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In Vitro Activity of CI-919 (AT-2266), an Oral Antipseudomonal Compound

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We tested CI-919 (AT-2266), a nalidixic acid analog, against 555 gram-positive and gram-negative bacteria, using microbroth or agar dilution methods. The activity of CI-919 was compared with those of cephalosporins, tobramycin, ticarcillin, dicloxacillin, rifampin, chloramphenicol, ampicillin, and trimethoprim-sulfamethoxazole. The minimal inhibitory concentrations of CI-919 for 90% of isolates were (in micrograms per milliliter): *Pseudomonas* spp. (including *Pseudomonas aeruginosa*), 4.0; *Enterobacteriaceae*, 0.5; *Staphylococcus* spp., 2.0; *Haemophilus influenzae*, 0.12; *Campylobacter jejuni*, 0.12; and enterococci, 16. The minimal inhibitory concentrations of CI-919 for 90% of 82 tobramycin-resistant, gram-negative strains was 4.0 μg/ml. CI-919 was bactericidal for most isolates, showing no cross-resistance with unrelated antimicrobial agents, and was stable for 11 weeks at temperatures ranging from 22 to −70°C. Inoculum size and media pH had little effect on the antibacterial activity of CI-919 for nine strains tested. CI-919 may be useful as an oral antibiotic for the treatment of infections due to diverse bacteria, including *P. aeruginosa*.

Current therapy of *Pseudomonas aeruginosa* infections requires parenteral administration of aminoglycosides, semisynthetic penicillins, or one of the newer broad-spectrum cephalosporins. The development of a drug that is effective after oral administration would represent a major therapeutic advance.

CI-919 (AT-2266; 1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-1,8-naphthyridine-3-carboxylic acid) is a new antimicrobial agent structurally related to nalidixic acid and pipemidic acid (Fig. 1). Preliminary reports have indicated that CI-919 is active against both gram-positive and gram-negative bacteria, including *P. aeruginosa* and aminoglycoside-resistant organisms (4, 5, 8). We previously showed that CI-919 was more active than tobramycin against systemic *P. aeruginosa* infections in mice (S. A. Chartrand, R. K. Scribner, M. I. Marks, and D. F. Welch, Cystic Fibrosis Club Abstr. 22nd, San Francisco, Calif., p. 45, 1981). We now report the results of our in vitro studies on the antimicrobial activity and stability of CI-919.

MATERIALS AND METHODS

Samples of CI-919 were supplied by Dainippon Pharmaceutical Co., Osaka, Japan. Other antimicrobial agents tested were obtained from the manufacturers. Stock solutions of the antimicrobial agents were either used immediately or stored at −70°C for up to 3 weeks.

Bacterial isolates were obtained from patients at Oklahoma Children's Memorial Hospital, Oklahoma City, and Montreal Children's Hospital, Montreal, Quebec, Canada, and from a stock collection of clinical and environmental isolates kindly provided by J. Flournoy, Veteran's Administration Hospital, Oklahoma City, Okla. *P. aeruginosa* (59 isolates), *Staphylococcus aureus* (33 isolates), and nontypable *Haemophilus influenzae* (3 isolates) were obtained from sputum cultures of patients with cystic fibrosis. Fifteen tobramycin-resistant organisms were provided by Michael T. Kelly, University of Texas Medical Branch, Galveston.

Minimal inhibitory concentrations (MICs) for all organisms except *H. influenzae* and *Campylobacter jejuni* were determined by the microbroth dilution method with Mueller-Hinton broth adjusted to a pH of 7.2 to 7.4. Calcium and magnesium concentrations were adjusted to 75 and 25 mg/liter, respectively, for gram-negative isolates. Preparation and inoculation of plates was carried out with the MIC-2000 system (Dynatech Laboratories Inc., Alexandria, Va.). Plates were used immediately or stored at −70°C for no longer than 2 weeks. Microbroth plates received an inoculum of approximately 10^5 CFU/ml. Plates were incubated for 18 h at 35°C in ambient air. The MIC was defined as the lowest concentration of drug that prevented visible growth. The minimal bactericidal concentration for each of 495 isolates was defined as the lowest concentration of drug that prevented growth after subculture of 1.5 μl from each well of the

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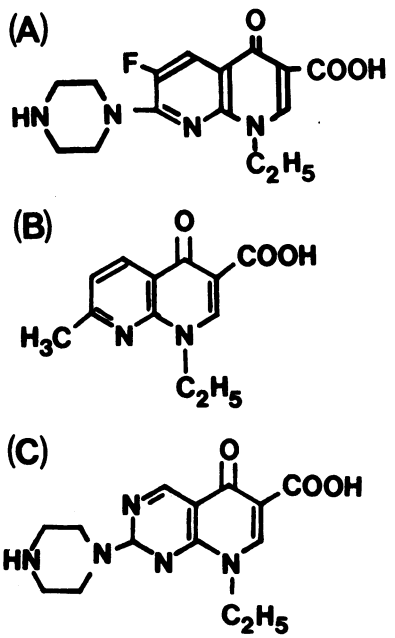

FIG. 1. Chemical structure of CI-919 (A), nalidixic acid (B), and pipemidic acid (C).

microbroth plate onto antibiotic-free Mueller-Hinton agar or sheep blood agar and overnight incubation at 35°C (99% killing rate). Control organisms (*Escherichia coli* ATCC 25922 and *S. aureus* ATCC 29213) were included in all sets of inoculations, and the MICs obtained were consistent within one twofold dilution of established values.

The MIC of each antibiotic for *Haemophilus* strains was determined by the twofold dilution method with Mueller-Hinton agar plus 1% supplement C (Difco Laboratories, Detroit, Mich.) The inoculum consisted of approximately 10^4 CFU, prepared from a log-phase culture and delivered to the agar surface with a Steers replicator. The plates were incubated for 18 h in ambient air at 35°C. The MIC was defined as the lowest concentration of drug at which no visible growth occurred.

Susceptibility testing of *C. jejuni* was performed by a modification of the standard agar dilution method. The inoculum was prepared from a 48-h culture in Mueller-Hinton broth. This suspension was adjusted to the density of a McFarland no. 1 turbidity standard. Approximately 10^5 CFU were delivered with a Steers replicator to the surface of Mueller-Hinton agar plates containing twofold dilutions of antimicrobial agents. The plates were then incubated in an atmosphere of 5% oxygen–10% carbon dioxide–85% nitrogen at 35°C for 48 h (1). The MIC was defined as the lowest concentration of antibiotic that completely inhibited growth.

The stability of CI-919 was tested as follows. Duplicate microbroth plates containing broth dilutions of CI-919 from 128 to 0.12 μg/ml were prepared, covered, and stored in the dark at 22, 4, −20, and −70°C for up to 11 weeks. At weekly intervals, a single plate from each group was inoculated with an *S. aureus* ATCC 29213 suspension of 10^5 CFU/ml as described above. Plates were incubated overnight at 35°C, and the MIC was determined as previously described.

The effect of inoculum density was studied with nine strains, three each of *P. aeruginosa*, *E. coli*, and *S. aureus*. Microbroth plates containing serial dilutions of CI-919 (pH 7.2) were inoculated in duplicate with 10-fold dilutions of each strain at organism densities ranging from 10^3 to 10^7 CFU/ml (quantified by subculture). The plates were incubated at 35°C overnight, and the MICs were determined as described above.

The effect of variation in medium pH was studied in the same nine isolates at a constant inoculum density of 10^5 CFU/ml. Peptone broth (2%) was adjusted to a pH of 5, 6, 7, 8, or 9 by the addition of K_2HPO_4 or KH_2PO_4. Serial dilutions of CI-919 in the various broths were prepared and added to microbroth plates, and MICs were determined as described above.

RESULTS

CI-919 was the most active agent tested against all *P. aeruginosa* strains, including 11 tobramycin-resistant cystic fibrosis isolates (MIC, ≥8.0 μg/ml) and six non-cystic fibrosis isolates selected for their high level of multiple-antibiotic resistance (Table 1). *Pseudomonas cepacia* isolates (50% MIC, ≤0.12 μg/ml) were somewhat more susceptible to CI-919 than were *Pseudomonas maltophilia* (50% MIC, 2.0 μg/ml).

CI-919 was also the most active agent against 11 multiply resistant *Serratia* strains. A total of 90% of *Achromobacter*, *Acinetobacter*, and *Flavobacterium* spp. were inhibited by ≤4.0 μg of the drug per ml. All 30 *Yersinia enterocolitica* isolates were inhibited by ≤0.12 μg of CI-919 per ml. Eight *Shigella* isolates that were resistant to chloramphenicol (MIC, 4.0 μg/ml) and seven that were resistant to ampicillin (MIC, >128 μg/ml) were inhibited by CI-919 at a concentration of ≤0.12 μg/ml. Of 82 tobramycin-resistant, gram-negative isolates (median MIC, 16 μg/ml), 90% were inhibited by ≤4.0 μg of CI-919 per ml.

All *H. influenzae* isolates (16 β-lactamase positive and 18 β-lactamase negative) were inhibited by CI-919 at a concentration of 0.12 μg/ml. Four strains were resistant to both ampicillin and chloramphenicol. Two *Staphylococcus epidermidis* strains that were resistant to dicloxacillin (MICs, 4 and 8 μg/ml, respectively) were susceptible to CI-919 (MIC, 0.5 μg/ml).

Table 2 shows a comparison of the in vitro activity of CI-919 with those of the related compounds norfloxacin and nalidixic acid for selected isolates. CI-919 was similar to norfloxacin in activity, and both were markedly superior to nalidixic acid.

Plates stored at temperatures ranging from 22 to −70°C showed no increase in MIC when inoculated weekly for 11 weeks. Alteration in

TABLE 1. In vitro activity of CI-919 compared with those of other antimicrobial agents

Bacterium (no. of isolates)	Antimicrobial agent	MIC (μg/ml)[a]		
		50%	90%	Range
Pseudomonas aeruginosa (59)	CI-919	1	2	≤0.06–4
	Ceftazidime	1	4	0.5–4
	Cefoperazone	2	8	0.25–64
	Ticarcillin	16	32	0.5–128
	Tobramycin	2	8	0.5–8
Pseudomonas aeruginosa (tobramycin resistant) (6)	CI-919	2	4	1–8
	Ceftazidime	16	16	2–128
	Cefoperazone	64	64	8–64
	Ticarcillin	>128	>128	32–>128
	Tobramycin	64	128	32–>128
Pseudomonas cepacia (15)	CI-919	≤0.12	2	≤0.12–4
	Ceftazidime	0.5	2	≤0.12–32
	Cefoperazone	4	16	1–128
	Ticarcillin	0.5	32	≤0.12–>128
	Tobramycin	4	16	2–>128
Pseudomonas maltophilia (21)	CI-919	2	4	1–8
	Ceftazidime	4	64	0.25–128
	Cefoperazone	8	32	1–64
	Ticarcillin	64	128	0.25–>128
	Tobramycin	4	32	1–>128
Pseudomonas spp. (12)	CI-919	≤0.12	0.5	≤0.12–1
	Ceftazidime	0.25	1	≤0.12–8
	Cefoperazone	4	16	2–64
	Ticarcillin	4	16	0.5–128
	Tobramycin	16	16	0.25–16
Escherichia coli (33)	CI-919	0.12	0.25	≤0.06–1
	Ampicillin	128	>128	1–>128
	Cefamandole	0.5	4	≤0.12–4
	Cefoperazone	0.25	1	≤0.12–2
	TMP-SMX	0.12-2.5	1-20	0.015-0.3–>8-160
	Ticarcillin	2	>128	0.5–>128
	Tobramycin	0.5	1	0.5–>128
Serratia spp. (32)	CI-919	0.25	0.25	≤0.06–8
	Cefoperazone	1	16	0.25–64
	Ticarcillin	4	>128	2–>128
	Tobramycin	2	128	0.5–>128
Yersinia enterocolitica (30)	CI-919	≤0.12	≤0.12	≤0.12
	Cefoperazone	1	2	0.5–4
	Ceftazidime	≤0.12	0.25	≤0.12–0.25
	Chloramphenicol	2	4	1–4
	TMP-SMX	0.03-0.6	0.06-1.2	0.03-0.6–0.06-1.2
	Tobramycin	0.5	0.5	0.25–0.5
Salmonella spp. (31)	CI-919	0.12	0.25	≤0.12–0.25
	Ampicillin	4	>128	1–>128
	Cefamandole	0.25	0.5	≤0.12–4
	Cefoperazone	0.25	2	≤0.12–16
	Chloramphenicol	4	4	4
	TMP-SMX	0.25-5	0.5-10	0.12-2.5–1-20
Shigella spp. (30)	CI-919	0.12	0.25	≤0.06–0.5
	Ampicillin	1	>128	0.25–>128
	Cefamandole	0.25	2	≤0.12–8
	Cefoperazone	≤0.12	1	≤0.12–2

TABLE 1—*Continued*

Bacterium (no. of isolates)	Antimicrobial agent	MIC (μg/ml)[a]		
		50%	90%	Range
	Chloramphenicol	2	4	4–16
	TMP-SMX	0.06-1.2	0.12-2.5	0.015-0.3–4-80
Other *Enterobacteriaceae* (128)[b]	CI-919	0.25	0.5	≤0.06–1
	Ampicillin	16	>128	0.5–>128
	Cefamandole	0.5	16	≤0.12–>128
	Cefoperazone	0.25	4	≤0.12–>128
	TMP-SMX	0.12-2.5	0.5-10	0.015-0.3–>8-160
	Ticarcillin	4	>128	0.25–>128
	Tobramycin	0.5	1	≤0.12–128
Miscellaneous gram-negative (tobramycin resistant) (82)[c]	CI-919	0.5	4	≤0.06–8
	Tobramycin	16	128	8–128
Nonfermentative bacilli (15)[d]	CI-919	0.25	4	0.06–8
	Cefamandole	4	64	≤0.12–64
	Cefoperazone	4	16	0.25–64
	Ceftazidime	0.5	4	0.12–4
	Ticarcillin	1	64	≤0.12–>128
	Tobramycin	16	64	0.5–>128
Campylobacter jejuni (26)	CI-919	≤0.06	0.12	≤0.06–0.25
	Erythromycin	0.25	1	≤0.12–2
	Chloramphenicol	1	4	0.25–8
	Tetracycline	1	16	≤0.12–32
	TMP-SMX	1-20	2-40	0.06-1.2–>4-80
Haemophilus influenzae (34)[e]	CI-919	0.12	0.12	≤0.06–0.12
	Chloramphenicol	0.5	0.5	0.25–16
	Cefamandole	0.25	0.5	0.06–0.5
	Moxalactam	≤0.03	≤0.03	≤0.03–0.06
	TMP-SMX	0.5-10	2-40	0.03-0.06–2-40
Staphylococcus aureus (33)	CI-919	0.5	1	0.5–2
	Cephalexin	2	2	1–4
	Cefoperazone	2	2	0.5–2
	Dicloxacillin	≤0.12	≤0.12	≤0.12–1
	Erythromycin	≤0.12	2	≤0.12–>128
	Gentamicin	8	16	≤0.12–16
	Rifampin	≤0.004	≤0.004	≤0.004
Staphylococcus spp. (coagulase negative) (28)	CI-919	0.5	2	0.25–8
	Cephalexin	2	32	≤0.12–64
	Cefoperazone	0.5	4	≤0.12–4
	Dicloxacillin	≤0.12	0.5	≤0.12–4
	Erythromycin	≤0.12	>128	≤0.12–>128
	Gentamicin	≤0.12	4	≤0.12–16
	Rifampin	≤0.004	0.008	≤0.004–0.008
Enterococci (22)	CI-919	8	16	4–16
	Ampicillin	1	2	0.125–4
	Cefoperazone	16	32	1–32
	Dicloxacillin	16	32	1–64
	Gentamicin	8	32	0.25–128
	Rifampin	0.125	2	≤0.007–8

[a] 50% and 90%, MICs inhibiting 50 and 90% of strains, respectively.

[b] Includes *Enterobacter* spp. (33), *Klebsiella* spp. (36), *Proteus* spp. (34), *Providencia* spp. (9), *Morganella morganii* (8), *Citrobacter* spp. (7), and *Edwardsiella tarda* (1).

[c] Includes *P. aeruginosa* (16), *Serratia marcescens* (12), *Proteus* spp. (10), *P. maltophilia* (9), *P. cepacia* (7), *Pseudomonas* spp. (7), *Enterobacter* spp. (4), *Acinetobacter* spp. (4), *Klebsiella* spp. (3), *Flavobacterium* sp. (3), *Achromobacter* sp. (2), *E. coli* (2), *Citrobacter* spp. (2), and *M. morganii* (1).

[d] Includes *Acinetobacter* spp. (7), *Achromobacter* spp. (4), *Flavobacterium* spp. (3), and *Aeromonas* sp. (1).

[e] Includes 18 β-lactamase-negative and 16 β-lactamase-positive strains. Four β-lactamase-positive strains were also acetyltransferase positive.

TABLE 2. In vitro activity of CI-919 compared with those of structurally related compounds

Bacterium (no. of isolates)	Antimicrobial agent	MIC (μg/ml)[a]		
		50%	80%	Range
Pseudomonas aeruginosa (6)	CI-919	1	2	0.25–2
	Norfloxacin	1	2	0.25–4
	Nalidixic acid	64	128	32–128
Escherichia coli (6)	CI-919	0.25	0.25	0.12–0.25
	Norfloxacin	0.25	0.25	0.12–0.25
	Nalidixic acid	4	8	2–8
Nonfermentative bacilli (6)	CI-919	0.25	4	0.12–4
	Norfloxacin	1	8	0.25–32
	Nalidixic acid	8	32	1–32
Staphylococcus aureus (6)	CI-919	1	1	1–2
	Norfloxacin	2	2	2
	Nalidixic acid	64	64	32–64
Staphylococcus spp. (coagulase negative) (6)	CI-919	1	2	0.5–4
	Norfloxacin	2	4	1–8
	Nalidixic acid	64	>128	64–>128

[a] 50% and 80%, MIC inhibiting 50 and 80% of isolates, respectively.

the inoculum density from 10^3 to 10^7 CFU/ml had no effect on the activity of CI-919. At a pH of 5.0 or less, there was a fourfold increase in MIC, whereas pH levels of up to 9.0 had no effect. Bactericidal concentrations of CI-919 were within one dilution of the MIC for 97% of all isolates tested. This was also true for strains tested at various pH ranges and at different inoculum densities.

DISCUSSION

In the search for an oral antimicrobial agent effective against systemic *Pseudomonas* infections, attention has recently been directed to nalidixic acid-related compounds (2, 5, 8). One of the first to be investigated was a pyridonecarboxylic acid derivative, pipemidic acid. Although it is well absorbed orally in experimental models, pipemidic acid is less active than aminoglycosides and penicillins in vitro (7, 9). Both 1,8-naphthyridine and 4-oxoquinoline analogs of pipemidic acid have broader in vitro antimicrobial spectra than that of the parent compound. Despite the somewhat increased in vitro activity of the quinolines, Matsumoto et al. (4) found the naphthyridines to be more active in vivo. Of the 1,8-naphthyridine preparations, he found the 6-fluoro-7-piperazinyl analog to be the most active against a wide range of bacteria (4). This compound, CI-919, differs from norfloxacin, a quinoline, by the presence of a nitrogen rather than a carbon atom at position 8.

CI-919 was active against the three major pulmonary pathogens in cystic fibrosis, *P. aeruginosa*, *S. aureus*, and *H. influenzae*. *P. cepacia*, which may also be an important pulmonary pathogen in certain patients with cystic fibrosis (A. Isles, H. Levison, C. Newth, M. Corey, and P. Fleming, Cystic Fibrosis Club Abstr. 23rd, Washington, D.C., p. 3, 1982), is particularly susceptible to CI-919. The lack of cross-resistance between CI-919 and the other available antipseudomonal antibiotics is encouraging, although the propensity for the emergence of bacterial resistance to CI-919 has not yet been examined extensively. In contrast to those of the aminoglycosides, the activity of CI-919 is not greatly diminished at the low pH levels often present in bronchial secretions of cystic fibrosis patients.

CI-919 was comparable in activity to trimethoprim-sulfamethoxazole and superior to ampicillin, erythromycin, and chloramphenicol against all of the recognized bacterial enteric pathogens. In contrast to trimethoprim-sulfamethoxazole, CI-919 was bactericidal against these strains. CI-919 showed relatively poor activity against enterococci, a trait shared by other nalidixic acid derivatives (8). The exact mode of action of CI-919 is unknown. It seems reasonable, however, to assume that CI-919 interferes with DNA replication, as does nalidixic acid (6).

The in vitro activity of CI-919 appears to be similar to that of the related quinoline derivative, norfloxacin (2, 3). The potential drawbacks to these nalidixic acid analogs are development of bacterial resistance, toxicity, and inadequate drug concentrations in serum and tissue. In preliminary studies, Shimizu was unable to demonstrate spontaneous mutants resistant to CI-919. Studies to evaluate long-term acquisition of

resistance are ongoing in our laboratory. The reported single 50% lethal dose for CI-919 in mice is greater than 5,000 mg/kg, while the 50% effective dose is 1.8 to 17.7 mg/kg, thus providing a wide toxic/therapeutic ratio (5). The major differences between CI-919 and norfloxacin may be in their pharmacokinetics and bioactivity in vivo, as suggested by Matsumoto et al. (4). In animal studies, CI-919 was well absorbed, concentrated in various tissues, including the lung, and was excreted unchanged in urine (5). Our early studies in mice show that peak serum concentrations after the administration of 100 mg of CI-919 per kg of body weight (mean, 7.6 μg/ml) consistently exceed the 90% MIC for the bacteria tested in this study by 2- to 64-fold (S. Chartrand, unpublished data). If similar therapeutic ratios are attainable in humans and toxicity and resistance are low, CI-919 may be a candidate for further study in the therapy of a number of infections, including many due to pseudomonads.

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