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In Vitro and In Vivo Antibacterial Activities of the Fluoroquinolone WIN 49375 (Amifloxacin)

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WIN 49375 (amifloxacin) is a synthetic antibacterial agent of the quinolone class. It is similar in chemical structure to pefloxacin but differs by containing a methylamino, rather than an ethyl, substituent at the 1-N position. The activity of WIN 49375 in vitro was comparable to those of norfloxacin and pefloxacin against *Enterobacteriaceae* and generally greater than those of tobramycin and cefotaxime. WIN 49375 was more active in vitro than carbenicillin and mezlocillin against *Pseudomonas aeruginosa* isolates and showed moderate activity against *Staphylococcus aureus*, with MICs of ≤2 μg/ml. The in vitro activity of WIN 49375 was not markedly affected by the presence of human serum, the size of the bacterial inoculum, or changes in pH between 6 and 8. Against systemic, gram-negative bacterial infections in mice, WIN 49375 was generally less active than cefotaxime but more active than gentamicin. WIN 49548, the major piperazinyl-*N*-desmethyl metabolite of WIN 49375, was as effective as the parent drug against experimental infections in mice when given parenterally. When administered orally, however, this metabolite was less potent than WIN 49375. WIN 49375 was highly active by the oral route, with 50% effective doses within two- to threefold of those obtained with parenteral medication.

The new fluorine-containing quinolones exhibit levels of antibacterial activity in vitro and in experimental infections that compare favorably with those of the beta-lactam and aminoglycoside antibiotics traditionally used in treating clinical infections. These newer fluoroquinolones (Fig. 1) include norfloxacin (4, 6, 13), pefloxacin (3, 18, 19), enoxacin (1, 7, 11, 17), ofloxacin (16), ciprofloxacin (2, 10, 21), and WIN 49375 (amifloxacin) (5, 14, 20). They are intrinsically resistant to bacterial enzymes that inactivate beta-lactam and aminoglycoside antibiotics and are well absorbed when administered orally. The present report describes the in vitro and in vivo antibacterial properties of WIN 49375.

MATERIALS AND METHODS

Chemicals. WIN 49375 [6-fluoro-1,4-dihydro-1-(methylamino)-7-(4-methyl-1-piperazinyl)-4-oxo-3-quinolinecarboxylic acid], its piperazinyl-*N*-desmethyl metabolite WIN 49548 [6-fluoro-1,4-dihydro-1-(methylamino)-7-(1-piperazinyl)-4-oxo-3-quinolinecarboxylic acid], rosoxacin, norfloxacin, and pefloxacin were synthesized at the Sterling-Winthrop Research Institute.

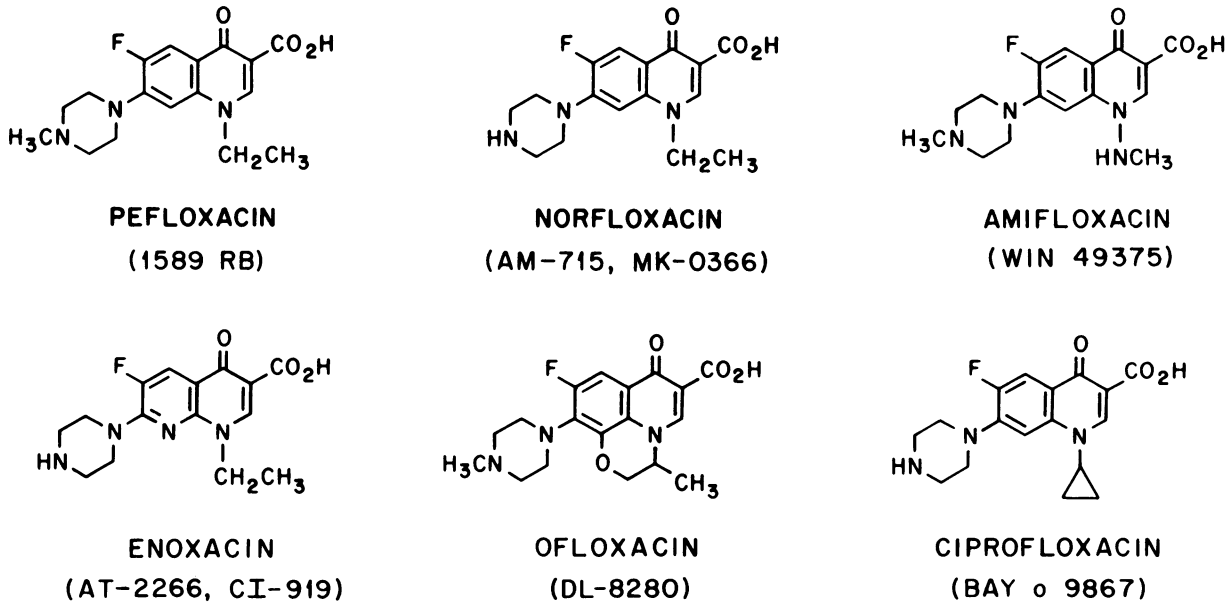

FIG. 1. Fluoroquinolone antibacterial agents.

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TABLE 1. In vitro activities of WIN 49375 and other antibacterial test agents

Test organism (no. tested)	Antibacterial test agent	MIC (μg/ml)[a]		
		Range	MIC_{50}	MIC_{90}
E. coli (13)	WIN 49375	0.06–8	0.125	0.125
	Norfloxacin	0.06–4	0.125	0.125
	Pefloxacin	0.06–4	0.125	0.125
	Rosoxacin	0.125–8	0.25	0.5
	Cefotaxime	0.06–>125	0.06	0.125
	Tobramycin	1–32	1	2
Klebsiella spp. (8)	WIN 49375	0.06–0.5	0.25	
	Norfloxacin	0.03–0.5	0.25	
	Pefloxacin	0.125–0.5	0.25	
	Rosoxacin	0.25–2	0.5	
	Cefotaxime	0.015–0.125	0.06	
	Tobramycin	0.5–1	0.5	
Proteus mirabilis (10)	WIN 49375	0.25–0.5	0.25	0.5
	Norfloxacin	0.06–0.25	0.125	0.25
	Pefloxacin	0.125–0.5	0.25	0.25
	Rosoxacin	0.5–2	1	1
	Cefotaxime	≤0.008–0.06	0.015	0.06
	Tobramycin	0.5–2	1	2
Morganella morganii (10)	WIN 49375	0.125–1	0.125	0.25
	Norfloxacin	0.06–1	0.125	0.25
	Pefloxacin	0.125–0.5	0.125	0.25
	Rosoxacin	0.5–1	0.5	1
	Cefotaxime	0.06–4	0.125	1
	Tobramycin	0.125–2	1	2
Serratia marcescens (3)	WIN 49375	0.125–0.25		
	Norfloxacin	0.06–0.25		
	Pefloxacin	0.25–0.5		
	Rosoxacin	0.25–0.5		
	Cefotaxime	0.125–0.25		
	Tobramycin	2–4		
Providencia spp. (9)	WIN 49375	0.06–0.25	0.25	0.25
	Norfloxacin	0.06–0.5	0.125	0.25
	Pefloxacin	0.06–0.5	0.25	0.5
	Rosoxacin	0.25–2	1	2
	Cefotaxime	0.03–0.25	0.125	0.25
	Tobramycin	0.5–32	4	16
Citrobacter spp. (4)	WIN 49375	0.125–0.5		
	Norfloxacin	0.06–0.5		
	Pefloxacin	0.125–0.5		
	Rosoxacin	0.5–2		
	Cefotaxime	0.015–4		
	Tobramycin	0.125–2		
Enterobacter spp. (13)	WIN 49375	0.125–1	0.125	1
	Norfloxacin	0.06–2	0.125	0.5
	Pefloxacin	0.125–2	0.25	0.5
	Rosoxacin	0.25–4	0.5	4
	Cefotaxime	0.015–>125	0.125	8
	Tobramycin	0.5–2	1	1
Salmonella enteritidis (9)	WIN 49375	0.125–0.25	0.125	0.125
	Norfloxacin	0.06–0.125	0.125	0.125
	Pefloxacin	0.125–0.25	0.125	0.25
	Rosoxacin	0.5–1	1	1
	Cefotaxime	0.125–0.25	0.125	0.25
	Tobramycin	1–8	2	4
P. aeruginosa (28)	WIN 49375	≤0.03–2.0	1.0	2.0
	Mezlocillin	1.0–>500	31.3	125
	Carbenicillin	0.5–>500	125	500
	Gentamicin	0.125–15.6	0.5	2.0
S. aureus (20)	WIN 49375	1.0–2.0	1.0	2.0
	Norfloxacin	0.5–4.0	2.0	2.0
	Pefloxcin	0.25–1.0	0.5	0.5
	Rosoxacin	0.5–1.0	0.5	1.0

[a] MICs were determined by the tube dilution procedure (see the text). MIC_{50} and MIC_{90} are the MICs required to inhibit 50 and 90% of strains, respectively.

Samples of cephalexin, cefotaxime, and cefonicid were generously provided by Eli Lilly & Co., Hoechst-Roussel Pharmaceuticals, Inc., and SmithKline Beckman Corp., respectively. Gentamicin sulfate, tobramycin sulfate, and carbenicillin disodium were purchased from Sigma Chemical Co.; mezlocillin, penicillin G, and penicillin V were obtained from local commercial suppliers.

Test organisms. The gram-negative bacilli used for in vitro susceptibility tests were clinical isolates obtained locally (Albany, N.Y.) during the last 2 years. Other test organisms were drawn from the Sterling-Winthrop Research Institute culture collection.

MIC test procedures. Susceptibility to test agents was measured by a broth dilution procedure. Isolates were grown overnight at 37°C in Mueller-Hinton broth (MHB, Difco Laboratories), and the cultures were adjusted to a constant turbidity of 0.1 absorbance unit at 650 nm (Bausch & Lomb Spectronic 20) in sterile distilled water before further dilution to the desired cell concentrations in MHB. (Double-strength MHB was used if the test drugs were diluted in water.) In tests with various amounts of inocula (for inoculum effect), the number of CFU per milliliter was confirmed by plate count.

Test agents were first dissolved in distilled water, aqueous NaOH, or dimethyl sulfoxide and sterilized by filtration (0.45-μm pore size) before further dilution in either sterile

TABLE 2. Effect of inoculum size on the MICs of WIN 49375 and cefotaxime for selected bacterial strains

Test organism[a]	Inoculum size (CFU/ml)	Median MIC (μg/ml)[b]	
		WIN 49375	Cefotaxime
E. coli	10^3	0.125	0.03
	10^5	0.125	0.125
	10^7	0.5	8
K. pneumoniae	10^3	0.125	≤0.015
	10^5	0.125	0.03
	10^7	0.25	4
Proteus mirabilis	10^3	0.5	0.03
	10^5	0.5	0.03
	10^7	1	>250
P. aeruginosa	10^3	1	8
	10^5	1	32
	10^7	4	>250

[a] Five isolates of each organism were tested.
[b] MICs were determined by the tube dilution procedure (see the text).

TABLE 3. Effect of 50% human serum on the in vitro activities of WIN 49375 and cefotaxime

Test organism[a]	Median (range) MIC (μg/ml)[b]			
	WIN 49375		Cefotaxime	
	Without serum	With serum	Without serum	With serum
E. coli	0.06 (0.03–0.06)	0.06 (0.06–0.125)	0.03 (0.03)	0.06 (0.06)
K. pneumoniae	0.06 (0.03–0.125)	0.06 (0.06–0.25)	0.03(0.004–0.03)	0.03 (0.008–0.06)
Proteus mirabilis	0.125 (0.06–0.125)	0.125 (0.125)	0.03 (0.03)	0.03 (0.03–0.06)
P. aeruginosa	1.0 (0.5–1.0)	4.0 (2.0–8.0)	8 (8–16)	8 (4–16)

[a] Five isolates of each organism were tested.
[b] MICs were determined by the microdilution procedure (see the text).

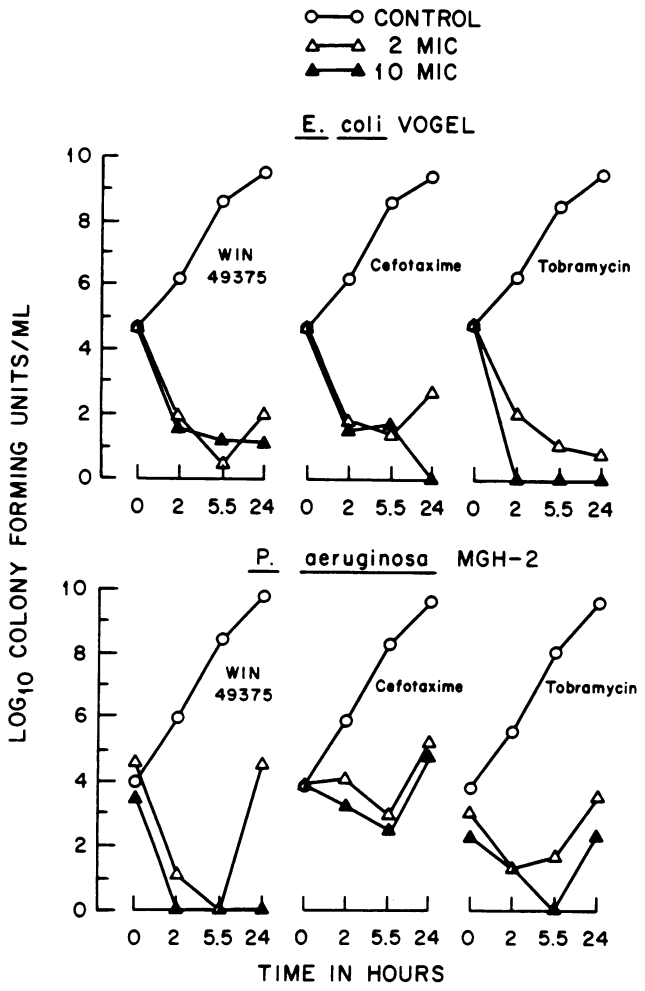

FIG. 2. In vitro bactericidal actions of WIN 49375, cefotaxime, and tobramycin against *E. coli* Vogel and *P. aeruginosa* MGH-2. Exponentially growing cultures were diluted to 10^5 CFU/ml in the absence of (○) or in the presence of the indicated drug equivalent to 2 (△) or 10 (▲) times the MIC. The cultures were incubated with agitation at 37°C and, at time intervals of 0, 2, 5.5, and 24 h, samples were removed, serially diluted, and plated for viable cells. The CFU were counted after 2 days of incubation at 37°C. The MIC for WIN 49375, cefotaxime, and tobramycin against *E. coli* Vogel were 0.25, 0.125, and 1.95 μg/ml, respectively, and 3.9, 31.3, and 0.5 μg/ml, respectively, against *P. aeruginosa* MGH-2, under the same growth conditions used for the test.

distilled water or MHB to the desired concentration. Portions (0.5 ml) of these solutions were added to tubes, and then 0.5 ml of the bacterial suspension was added to provide a final inoculum of 10^5 CFU/ml unless stated otherwise. A microdilution procedure was also used in which the same inoculum was achieved by the addition of 0.05 ml of the bacterial suspension in double-strength MHB to microtiter wells containing 0.05 ml of the test agent in water. The MIC was defined as the lowest concentration of test agent that prevented visible growth after 18 h of incubation at 37°C.

The effect of human serum (Control no. 28N5415, GIBCO Diagnostics) was determined by microdilution tests. The test agents were mixed with 50% serum (final concentration) in the wells of microtiter trays (0.1-ml final volume) to which 0.0015 ml of the bacterial suspension was added to provide a final inoculum of 10^5 CFU/ml.

The effect of pH on the MIC was measured by the broth dilution procedure described above. Solutions of test agents and suspensions of the bacterial test strains were diluted in MHB previously adjusted to pH 5, 6, 7, or 8 with 1 N NaOH or 1 N HCl.

Mouse protection tests. Gentamicin sulfate, cefotaxime sodium, piperacillin sodium, or WIN 49375 mesylate was dissolved in sterile distilled water. WIN 49548 was dissolved in 0.1 ml of 0.5 N NaOH to give a concentration of 100 mg/ml and then diluted with sterile distilled water to provide stock solutions.

The stock solutions of all test isolates except *Escherichia coli* Vogel were streaked onto brain heart infusion agar

TABLE 4. Effect of pH on the activity of WIN 49375, norfloxacin, and cephalexin

Test organism and test agent	MIC (μg/ml)[a] at pH:			
	5	6	7	8
E. coli Vogel				
WIN 49375	2	0.5	0.125	0.125
Norfloxacin	4	1	0.25	0.06
Cephalexin	125	32	8	16
Proteus mirabilis MGH-1				
WIN 49375	4	1	0.5	0.5
Norfloxacin	4	1	0.25	0.125
Cephalexin	>125	64	32	32
P. aeruginosa MGH-2				
WIN 49375	8	1	1	2
Norfloxacin	32	2	1	0.5
Cephalexin	>125	>125	>125	>125

[a] MICs were determined by the tube dilution procedure (see the text).

TABLE 5. Protective effect of WIN 49375 and other antibiotics against experimental infections in mice

Test organism (inoculum size, CFU/mouse)[a]	Drug[b]	Route	MIC[c] (μg/ml)	ED_{50} (mg/kg per dose) (95% confidence limits)[d]
Citrobacter freundii 15347 (10^6)	WIN 49375	p.o.	0.25	0.7 (0.4–0.9)
	WIN 49375	s.c.	0.25	0.5 (0.3–0.7)
	Cefotaxime	s.c.	0.015	0.02 (0.01–0.03)
	Gentamicin	s.c.	0.5	0.9 (0.7–1.2)
E. coli Vogel (10^7)	WIN 49375	p.o.	0.03	1.0 (0.8–1.3)
	WIN 49375	s.c.	0.03	0.5 (0.2–1.6)
	Cefotaxime	s.c.	0.125	1.5 (0.9–2.4)
	Gentamicin	s.c.	0.25	0.3 (0.2–0.4)
E. coli 15323 (5×10^5)	WIN 49375	p.o.	0.03	0.8 (0.6–1.1)
	WIN 49375	s.c.	0.03	0.3 (0.2–0.4)
	Cefotaxime	s.c.	0.06	0.04 (0.03–0.05)
	Gentamicin	s.c.	0.25	0.7 (0.5–0.9)
E. coli 15352 (4×10^5)	WIN 49375	p.o.	0.03	0.8 (0.6–1.1)
	WIN 49375	s.c.	0.03	0.3 (0.2–0.3)
	Cefotaxime	s.c.	0.125	0.07 (0.06–0.09)
	Gentamicin	s.c.	0.125	0.4 (0.3–0.6)
Enterobacter agglomerans 14866 (5×10^5)	WIN 49375	p.o.	0.06	0.9 (0.6–1.2)
	WIN 49375	s.c.	0.06	0.3 (0.2–0.4)
	Cefotaxime	s.c.	0.06	0.02 (0.01–0.02)
	Gentamicin	s.c.	0.125	0.2 (0.1–0.3)
Enterobacter cloacae 905 (10^6)	WIN 49375	p.o.	0.03	1.0 (0.7–1.3)
	WIN 49375	s.c.	0.03	0.3 (0.2–0.4)
	Cefotaxime	s.c.	8	15.2 (11.2–22.4)
	Gentamicin	s.c.	0.125	0.4 (0.3–0.5)
P. aeruginosa MGH-2 (2×10^6)	WIN 49375	p.o.	1	3.2 (2.0–4.7)
	WIN 49375	s.c.	1	2.2 (1.7–2.8)
	Cefotaxime	s.c.	32	22 (12–32)
	Gentamicin	s.c.	2	5.4 (4.0–7.5)
P. aeruginosa 12-4-4 (2×10^6)	WIN 49375	p.o.	2	6.4 (4.7–8.5)
	WIN 49375	s.c.	2	4.2 (3.3–5.6)
	Gentamicin	s.c.	1	7.7 (5.8–10)
P. aeruginosa PD-05141 (2×10^6)	WIN 49375	p.o.	1	4.3 (3.0–6.4)
	WIN 49375	s.c.	1	3.6 (2.7–4.9)
	Gentamicin	s.c.	0.5	7.3 (5.3–10)
S. aureus Smith (9×10^3)	WIN 49375	p.o.		12.8 (9.3–16.9)
	WIN 49375	s.c.		6.8 (4.8–9.3)
	Penicillin G	s.c.		0.5 (0.3–0.7)
	Penicillin V	p.o.		0.9 (0.7–1.3)

[a] Mice were infected intraperitoneally with 0.5 ml of a bacterial suspension in 5% mucin or in saline (*E. coli* Vogel).

[b] Medications were given in volumes of 0.5 ml perorally (p.o.) or 0.2 ml subcutaneously (s.c.). For *E. coli* Vogel infections, oral medication was given as a single dose 0.5 h postinfection; subcutaneous medications were given at 0.5, 3.5, and 20 h postinfection. For *P. aeruginosa* infections, doses were given at 0.5, 4, and 7 h postinfection. For *S. aureus* infections, single doses were given at 0.5 h postinfection. For the remaining bacterial infections, two doses were given at 0.5 and 3.5 to 4 h postinfection.

[c] MICs were determined by the microdilution procedure (see the text).

[d] The number of survivors at day 7 postinfection was used to calculate the ED_{50} by probit analysis.

plates and incubated for 16 to 18 h at 37°C. The resulting growth was washed from the plate with sterile saline, disaggregated by shaking vigorously with glass beads, diluted further in saline, and finally diluted in 5% gastric mucin to provide test inocula. *E. coli* Vogel was incubated in tryptose phosphate broth (Difco Laboratories) at 37°C for 5 h and then diluted with saline to provide the test inoculum. The various inoculum sizes tested are given in the tables.

ICR female mice weighing 18 to 20 g were inoculated intraperitoneally with 0.5 ml of the bacterial suspension and then distributed into subgroups of 10 to receive serial twofold doses of the test agents and a subgroup of 20 to serve as untreated controls. The infected mice were medicated orally or subcutaneously at various times postinfection as noted in the tables. The number of survivors was determined daily for 7 days, at which time the 50% effective dose (ED_{50}) values were determined by probit analysis (15). When survivor values of 0 to 100% were required to calculate confidence limits, we used the approximation method of Miller and Tainter (9).

RESULTS

In vitro susceptibility. The MICs (where attainable) required to inhibit 50 and 90% of strains for the various test agents are shown in Table 1. The fluoroquinolones were more active than the other agents against the *Escherichia*, *Morganella*, *Citrobacter*, *Enterobacter*, and *Salmonella* isolates. Cefotaxime was the most potent agent against *Proteus* and *Klebsiella* spp. and was as active as the fluoroquinolones against *Serratia* and *Providencia* spp. Among the fluoroquinolones, comparable MICs required to inhibit 90% of strains were observed for all of the bacterial test strains. As a group they were substantially more active than tobramycin against all genera tested except the *Klebsiella* and *Enterobacter* spp.

WIN 49375 was substantially more active than the antipseudomonal penicillins, mezlocillin, and carbenicillin and was comparable to gentamicin against isolates of *Pseudomonas aeruginosa* from patients with cystic fibrosis. *Staphylococcus aureus* was uniformly susceptible to the quinolones tested, with MICs required to inhibit 90% of strains ranging from 0.5 to 2 μg/ml (Table 1).

The bactericidal activity of WIN 49375 was compared with that of cefotaxime and tobramycin against two representative test organisms (Fig. 2). All three of these test agents were initially bactericidal at 2 and 10 times the MIC. Only tobramycin prevented regrowth of the *E. coli* isolate at 2 times the MIC, and only WIN 49375 prevented regrowth of the *P. aeruginosa* isolate at 10 times the MIC.

When the inoculum was increased from 10^3 to 10^7 CFU/ml, the median MICs for WIN 49375 for 20 strains of gram-negative bacilli, including *P. aeruginosa*, increased from two- to fourfold (Table 2). In these tests, the median MICs for cefotaxime increased from 32 to >8,000-fold. The in vitro activity of WIN 49375 or cefotaxime against these 20 bacterial strains was little affected by the presence of human serum (50%, vol/vol) (Table 3). The MICs for WIN 49375 were generally not affected by changes in pH between 6 and 8 against single strains of *E. coli*, *Proteus mirabilis*, and *P. aeruginosa* (Table 4). At pH 5 the MICs for WIN 49375 increased from 8- to 16-fold over those obtained at pH 7. These responses were similar to those of two other oral antibacterial agents, norfloxacin and cephalexin.

Experimental infections. The capacity of WIN 49375 to protect mice against otherwise lethal systemic infections was tested against those of cefotaxime and gentamicin. Cefotaxime was the most active parenteral agent against all the *Enterobacteriaceae* tested except *Enterobacter cloacae* (Table 5). WIN 49375 showed a consistently high protective effect against these bacterial infections, with ED_{50}s of 0.3 to 0.5 mg/kg, which were comparable to or slightly more effective than those for gentamicin. Similar results for WIN 49375 relative to gentamicin were also observed with *P. aeruginosa* infections.

The activity of WIN 49375 against these gram-negative bacterial infections was retained when the medication was administered orally (Table 5). The ED_{50}s obtained with oral medication were only two- to three-fold higher than when WIN 49375 was given subcutaneously. This was especially evident against *P. aeruginosa* infections, where the ED_{50}s of WIN 49375 given orally and parenterally differed by less than twofold.

The effectiveness of WIN 49375 with various routes of medication was demonstrated with these experimental infections. When mice infected intraperitoneally with *E. coli* Vogel were medicated at 0.5-h postinfection subcutaneously, intravenously, or orally the ED_{50}s for WIN 49375 were 0.6, 0.8, and 1.0 mg/kg, respectively.

WIN 49375 was compared with cefonicid for prophylactic action in mice infected with *E. coli* Vogel. Mice were given a subcutaneous injection with either of these test drugs at various times before, or 0.5 h after, intraperitoneal inoculation with the challenge bacteria. The data showed that WIN 49375 was actually more potent than cefonicid in this test (Table 6).

When given subcutaneously or orally, WIN 49375 also protected mice from otherwise lethal *S. aureus* Smith infections. In this test penicillin G (subcutaneous medication) and penicillin V (oral medication) were both more effective than WIN 49375 against *S. aureus* (Table 5).

Metabolite activity. The major urinary metabolite found after intravenous administration of WIN 49375 to monkeys was the piperazinyl-*N*-desmethyl analog (WIN 49548) (D. P. Benziger, L. F. McCoy, M. F. Kuhrt, R. A. Dobson, R. G. Ferraino, C. H. Nash, and J. B. Cornett, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 23rd, Las Vegas, Nev., abstr. no. 706, p. 214, 1983). This compound was synthesized and tested for in vitro and in vivo antibacterial activities. The test results (Table 7) showed that WIN 49548 was as active as the parent compound in vitro against selected gram-negative bacilli. Against experimental bacterial infections in mice, this metabolite, when given subcutaneously, was at least as effective as its parent compound. However, when given orally, WIN 49548 was consistently less active than WIN 49375 against infection (Table 7).

TABLE 6. Prophylaxis against an *E. coli* infection[a]

Time of medication relative to infection (h)[b]	Mean ± SD ED_{50} (mg/kg per dose)	
	WIN 49375	Cefonicid
+0.5	0.9 ± 0.2	3.4 ± 1.2
−1	3.5 ± 1.0	7.4 ± 0.8
−4	33 ± 11	54 ± 5
−6	65 ± 17	469

[a] All mice were inoculated intraperitoneally.

[b] Groups of female mice (10 per dose level) were given a single medication subcutaneously within a twofold serial dose series. The MICs for cefonicid and WIN 49375 against *E. coli* Vogel were 0.125 and 0.06 μg/ml, respectively, as determined by the microtiter procedure.

[c] Mean values from three separate experiments, except the 0.5-h postinfection dose was included in only two tests, and an end point was achieved in only one test with cefonicid medication at −6 h.

DISCUSSION

The in vitro activity of WIN 49375 was comparable to that of cefotaxime and tobramycin against *Enterobacteriaceae*. Our results are similar to those from comparative tests with other fluoroquinolones (1, 2, 6, 11, 13, 21). These published in vitro studies show that the fluoroquinolones are most active against gram-negative bacteria and moderately active against staphylococci. WIN 49375 showed a similar activity, both in this study and elsewhere (14), but little activity against anaerobic bacteria (5). The in vitro activity of WIN 49375 was not markedly affected by changes in pH between 6 and 8, the size of the bacterial inoculum, or the presence of human serum. WIN 49375 was approximately 50% bound to human serum (data not shown), a somewhat higher value than those obtained for ciprofloxacin (21) and enoxacin (12).

When administered parenterally to experimentally infected mice, WIN 49375 was generally less active than cefotaxime. When the infecting bacterial strain was less

TABLE 7. Comparative activities of WIN 49375 and its major piperazinyl-*N*-desmethyl metabolite, WIN 49548

Test organism	Inoculum size (CFU/mouse)[a]	Drug[b]	Route	MIC[c] (μg/ml)	ED_{50} (mg/kg per dose) (95% confidence limits)[d]
Enterobacter aerogenes 286	9×10^6	WIN 49375	s.c.	0.015	0.3 (0.2–0.4)
		WIN 49548	s.c.	0.015	0.4 (0.3–2.5)
Providencia stuartii SP	2×10^6	WIN 49375	s.c.	0.06	0.3 (0.2–0.4)
		WIN 49548	s.c.	0.125	0.2 (0.2–0.3)
S. typhi ATCC 6539	8×10^6	WIN 49375	s.c.	0.03	0.2 (0.2–0.3)
		WIN 49548	s.c.	0.03	0.2 (0.1–0.2)
Shigella flexneri ATCC 11836	2×10^6	WIN 49375	s.c.	0.06	0.4 (0.3–0.5)
		WIN 49548	s.c.	0.06	0.2 (0.2–0.3)
E. coli Vogel	3×10^6	WIN 49375	s.c.	0.03	0.4 (0.2–0.6)
		WIN 49548	s.c.	0.03	0.3 (0.2–0.4)
	3×10^6	WIN 49375	p.o.	0.03	1.0 (0.8–1.3)
		WIN 49548	p.o.	0.03	5.4 (1.7–2.9)
P. aeruginosa MGH-2	3×10^6	WIN 49375	s.c.	1	2.3 (1.6–3.3)
		WIN 49548	s.c.	2	0.5 (0.2–0.7)
	4×10^6	WIN 49375	p.o.	1	3.2 (2.2–4.8)
		WIN 49548	p.o.	2	7.7 (5.2–11)

[a] Mice were infected intraperitoneally with 0.5 ml of the bacterial suspension in 5% mucin or in saline (*E. coli* Vogel).
[b] Single medications were given either subcutaneously (0.5 ml, s.c.) or perorally (0.2 ml, p.o.) 0.5 h postinfection for all bacterial infections except those with *P. aeruginosa* where medications were given at 0.5, 4, and 7 h postinfection.
[c] MICs were determined by the microdilution procedure (see the text).
[d] The number of survivors at day 7 postinfection was used to calculate the ED_{50} by probit analysis.

susceptible to cefotaxime than to WIN 49375 (e.g., *E. coli* Vogel, *Enterobacter cloacae* 905, or *P. aeruginosa* MGH-2), then the fluoroquinolone was the more active agent. WIN 49375 generally showed greater efficacy than equal weight doses of gentamicin against the same experimental infections. This was especially true with *P. aeruginosa* infections.

In contrast to the aminoglycosides and most cephalosporin antibiotics, WIN 49375 showed nearly equal efficacy, whether given orally or parenterally, against these experimental infections. This attribute of WIN 49375 is shared by enoxacin and ofloxacin which, when given orally, are as active against experimental infections as gentamicin (8, 11, 17).

In comparative tests, norfloxacin has been reported to be less active than either enoxacin (7, 11) or ofloxacin (16) by the oral medication route. Since the bacterial strains used in those reported experiments were equally susceptible in vitro to these fluoroquinolones, the results suggest that norfloxacin was either less well orally absorbed, less metabolically stable, or more rapidly excreted. The pharmacokinetics of norfloxacin and enoxacin were compared for several animal species, and the results indicated that norfloxacin was not absorbed as well as enoxacin when given orally (12). We obtained analogous results with WIN 49375, a 1-methylamino analog of pefloxacin, and its metabolite WIN 49548, a 1-methylamino analog of norfloxacin. When administered parenterally, this metabolite was as active as its parent WIN 49375; but when administered orally, it was less effective. However, the metabolite probably does contribute to the overall efficacy of WIN 49375 against these experimental infections.

If our results are predictive of clinical situations, then WIN 49375 should be effective against gram-negative bacterial sepsis in humans. The effectiveness of oral therapy may also encourage clinical acceptance of antibacterial fluoroquinolones for the treatment of bacterial infections beyond their traditional use in treating only urinary tract infections.

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