect ciprofloxacin or ceftazidime resistance are above the breakpoints for each drug (ceftazidime, 32 μg/ml; ciprofloxacin, 2 to 4 μg/ml [10; R. Tilton, Proc. 1st Int. Ciprofloxacin Workshop, 1985, in press.]). In addition, 20 selected colonies from antibiotic-free culture plates of animal receiving ciprofloxacin were retested for ciprofloxacin MIC and MBC by the broth macrodilution technique. In calculating mean bacterial densities in vegetations, culture-negative vegetations were considered to contain <2 $\log_{10}$ CFU/g based on average vegetation weight of 0.01 g (5). To delineate the relative proportions of ceftazidime-resistant and ceftazidime-susceptible isolates contained within individual vegetations, a resistance ratio was calculated (6). This is defined as the $\log_{10}$ of the ratio of the number of resistant organisms to the total number of organisms within a vegetation ($\log_{10}$ [number of resistant isolates/total number of isolates]).

Antibiotic levels. Antibiotic trough and peak levels were determined in serum samples obtained just before and 1 h after drug administrations on day 2 or 3 of therapy. Ceftazidime levels were determined by high-pressure liquid chromatography; ciprofloxacin levels were determined by bioassay. The indicator organism for the ciprofloxacin bioassay was *Klebsiella pneumoniae* ATCC 10031. The sensitivity of the assay for ceftazidime is 5 μg/ml; that for ciprofloxacin is 0.02 μg/ml. The day-to-day variation for the bioassay is 9.49%; that for high-pressure liquid chromatography is 1.4 to 5.1%.

SBTs. All serum bactericidal titers (SBTs) were determined in sera obtained at the 1-h-posttreatment period referred to above, with the microtiter method (12). The diluent was heat-inactivated (56°C for 30 min), freshly pooled normal rabbit serum. The SBT was defined as the highest serum dilution yielding zero colonies on subculture (>99.9% killing of the original inoculum [12]).

Statistical evaluation. The chi-square test with Yates correction factor was used for comparing differences in proportions between groups of animals; the two-tailed Student *t* test was used for comparing the $\log_{10}$ CFU per gram of vegetation in the different therapy groups. A *P* value of <0.05 was considered significant.

RESULTS

Mortality. Nine animals, four from the control group, three from the ciprofloxacin group, and two from the ceftazidime group, died within 12 h of inoculation and were not included in the data analysis. Mortality rates in the remaining animals, given as the number of animals dying before the assigned sacrifice date/total number analyzed, were as follows: controls, 5/13 (38%); ceftazidime, 4/15 (26%); ciprofloxacin, 2/14 (14%). There were no significant differences among these mortality rates.

Blood cultures. Blood cultures taken 24 h after inoculation were positive for *P. aeruginosa* in all 42 surviving animals, presumptively confirming induction of endocarditis (1). Blood cultures taken at time of sacrifice were uniformly positive in control animals and remained positive in 9 of the 11 animals (81%) receiving ceftazidime. In contrast, terminal blood cultures were negative in all 12 sacrificed recipients of ciprofloxacin. These frequencies of positive blood cultures were significantly lower in ciprofloxacin-treated animals than in controls or animals receiving ceftazidime ($P < 0.005$).

Densities of *P. aeruginosa* in vegetations. All vegetations from untreated controls ($n = 30$) yielded *P. aeruginosa* on cultures (no spontaneous cures). The range of bacterial densities in vegetations from these untreated controls (7 to 11 $\log_{10}$ CFU/g) was similar to those previously reported in this model (Table 1) (1–3, 7).

Ciprofloxacin exerted a rapid bactericidal effect in vivo. By day 2 of therapy, mean vegetation densities from ciprofloxacin-treated animals were <3 $\log_{10}$ CFU/g, as com-

pared with >8 $\log_{10}$ CFU/g for animals receiving no therapy or ceftazidime ($P < 0.0005$, ciprofloxacin versus the latter two regimens). This salutary effect of ciprofloxacin in reducing vegetation densities of *P. aeruginosa* was also seen at days 6 and 11 of therapy. Ciprofloxacin was also very effective at rendering individual vegetations culture negative (Table 1). Over the 11-day therapy period, 29 of 34 vegetations (85%) in animals given ciprofloxacin were culture negative versus 0 of 33 in animals receiving ceftazidime ($P < 0.0005$). Moreover, ciprofloxacin rendered all individual vegetations culture negative in 8 of 12 animals (67%) (Table 1).

At 6 days after discontinuing antibiotic therapy, all vegetations from ciprofloxacin-treated animals were culture negative; those from animals previously receiving ceftazidime were >7 $\log_{10}$ CFU/g (mean, >9 $\log_{10}$ CFU/g).

Persistence of ceftazidime resistance in vivo. Ceftazidime resistance was stable in vivo. Resistance ratios among *P. aeruginosa* isolated from cardiac vegetations were low at each sacrifice time (mean ± standard error of the mean = $-0.65 + 0.15$; range, 0.0 to -4.07). The low resistance ratios confirm that most of the bacterial population within vegetations remained ceftazidime resistant (6). There were no *P. aeruginosa* isolated on subculture plates containing 8 μg of ciprofloxacin per ml. In addition, in 17 of 20 isolates from antibiotic-free culture plates, retested for ciprofloxacin MIC and MBC, these values remained at pretherapy levels (0.195 μg/ml). For the remaining three isolates, the MIC and MBC were each 0.39 μg/ml.

SBTs. No serum bactericidal activity was detectable in six of nine serum samples from animals receiving ceftazidime; bactericidal activity was low in the remaining three samples (one each = undiluted, 1:2, and 1:4 SBTs). In contrast, all five serum samples from animals receiving ciprofloxacin exhibited bactericidal activity at ≥1:8 titer (two at 1:8, three at >1:128 titers).

Serum antibiotic levels. Serum ciprofloxacin levels at 1 h postdosage exceeded the MBC for the infecting *Pseudomonas* strain by greater than twofold in all determinations (mean ± standard error of the mean = 3.46 ± 1.01 μg/ml; range, 0.92 to 8.7 μg/ml). Such peak serum concentrations are attainable in humans given 500 to 1,000 mg of ciprofloxacin orally (20). Mean ceftazidime concentrations were 49 ± 8.3 μg/ml (range, 25 to 78 μg/ml). No ceftazidime or ciprofloxacin was detectable in serum samples obtained approximately 12 h postdosage (trough samples).

DISCUSSION

Over the last decade, a variety of β-lactamase-stable, extended-spectrum cephalosporins have been developed and put into clinical use, including cefoxitin, cefotaxime, moxalactam, cefoperazone, and ceftazidime (9). Recently, a number of reports have documented the emergence of resistance during therapy with these agents related to inducible or mutationally derepressed β-lactamase production (4, 11, 17, 19). A disturbing feature of the acquired resistance to these newer β-lactamase-stable cephalosporins has been the development of cross-resistance to multiple other β-lactam antibiotics, as well as the extension of resistance across antibiotic class lines to the aminoglycosides (11). Of note, this phenomenon has been most commonly observed among genera within the expanded spectrum of newer cephalosporins, especially *Enterobacter*, *Serratia* and *Pseudomonas* species (11, 19).

Infective endocarditis caused by *P. aeruginosa* would appear to be an ideal setting for development of such resistance to newer β-lactam antibiotics, because of the high in vivo inoculum within cardiac vegetations (>7 to 10 $\log_{10}$ CFU/g), as well as the possibility for subinhibitory drug levels to exist in certain parts of the vegetation (17). We have recently encountered such problems with emergence of resistance during therapy of experimental aortic valve *Pseudomonas* endocarditis (1a, 3). In one study (3), 5 animals of 27 (19%) receiving ceftazidime monotherapy or combined with amikacin developed refractory endocarditis with ceftazidime-resistant *Pseudomonas* variants isolated from cardiac vegetations. Detailed analyses of these strains showed that they were cross resistant to most β-lactams and stably derepressed for chromosomally mediated β-lactamases, with enzyme production rates 100 to 300 times the parental strain (A. S. Bayer, D. C. Norman, and K. S. Kim, Abstr. Annu. Meet. Am. Soc. Microbiol. 1985, A42, p. 8). Additionally, this β-lactamase was able to competitively and avidly bind to ceftazidime by nonhydrolytic mechanisms. Moreover, the mechanism of ceftazidime resistance appeared to be multifactorial, as a significant reduction in ceftazidime uptake was also delineated. We have shown that these β-lactam-resistant *Pseudomonas* isolates remain susceptible in vitro to the killing action of ciprofloxacin, a new carboxyquinolone agent (3). However, the in vivo efficacy of ciprofloxacin against such multiresistant organisms has not been documented. The present investigation examines the efficacy of ciprofloxacin in experimentally induced *Pseudomonas* endocarditis caused by a multiply β-lactam-resistant strain isolated from vegetations during the latter study above (3).

Ciprofloxacin was chosen as an important candidate agent to evaluate in this model of *Pseudomonas* endocarditis for several reasons. The MBC for this *P. aeruginosa* strain (PA-48) to ciprofloxacin remains the same (0.195 μg/ml) whether testing is performed at 10^5 or 10^7 inoculum, or whether testing is carried out at logarithmic or stationary growth phases (3). The higher inoculum and stationary growth phases may represent conditions extant within cardiac vegetations (1, 2, 8). Also, we have previously documented the excellent in vivo efficacy of ciprofloxacin in experimental right- and left-sided *Pseudomonas* endocarditis caused by the parental strain (PA-96) from which PA-48 was derived (1a, 1b).

The present study showed that: ciprofloxacin exerted a rapid bactericidal effect in vivo by sterilizing all blood cultures and reducing mean vegetation counts of *Pseudomonas* >5 $\log_{10}$ CFU/g below those seen in untreated controls and animals receiving ceftazidime; ciprofloxacin prevented bacteriologic relapse in animals evaluated at 6 days after discontinuation of therapy; ciprofloxacin effected an overall bacteriologic cure in two-thirds of animals receiving this agent; and no ciprofloxacin resistance developed in vivo. This lack of development of ciprofloxacin resistance in vivo was also noted in our previous studies of the efficacy of ciprofloxacin in experimental *Pseudomonas* endocarditis caused by the parental strain (PA-96) from which PA-48 was derived (1a, 1b).

With the increasing reports of multi-β-lactam and aminoglycoside resistances emerging during therapy with extended-spectrum cephalosporins, new quinolone antibiotics may become important components of the therapeutic arsenal. One note of caution, however, emanates from the recent study of Sanders, who observed β-lactam-quinolone cross resistances in laboratory-derived mutants of *K. pneumoniae*

(18). The frequency of such β-lactam-quinolone or β-lactam-aminoglycoside-quinolone cross resistance needs to be further delineated among clinically derived isolates.

ACKNOWLEDGMENTS

We thank Andrew Finn for performance of ceftazidime level determinations and Barbara Painter for performance of ciprofloxacin level determinations.

LITERATURE CITED

1. **Archer, G., and F. R. Fekety.** 1976. Experimental endocarditis due to *Pseudomonas aeruginosa*. I. Description of a model. J. Infect. Dis. **134**:1–7.
1a. **Bayer, A. S., I. K. Blomquist, and K. S. Kim.** 1986. Ciprofloxacin in experimental aortic valve endocarditis due to *Pseudomonas aeruginosa*. J. Antimicrob. Chemother. **17**:641–649.
1b. **Bayer, A. S., and K. S. Kim.** 1986. In vivo efficacy of azlocillin and amikacin versus ciprofloxacin with and without amikacin in experimental right-sided endocarditis due to *Pseudomonas aeruginosa*. Chemotherapy (Basel) **32**:364–373.
2. **Bayer, A. S., K. Lam, D. Norman, K. S. Kim, and J. Morrison.** 1985. Amikacin + ceftazidime therapy of experimental right-sided *Pseudomonas aeruginosa* endocarditis in rabbits. Chemotherapy (Basel) **31**:351–361.
3. **Bayer, A. S., D. Norman, and K. S. Kim.** 1985. Efficacy of amikacin and ceftazidime in experimental aortic valve endocarditis due to *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. **28**:781–785.
4. **Beckwith, D. G., and J. A. Jahre.** 1980. Role of a cefoxitin-inducible beta-lactamase in a case of breakthrough bacteremia. J. Clin. Microbiol. **12**:517–520.
5. **Carrizosa, J., and D. Kaye.** 1976. Antibiotic synergism in enterococcal endocarditis. J. Lab. Clin. Med. **88**:132–141.
6. **Chambers, H. F., C. J. Hackbarth, T. A. Drake, M. G. Rusnak, and M. A. Sande.** 1984. Endocarditis due to methicillin-resistant *Staphylococcus aureus* in rabbits: expression of resistance to β-lactam antibiotics *in vivo* and *in vitro*. J. Infect. Dis. **149**:894–903.
7. **Choi, C., A. S. Bayer, N. K. Fujita, K. Lam, L. B. Guze, and T. T. Yoshikawa.** 1983. Therapy of experimental *Pseudomonas* endocarditis with high dose amikacin and ticarcillin. Chemotherapy (Basel) **29**:303–312.
8. **Durack, D. T., and P. B. Beeson.** 1972. Experimental bacterial endocarditis. II. Survival of bacteria in endocardial vegetations. Br. J. Exp. Pathol. **53**:50–53.
9. **Hewitt, W. L.** 1983. The third-generation cephalosporins, p. 403–423. *In* J. S. Remington, M. N. Swartz (ed.), Current clinical topics in infectious diseases, 4th ed. McGraw-Hill Book Co., New York.
10. **National Committee for Clinical Laboratory Standards.** 1983. Standard method for dilution antimicrobial susceptibility tests for bacteria which grow aerobically. National Committee for Clinical Laboratory Standards, Villanova, Pa.
11. **Preheim, L. C., R. G. Penn, C. C. Sanders, R. V. Goering, and D. K. Giger.** 1982. Emergence of resistance to β-lactam and aminoglycoside antibiotics during moxalactam therapy of *Pseudomonas aeruginosa* infections. Antimicrob. Agents Chemother. **22**:1037–1041.
12. **Prober, C. G., S. S. Dougherty, K. L. Vosti, and A. S. Yeager.** 1979. Comparison of a micromethod for performance of the serum bactericidal test with the standard tube dilution method. Antimicrob. Agents Chemother. **16**:46–48.
13. **Reyes, M. P., W. J. Brown, and A. M. Lerner.** 1978. Treatment of patients with *Pseudomonas* endocarditis with high-dose aminoglycoside and carbenicillin therapy. Medicine (Baltimore) **57**:57–58.
14. **Reyes, M. P., and A. M. Lerner.** 1983. Current problems in the treatment of infective endocarditis due to *Pseudomonas aeruginosa*. Rev. Infect. Dis. **5**:304–321.
15. **Reyes, M. P., W. A. Palutke, R. F. Wylin, and A. M. Lerner.** 1973. *Pseudomonas aeruginosa* endocarditis in the Detroit Medical Center 1969–1972. Medicine (Baltimore) 51:73–194.
16. **Sande, M. A., and M. L. Johnson.** 1975. Antimicrobial therapy of experimental endocarditis caused by *Staphylococcus aureus*. J. Infect. Dis. **131**:367–375.
17. **Sanders, C. C.** 1983. Novel resistance selected by the new expanded spectrum cephalosporins: a concern. J. Infect. Dis. **147**:585–589.
18. **Sanders, C. C., W. E. Sanders, Jr., R. V. Goering, and V. Werner.** 1984. Selection of multiple antibiotic resistance by quinolones, β-lactams, and aminoglycosides with special reference to cross-resistance between unrelated drug classes. Antimicrob. Agents Chemother. **26**:797–801.
19. **Sanders, C. C., and W. E. Sanders, Jr.** 1983. Emergence of resistance during therapy with the newer β-lactam antibiotics: role of inducible β-lactamases and implications for the future. Rev. Infect. Dis. **5**:639–648.
20. **Tartaglione, T. A., A. C. Raffalovich, W. J. Poynor, A. Espinel-Ingroff, and T. M. Kerkering.** 1986. Pharmacokinetics and tolerance of ciprofloxacin after sequential increasing oral doses. Antimicrob. Agents Chemother. **29**:62–66.