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In Vitro Activity Of Ciprofloxacin, a New Carboxyquinoline Antimicrobial Agent

G. M. ELIOPOULOS,* A. GARDELLA, AND R. C. MOELLERING, JR.

Department of Medicine, New England Deaconess Hospital, and Harvard Medical School, Boston, Massachusetts 02215

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The in vitro activity of ciprofloxacin (Bay o 9867), a new carboxyquinoline antimicrobial agent, was compared with those of norfloxacin, nalidixic acid, and several other oral and parenteral antimicrobial agents. Ciprofloxacin was substantially more active than nalidixic acid or cinoxacin against all gram-negative bacteria tested. Virtually all strains of *Enterobacteriaceae* were inhibited by the new drug at concentrations of ≤0.125 μg/ml. Ciprofloxacin was more active than norfloxacin against *Klebsiella* sp., *Enterobacter* sp., and *Serratia marcescens*, and it was the most active agent tested against *Pseudomonas aeruginosa* (MIC_{90}, 0.5 μg/ml). The new drug also demonstrated significant activity against gram-positive cocci, inhibiting all strains of staphylococci at concentrations of ≤1.0 μg/ml. Ciprofloxacin was bactericidal at concentrations near the MIC against most isolates tested. Although stepwise increases in resistance were seen with *Escherichia coli* and *P. aeruginosa* during serial passage on plates containing incremental concentrations of the drug, significant resistance did not emerge during incubation of strains in broth containing concentrations of ciprofloxacin above the MBC.

Ciprofloxacin (Bay o 9867) is a recently developed carboxyquinoline antimicrobial compound which is structurally related to nalidixic acid (20). Although older drugs of this class, such as nalidixic acid or cinoxacin, are active against a wide range of gram-negative bacteria, they are relatively inactive against *Pseudomonas aeruginosa* and gram-positive cocci (6). The MICs of the older agents against most enteric gram-negative organisms relative to achievable drug concentrations are such that use of those drugs has been generally limited to the treatment of urinary tract infections (1). Several new quinoline derivatives, including norfloxacin (7, 8, 10, 11), AT-2266 (2, 9), and ofloxacin (14), are not only more potent than nalidixic acid against susceptible enteric gram-negative bacteria, but also demonstrate significant activity against organisms resistant to the older drugs, including *P. aeruginosa* and many gram-positive bacteria. The present study examines the in vitro activity of ciprofloxacin against routine clinical isolates of gram-negative bacteria and against selected gram-positive organisms in comparison with those of other orally or parenterally administered antimicrobial agents.

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MATERIALS AND METHODS

Bacterial strains. Gram-negative bacteria used in this study were routine clinical isolates recently collected in our hospital. Duplicate isolates from individual patients were excluded, but organisms were otherwise unselected. Strains of *Campylobacter jejuni* and routine gram-positive isolates had been collected earlier at the Massachusetts General Hospital. Penicillin-resistant pneumococci and viridans streptococci were obtained as previously reported (4).

Antimicrobial agents. Standard antimicrobial reference powders were provided by the following sources: ciprofloxacin, Miles Pharmaceuticals, West Haven, Conn.; norfloxacin, Merck Sharp & Dohme Research Laboratories, Rahway, N.J.; cephalexin, moxalactam, and cinoxacin, Eli Lilly & Co., Indianapolis, Ind.; and cloxacillin, Bristol Laboratories, Syracuse, N.Y. Amikacin sulfate was obtained from Bristol Laboratories. Tetracycline hydrochloride and nalidixic acid were purchased from Sigma Chemical Co., St. Louis, Mo. Sultamicillin was simulated using ampicillin and sulbactam susceptibility powders (Pfizer, Inc., Groton, Conn.) in a mixture of 1.6:1.0; activity was expressed in terms of the ampicillin component. Antibiotic solutions were prepared on the day of use.

Agar dilution susceptibility studies. Susceptibility testing was performed by a standard agar dilution technique (18) using Mueller-Hinton agar (BBL Microbiology Systems, Cockeysville, Md.), which was supplemented with 5% defibrinated sheep blood when testing streptococci. Brucella agar (Difco Laboratories, Detroit, Mich.) supplemented with 10% sheep blood was used for *C. jejuni*. Overnight cultures of test organisms in Mueller-Hinton broth (BBL), Todd-Hewitt broth (BBL; for streptococci), or thioglycolate medium (GIBCO Diagnostics, Madison, Wis.; for *C. jejuni*) were diluted in Mueller-Hinton broth to approximately 10^7 CFU/ml. Final inocula of approximately 10^4 CFU were applied to plates by means of a 32-prong inoculator. Plates were examined after 24 h of incubation at 37°C. *C. jejuni* was incubated in a microaerophilic atmosphere (Campy-Pak; BBL); other organisms were incubated in room air.

Broth dilution studies. Susceptibility to ciprofloxacin of six representative isolates from each of several bacterial species was determined by a broth dilution technique. Tubes containing serial twofold dilutions of ciprofloxacin in Mueller-Hinton broth were inoculated beneath the surface with log-phase suspensions of test organisms to yield a final inoculum of 5×10^5 to 10^6 CFU/ml. Tubes were swirled on a Vortex mixer after 20 h of incubation and reincubated for 4 h. MICs were determined by visual inspection for lack of turbidity. Samples of 0.01 ml were removed to antibiotic-free plates which were incubated for 24 h at 37°C. The MBC, as defined by a 99.9% reduction in the initial inoculum, was determined by the method of Pearson et al. (12), assuming a 5% pipetting error.

* Corresponding author.

Time-kill curve studies. The bactericidal activity of ciprofloxacin over time was examined in the following manner. Several 250-ml flasks containing desired quantities of the antimicrobial agent in 19 ml of Mueller-Hinton broth were prepared. Each flask was then inoculated with 1 ml of bacterial suspension, prepared by diluting an overnight culture of the test organism in fresh broth to yield an inoculum of approximately 10^5 CFU/ml. Samples of 0.5 ml were removed immediately, and serial 10-fold dilutions in normal saline were prepared for colony counts. Flasks were then incubated at 37°C without agitation. Further samples for colony counts were obtained at 4, 24, and 48 h.

Selection of resistant organisms. The method described by Tenney et al. (17) was used to determine whether organisms resistant to ciprofloxacin could be selected. Briefly, heavy inocula of *P. aeruginosa* ATCC 27853 or *Escherichia coli* ATCC 29522 were applied to agar plates containing the antimicrobial agent at a concentration equal to one-half the MIC. Colonies arising after 24 h were then serially transferred to plates containing twofold incremental concentrations of the drug until a concentration was reached which prevented further growth.

RESULTS

Agar dilution MICs. Results of agar dilution susceptibility studies are shown in Table 1. Ciprofloxacin was substantially more active than either nalidixic acid or cinoxacin against all gram-negative bacteria tested, inhibiting all but four strains of *Enterobacteriaceae* at concentrations of ≤0.125 μg/ml. The activities of ciprofloxacin and norfloxacin were comparable against most *Enterobacteriaceae*, but several strains of *Klebsiella pneumoniae*, *Enterobacter cloacae*, and *Serratia marcescens* were inhibited by significantly lower concentrations of ciprofloxacin. Ciprofloxacin was also the most active drug tested against *P. aeruginosa*, inhibiting 38 of 39 strains at concentrations of ≤0.5 μg/ml.

In contrast to nalidixic acid or cinoxacin, ciprofloxacin was active against both methicillin-susceptible and -resistant *Staphylococcus aureus*. Ciprofloxacin was more active than norfloxacin against the streptococcal isolates, including penicillin-resistant pneumococci and viridans streptococci. Against enterococci, the activity of ciprofloxacin was comparable to that of sultamicillin.

Broth dilution studies. MICs of ciprofloxacin against representative strains of *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *S. aureus*, and *Streptococcus faecalis* determined by a broth dilution technique were comparable to those determined by agar dilution. Ciprofloxacin was bactericidal at concentrations less than or equal to four times the MIC against 29 of 30 strains. Against one strain of *S. aureus*, the MBC of ciprofloxacin (16 μg/ml) was 16-fold the corresponding MIC.

Time-kill studies. At concentrations above the MIC, ciprofloxacin was bactericidal against one strain each of *E. coli* and *P. aeruginosa*. The bactericidal effect was sustained at concentrations of the drug as high as 1,000 μg/ml. The possibility that antibiotic carryover resulted in spuriously high levels of killing at high concentrations of the antimicrobial agent was excluded by washing these samples over a 0.45-μm Millipore filter with 5 ml of physiologic saline before counting.

There was no evidence of regrowth of either strain at 24 or 48 h of incubation in ciprofloxacin. Several colonies surviving after 48 h of incubation in various concentrations of the drug were retested for susceptibility to ciprofloxacin; none was found to have become resistant. The bactericidal activi-

TABLE 1. Comparative in vitro activity of ciprofloxacin against clinical isolates

Strain (no.)	Antibiotic	MIC (μg/ml) for the following % of strains:		MIC range (μg/ml)
		50	90	
E. coli (40)	Ciprofloxacin	≤0.06	≤0.06	≤0.06
	Norfloxacin	≤0.06	≤0.06	≤0.06–0.125
	Nalidixic acid	2	2	0.25–8
	Cinoxacin	2	2	0.5–16
	Cephalexin	4	8	2–8
	Tetracycline	2	128	0.25–≥256
	Sultamicillin	2	16	0.25–32
	Moxalactam	≤0.06	0.125	≤0.06–0.125
	Amikacin	1	2	0.25–4
K. pneumoniae (34)	Ciprofloxacin	≤0.06	≤0.06	≤0.06–0.125
	Norfloxacin	0.125	0.25	≤0.06–1.0
	Nalidixic acid	4	8	2–32
	Cinoxacin	4	8	2–16
	Cephalexin	4	8	2–8
	Tetracycline	2	4	0.5–32
	Sultamicillin	4	4	2–32
	Moxalactam	0.125	0.125	≤0.06–0.125
	Amikacin	1	2	0.5–2
Proteus mirabilis (40)	Ciprofloxacin	≤0.06	≤0.06	≤0.06–0.125
	Norfloxacin	≤0.06	0.125	≤0.06–1.0
	Nalidixic acid	4	8	2–64
	Cinoxacin	4	4	1–16
	Cephalexin	8	16	8–128
	Tetracycline	64	128	2–128
	Sultamicillin	1	1	0.25–8
	Moxalactam	≤0.06	0.125	≤0.06–0.25
	Amikacin	4	8	1–16
Proteus vulgaris (10)	Ciprofloxacin	≤0.06	0.125	≤0.06–0.125
	Norfloxacin	≤0.06	≤0.06	≤0.06–0.125
	Nalidixic acid	2	2	2–4
	Cinoxacin	2	4	1–4
	Cephalexin	≥256	≥256	128–≥256
	Tetracycline	32	128	4–128
	Sultamicillin	8	16	2–16
	Moxalactam	≤0.06	0.125	≤0.06–0.25
	Amikacin	1.0	2	0.5–2
Morganella morganii (10)	Ciprofloxacin	≤0.06	≤0.06	≤0.06
	Norfloxacin	≤0.06	≤0.06	≤0.06
	Nalidixic acid	2	2	1.0–2
	Cinoxacin	1.0	2	1.0–2
	Cephalexin	≥256	≥256	16–≥256
	Tetracycline	2	4	2–128
	Sultamicillin	16	16	1.0–16
	Moxalactam	≤0.06	0.125	≤0.06–0.25
	Amikacin	4	4	1.0–8
E. cloacae (39)	Ciprofloxacin	≤0.06	≤0.06	≤0.06–0.25
	Norfloxacin	0.25	0.5	≤0.06–16
	Nalidixic acid	4	4	2–64
	Cinoxacin	4	8	2–128
	Cephalexin	≥256	≥256	32–≥256
	Tetracycline	4	4	1.0–32
	Sultamicillin	16	64	2–128
	Moxalactam	≤0.06	8	≤0.06–16
	Amikacin	1.0	1.0	0.5–2
Enterobacter aerogenes (25)	Ciprofloxacin	≤0.06	≤0.06	≤0.06
	Norfloxacin	0.125	0.125	≤0.06–0.125
	Nalidixic acid	4	4	2–16
	Cinoxacin	4	8	4–16
	Cephalexin	128	≥256	8–≥256
	Tetracycline	2	4	2–16

TABLE 1—*Continued*

Strain (no.)	Antibiotic	MIC (μg/ml) for the following % of strains: 50	90	MIC range (μg/ml)
	Sultamicillin	32	32	1.0–64
	Moxalactam	0.125	4	≤0.06–8
	Amikacin	1.0	2	0.5–2
Citrobacter freundii (24)	Ciprofloxacin	≤0.06	≤0.06	≤0.06–0.125
	Norfloxacin	≤0.06	0.125	≤0.06–0.25
	Nalidixic acid	4	8	2–16
	Cinoxacin	4	8	2–64
	Cephalexin	64	≥256	8–≥256
	Tetracycline	2	2	1–≥256
	Sultamicillin	8	64	2–64
	Moxalactam	≤0.06	4	≤0.06–16
	Amikacin	1.0	2	1.0–2
S. marcescens (20)	Ciprofloxacin	≤0.06	1.0	≤0.06–2
	Norfloxacin	0.125	4	≤0.06–8
	Nalidixic acid	2	≥256	0.5–≥256
	Cinoxacin	8	≥256	8–≥256
	Cephalexin	≥256	≥256	≥256
	Tetracycline	128	≥256	16–≥256
	Sultamicillin	32	256	16–≥256
	Moxalactam	0.25	16	≤0.06–32
	Amikacin	2	2	0.5–2
P. aeruginosa (39)	Ciprofloxacin	0.25	0.5	≤0.06–2
	Norfloxacin	1.0	1.0	0.25–8
	Nalidixic acid	64	128	32–≥256
	Cinoxacin	≥256	≥256	128–≥256
	Cephalexin	≥256	≥256	≥256
	Tetracycline	32	32	8–32
	Sultamicillin	≥256	≥256	64–≥256
	Moxalactam	16	32	8–32
	Amikacin	4	16	2–32
C. jejuni (10)	Ciprofloxacin	0.25	0.5	0.125–0.5
	Norfloxacin	1.0	2	0.25–2
S. aureus (methicillin susceptible) (10)	Ciprofloxacin	0.5	0.5	0.25–0.5
	Norfloxacin	1.0	2	0.5–2
	Nalidixic acid	32	32	16–32
	Cinoxacin	128	≥256	64–≥256
	Cephalexin	4	4	2–8
	Tetracycline	0.25	0.5	0.125–0.5
	Sultamicillin	1.0	1.0	0.25–2
	Cloxacillin	0.125	0.25	≥0.06–0.25
	Moxalactam	8	8	4–8
S. aureus (methicillin resistant) (10)	Ciprofloxacin	0.5	1.0	0.5–10
	Norfloxacin	1.0	2	0.5–2
	Nalidixic acid	32	32	32
	Cinoxacin	≥256	≥256	128–≥256
	Cephalexin	≥256	≥256	128–≥256
	Tetracycline	0.5	0.5	0.125–0.5
	Sultamicillin	16	16	8–16
	Cloxacillin	16	16	0.5–16
	Moxalactam	128	≥256	128–≥256
Staphylococcus epidermidis (10)	Ciprofloxacin	0.25	0.25	0.125–0.25
	Nalidixic acid	64	64	32–64
	Cephalexin	16	64	1.0–64
	Tetracycline	2	128	0.25–128
	Sultamicillin	2	4	0.25–4
	Cloxacillin	4	128	0.125–128
	Moxalactam	64	128	4–128

TABLE 1—*Continued*

Strain (no.)	Antibiotic	MIC (μg/ml) for the following % of strains: 50	90	MIC range (μg/ml)
Streptococcus pneumoniae (penicillin resistant) (9)	Ciprofloxacin	0.5	1.0	0.5–2
	Norfloxacin	4	8	2–16
	Naladixic acid	≥256	≥256	≥256
Viridans streptococci (penicillin susceptible (8)	Ciprofloxacin	0.5	0.5	0.25–1.0
	Norfloxacin	4	8	1.0–8
	Nalidixic acid	128	≥256	64–≥256
Viridans streptococci (penicillin resistant) (10)	Ciprofloxacin	0.5	4	0.25–4
	Norfloxacin	4	16	2–32
	Nalidixic acid	≥256	≥256	≥256
Group B streptococci (10)	Ciprofloxacin	0.5	1.0	0.5–1.0
	Norfloxacin	2	4	2–4
	Nalidixic acid	≥256	≥256	≥256
	Cinoxacin	≥256	≥256	≥256
	Cephalexin	4	4	1.0–8
	Tetracycline	32	64	0.25–64
	Sultamicillin	0.125	0.125	≤0.06–0.125
	Cloxacillin	1.0	1.0	0.5–2
S. faecalis (10)	Ciprofloxacin	1.0	2	0.5–2
	Norfloxacin	4	8	2–8
	Nalidixic acid	≥256	≥256	≥256
	Cinoxacin	≥256	≥256	128–≥256
	Cephalexin	≥256	≥256	32–≥256
	Tetracycline	2	128	1.0–128
	Sultamicillin	1.0	2	0.5–2
	Cloxacillin	64	128	64–128
	Moxalactam	≥256	≥256	≥256
S. faecium (10)	Ciprofloxacin	4	8	1.0–16
	Norfloxacin	4	8	2–32
	Nalidixic acid	≥256	≥256	≥256
	Cinoxacin	≥256	≥256	≥256
	Cephalexin	≥256	≥256	≥256
	Tetracycline	64	≥256	1.0–≥256
	Sultamicillin	8	8	2–8
	Cloxacillin	≥256	≥256	≥256
	Moxalactam	≥256	≥256	≥256

ties of ciprofloxacin, norfloxacin, and nalidixic acid against these bacterial strains were compared after 4 h of incubation (Fig. 1). The maximum bactericidal effect of nalidixic acid against the *E. coli* isolate occurred at concentrations of 10 and 100 μg/ml. In contrast, ciprofloxacin and norfloxacin demonstrated sustained bactericidal activities to the highest concentrations tested.

Stepwise selection of resistance. By serial passage of *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 on agar plates containing twofold incremental concentrations of ciprofloxacin or norfloxacin, colonies of each strain were selected which were substantially more resistant than the initial strain. Resistance to both drugs at the highest concentrations used (64 μg/ml) was readily produced in the *P. aeruginosa* strain. With the *E. coli* strain, resistance beyond 8 μg of ciprofloxacin per ml or 1.0 μg of norfloxacin per ml was not observed.

DISCUSSION

Ciprofloxacin, like several other recently developed nalidixic acid analogs (8, 9, 14), was found to be highly active

against a broad range of gram-negative bacteria, including *P. aeruginosa*. Only 4 of approximately 250 strains of *Enterobacteriaceae* tested failed to be inhibited by concentrations of ≤0.125 μg of ciprofloxacin per ml.

In contrast to nalidixic acid, ciprofloxacin was active against several species of gram-positive organisms. Interestingly, the drug was slightly less active against penicillin-resistant viridans streptococci than it was against penicillin-susceptible strains and against *Streptococcus faecium* in comparison with *S. faecalis*. Thus, the activity of ciprofloxacin appeared to parallel that of penicillin against these streptococci. Why this should occur is unclear since relative resistance to penicillin in viridans streptococci appears to be due to alterations in penicillin-binding proteins (4a), whereas nalidixic acid and its analogs are thought to exert their antibacterial effects by inhibition of the enzyme DNA-gyrase (5). Although permeability mutants with increased resistance to both nalidixic acid and β-lactam antibiotics have been described in gram-negative bacteria (13), no evidence of permeability barriers has been found in enterococci (19). An alternative explanation for these observations is that genetic determinants of relative ciprofloxacin resistance in streptococci are linked to those mediating penicillin resistance in these strains.

Ciprofloxacin was bactericidal at concentrations near the MIC against 29 of 30 strains tested. We found no evidence of a "paradoxical" bactericidal effect of this drug analogous to that which has been described previously (and confirmed in this study) with nalidixic acid (3).

Since strains resistant to nalidixic acid have been noted to emerge during therapy of urinary tract infections with this agent (16), consideration must be given to the ease with which bacterial resistance to the newer quinoline derivatives develops. By serial passage on plates containing incremental concentrations of ciprofloxacin, we were able easily to select colonies of *P. aeruginosa* ATCC 27853 which were resistant to the highest concentration of the antimicrobial tested (64 μg/ml). In contrast, we were unable to select colonies of *E. coli* ATCC 25922 which were capable of growth on plates containing 8 μg of ciprofloxacin per ml. These results are similar to those of Tenney et al. (17), who were able to select resistance to norfloxacin in *E. coli* and *P. aeruginosa* to concentrations as high as 8 and ≥256 μg/ml, respectively.

In view of its broad range of activity against a variety of gram-negative and gram-positive organisms, ciprofloxacin appears to be a potentially useful agent in the treatment of bacterial urinary tract infections or enteritis due to *C. jejuni*. In addition, because it is substantially more potent than the previously available quinoline antimicrobial agents, it is possible that achievable serum or tissue concentrations of ciprofloxacin may be adequate to permit use of the drug for infections beyond the urinary or gastrointestinal tracts.

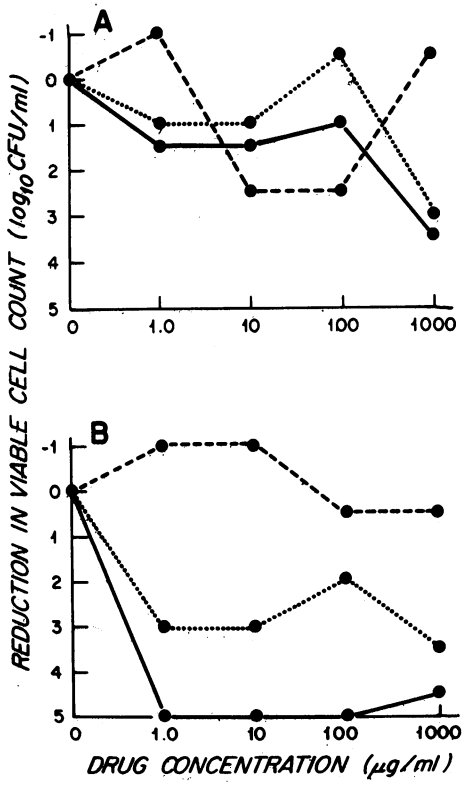

FIG. 1. Bactericidal activity of quinoline derivatives by time-kill studies. Bactericidal activities of ciprofloxacin (————), norfloxacin (· · · · · · ·), and nalidixic acid (– – – – –) were determined in broth cultures of *E. coli* (A) and *P. aeruginosa* (B) after 4 h of incubation.

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