ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, June 1990, p. 1154–1159
0066-4804/90/061154-06$02.00/0

Vol. 34, No. 6

Comparative In Vitro Activity of WIN 57273, a New Fluoroquinolone Antimicrobial Agent

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Received 8 November 1989/Accepted 22 March 1990

The in vitro activity of WIN 57273, a new fluoroquinolone antimicrobial agent, was evaluated against approximately 600 bacterial isolates. The new drug was 4- to 128-fold more active than ciprofloxacin against a broad range of gram-positive organisms, with the new drug inhibiting 90% of strains of each species except *Enterococcus faecium* at concentrations of ≤0.25 μg/ml. WIN 57273 was four- to eightfold less active than ciprofloxacin against many members of the family *Enterobacteriaceae*, but the MICs of the new drug for 90% of strains tested (MIC_{90}s) were ≤8 μg/ml (range, 0.25 to 8 μg/ml) for all species. *Branhamella catarrhalis*, *Haemophilus influenzae*, *Neisseria gonorrhoeae*, and *Legionella* spp. were highly susceptible (MIC_{90}s, ≤0.06 μg/ml). WIN 57273 demonstrated excellent activity against anaerobes (MIC_{90}s, ≤0.25 μg/ml), and the drug was also more active than ciprofloxacin against 30 strains of *Mycobacterium avium-M. intracellulare* (MIC, 0.1 to 1.0 μg/ml). The activity of WIN 57273 against gram-positive organisms was minimally affected by pH and increased at low pH (5.4) against gram-negative organisms. The bactericidal activity of WIN 57273 was demonstrated by time-kill techniques against selected organisms. The frequencies of spontaneous resistance to the new agent were low, but resistant colonies could be selected after serial passage of initially susceptible organisms through incremental concentrations of the drug.

Activity against gram-positive organisms represents a major advantage of the new fluoroquinolone group of antimicrobial agents compared with older agents such as nalidixic acid (3). Nevertheless, the majority of the new compounds which have been subjected to extensive evaluation demonstrate less potent activities against most commonly encountered gram-positive and anaerobic pathogens than they do against members of the family *Enterobacteriaceae* (3).

WIN 57273 [1-cyclopropyl-7-(2,6-dimethyl-4-pyridinyl)-6-fluoro-1,4-dihydro-4-oxo-3-quinolonecarboxylic acid] is a recently developed fluoroquinolone which has demonstrated particularly good activity against gram-positive organisms (9). In the present study we examined the in vitro activity of this new agent in comparison with those of other antimicrobial agents against approximately 600 bacterial isolates. The bactericidal activity of WIN 57273 against representative strains was tested by time-kill techniques. Frequencies of spontaneous resistance to the drug and the extent to which resistance could be selected by serial transfer in incremental concentrations of drug were also examined.

MATERIALS AND METHODS

Bacterial strains. Most bacteria used in this study were routine clinical isolates collected at the New England Deaconess Hospital or Massachusetts General Hospital. Additional gram-positive bacteria with specific resistance traits have been collected in our laboratory from numerous sources (1). Strains were stored frozen at −70°C.

Antimicrobial agents. WIN 57273 was provided by Sterling-Winthrop Research Institute, Rensselaer, N.Y. Other standard antimicrobial reference powders were generously provided by the indicated pharmaceutical companies: ciprofloxacin, Miles Pharmaceuticals, West Haven, Conn.; imipenem, Merck Sharp & Dohme Research Laboratories, Rahway, N.J.; vancomycin, ceftazidime, and cephalexin, Eli Lilly & Co., Indianapolis, Ind.; clindamycin, The Upjohn Co., Kalamazoo, Mich.; ampicillin, Pfizer, Inc., Groton, Conn.; piperacillin, Lederle Laboratories, Pearl River, N.Y.; and oxacillin, Bristol-Myers Co., Syracuse, N.Y.

Susceptibility studies. MICs against most organisms were determined by agar dilution methods (12). Mueller-Hinton agar (BBL Microbiology Systems, Cockeysville, Md.) was used for testing most aerobic and facultative organisms. This was supplemented with 5% defibrinated sheep blood when testing streptococci and diphtheroids. Kellogg supplement (1%; vol/vol) (12) was added to chocolatized blood agar for testing gonococci (GC agar base; BBL) and *Haemophilus influenzae* and *Branhamella catarrhalis* (Mueller-Hinton agar). *Bacteroides fragilis* was tested on Wilkins-Chalgren medium (Oxoid Ltd., Basingstoke, England); 5% sheep blood was added for other anaerobes. *Legionella* spp. were tested on the buffered starch yeast extract medium of Saito et al. (14). Bacterial suspensions were prepared from fresh plates into liquid media and applied with a multipronged inoculating device to yield final inocula of approximately 10^5 CFU per spot for anaerobes (11) and 10^4 CFU per spot for other organisms. Plates were incubated at 35°C in room air (most organisms), 5% CO_2 (gonococci), a microaerophilic atmosphere (Campylo-Pak; BBL) (*Campylobacter jejuni*), or an anaerobic atmosphere (GasPak; BBL) (anaerobes). MICs were determined after incubation periods of 18 h (most organisms) or 48 h (anaerobes). *Legionella* spp. were evaluated at 48 h of incubation. For determination of pH effects on WIN 57273 activity, the pH of Mueller-Hinton agar was adjusted with hydrochloric acid or sodium hydroxide to desired levels.

The activity of WIN 57273 against *Mycobacterium avium-M. intracellulare* complex organisms was determined by the broth microdilution method in 7H11 broth as described by Yajko et al. (18). Plates were examined after 4 and 7 days of incubation at 37°C in 9% CO_2.

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TABLE 1. Comparative in vitro activity of WIN 57273

Organism (no.)	Antimicrobial agent	MIC (μg/ml)[a]		
		Range	50%	90%
Staphylococcus aureus, methicillin susceptible (15)	WIN 57273	≤0.003–0.03	0.015	0.03
	Ciprofloxacin	0.25–0.5	0.5	0.5
	Vancomycin	1–2	1	2
	Clindamycin	0.25	0.25	0.25
	Cephalexin	4–32	8	32
	Oxacillin	0.25–1	0.5	0.5
	Imipenem	0.03–0.06	0.06	0.06
Staphylococcus aureus, methicillin resistant (15)	WIN 57273	0.008–0.03	0.015	0.03
	Ciprofloxacin	0.25–1	0.5	0.5
	Vancomycin	1–2	1	2
	Clindamycin	≥128	>128	>128
	Cephalexin	≥128	>128	>128
	Imipenem	8–32	8	32
Coagulase-negative staphylococci (30)	WIN 57273	0.008–0.25	0.015	0.03
	Ciprofloxacin	0.03–1	0.25	0.5
	Vancomycin	1–4	2	4
	Clindamycin	0.12–>128	0.25	>128
	Cephalexin	1–128	8	64
	Oxacillin	0.25–128	2	64
	Imipenem	0.03–32	0.12	32
Streptococcus pyogenes (10)	WIN 57273	0.06–0.12	0.12	0.12
	Ciprofloxacin	0.5–1	0.5	0.5
	Vancomycin	0.5	0.5	0.5
	Clindamycin	≤0.06	≤0.06	≤0.06
	Cephalexin	0.5–1	1	1
	Oxacillin	≤0.06–0.12	≤0.06	0.12
	Ampicillin	≤0.06–0.12	≤0.06	0.12
	Imipenem	0.008–0.03	0.008	0.03
Streptococcus agalactiae (10)	WIN 57273	0.06–0.12	0.06	0.12
	Ciprofloxacin	0.5–1	0.5	0.5
	Vancomycin	0.5	0.5	0.5
	Clindamycin	≤0.06–0.12	≤0.06	≤0.06
	Cephalexin	4–8	4	8
	Oxacillin	0.5–2	0.5	2
	Ampicillin	0.5–1	0.5	1
	Imipenem	0.03–0.06	0.03	0.06
Streptococci, groups C and G (10)	WIN 57273	0.06–0.12	0.06	0.12
	Ciprofloxacin	0.5	0.5	0.5
	Vancomycin	0.5	0.5	0.5
	Clindamycin	≤0.06–0.12	≤0.06	≤0.06
	Cephalexin	0.5–1	1	1
	Oxacillin	≤0.06–0.25	0.12	0.12
	Ampicillin	0.12–0.25	0.12	0.12
	Imipenem	0.008–0.015	0.015	0.015
Streptococcus pneumoniae penicillin susceptible (10)	WIN 57273	0.03–0.06	0.03	0.03
	Ciprofloxacin	1–4	2	4
	Vancomycin	0.5	0.5	0.5
	Clindamycin	0.12–0.25	0.12	0.25
	Cephalexin	2	2	2
	Oxacillin	0.12	0.12	0.12
	Ampicillin	≤0.06	≤0.06	≤0.06
	Imipenem	0.008–0.015	0.015	0.015
Streptococcus pneumoniae, penicillin resistant (7)	WIN 57273	0.015–0.06	0.03	
	Ciprofloxacin	1–4	2	
	Vancomycin	0.5–1	0.5	
	Clindamycin	0.25–8	1	
	Cephalexin	2–64	32	
	Oxacillin	4–32	16	
	Ampicillin	0.12–8	2	
	Imipenem	0.06–1	0.5	

Continued

TABLE 1—*Continued*

Organism (no.)	Antimicrobial agent	MIC (μg/ml)[a]		
		Range	50%	90%
Viridans group streptococci, penicillin susceptible (10)	WIN 57273	0.008–0.06	0.03	0.06
	Ciprofloxacin	0.008–1	0.5	1
	Vancomycin	1	1	1
	Clindamycin	≤0.06–0.12	≤0.06	0.12
	Cephalexin	2–16	4	8
	Oxacillin	0.12–2	1	1
	Ampicillin	≤0.06–1	0.25	0.5
	Imipenem	0.015–0.25	0.06	0.12
Viridans group streptococci, penicillin resistant (10)	WIN 57273	0.015–0.03	0.015	0.06
	Ciprofloxacin	1–4	1	4
	Vancomycin	0.5–1	0.5	1
	Clindamycin	≤0.06–8	0.12	8
	Cephalexin	16–>128	128	>128
	Oxacillin	8–32	32	32
	Ampicillin	0.5–16	4	4
	Imipenem	0.12–2	1	1
Enterococcus avium (10)	WIN 57273	0.12–0.25	0.25	0.25
	Ciprofloxacin	2–4	2	2
	Vancomycin	1–2	1	2
	Clindamycin	4–>128	8	>128
	Cephalexin	8–>128	16	64
	Oxacillin	8–>128	16	>128
	Ampicillin	0.5–16	1	2
	Imipenem	0.25–16	0.5	4
Enterococcus faecalis (10)	WIN 57273	0.06–0.12	0.12	0.12
	Ciprofloxacin	0.5–4	1	4
	Vancomycin	1–4	1	4
	Clindamycin	32–>128	32	>128
	Cephalexin	≥128	128	>128
	Ampicillin	0.5–2	1	2
	Imipenem	1–4	2	2
Enterococcus faecium (10)	WIN 57273	0.25–8	0.25	4
	Ciprofloxacin	1–16	2	8
	Vancomycin	0.5–4	1	4
	Clindamycin	1–>128	2	>128
	Cephalexin	≥128	>128	>128
	Ampicillin	4–8	4	8
	Imipenem	1–32	8	32
JK group diphtheroids (10)	WIN 57273	0.015–0.03	0.015	0.03
	Ciprofloxacin	0.25–2	0.25	0.5
	Vancomycin	1	1	1
	Clindamycin	1–>128	8	>128
	Cephalexin	16–>128	32	>128
	Ampicillin	4–>128	8	>128
	Imipenem	0.5–128	1	128
Listeria monocytogenes (10)	WIN 57273	0.12–0.25	0.25	0.25
	Ciprofloxacin	1–4	1	1
	Vancomycin	1	1	1
	Clindamycin	0.5–2	2	2
	Cephalexin	16–64	64	64
	Ampicillin	≤0.06–1	0.06	0.25
	Imipenem	0.03–0.25	0.06	0.25
Acinetobacter calcoaceticus subsp. *anitratus* (10)	WIN 57273	0.12–0.25	0.25	0.25
	Ciprofloxacin	0.25–0.5	0.25	0.5
	Cephalexin	>128	>128	>128
	Piperacillin	16–64	16	32
	Ceftazidime	4–16	8	8
	Imipenem	0.25–1	0.25	0.5

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TABLE 1—*Continued*

Organism (no.)	Antimicrobial agent	MIC (μg/ml)[a] Range	50%	90%
Aeromonas hydrophila (9)	WIN 57273	0.015–0.25	0.06	
	Ciprofloxacin	≤0.003–0.06	0.015	
	Cephalexin	64–>128	>128	
	Piperacillin	4–>128	8	
	Ceftazidime	0.25–4	0.5	
	Imipenem	0.25–16	4	
Branhamella catarrhalis (10)	WIN 57273	0.008–0.12	0.015	0.03
	Ciprofloxacin	0.12–2	0.12	0.12
	Cephalexin	4–32	8	32
	Ampicillin	2–128	32	64
	Imipenem	0.12–2	0.5	0.5
Campylobacter jejuni (10)	WIN 57273	0.03–0.5	0.12	0.25
	Ciprofloxacin	0.06–0.5	0.25	0.5
	Clindamycin	0.25–2	1	2
	Erythromycin	0.5–4	2	4
	Imipenem	0.03–0.12	0.06	0.12
Citrobacter freundii (20)	WIN 57273	0.25–8	0.5	4
	Ciprofloxacin	0.06–0.5	0.06	0.5
	Cephalexin	32–>128	>128	>128
	Piperacillin	1–>128	1	64
	Imipenem	0.25–8	1	4
Enterobacter aerogenes (20)	WIN 57273	0.25–1	0.5	1
	Ciprofloxacin	0.06–0.25	0.12	0.12
	Cephalexin	>128	>128	>128
	Piperacillin	1–>128	2	64
	Imipenem	0.5–8	1	4
Enterobacter cloacae (20)	WIN 57273	0.25–0.5	0.25	0.5
	Ciprofloxacin	0.06–0.12	0.12	0.12
	Cephalexin	>128	>128	>128
	Piperacillin	1–>128	2	16
	Imipenem	0.25–2	0.5	2
Escherichia coli (30)	WIN 57273	0.12–0.5	0.12	0.25
	Ciprofloxacin	0.015–0.06	0.03	0.03
	Cephalexin	4–64	16	32
	Piperacillin	2–>128	2	64
	Imipenem	0.25–1	0.5	0.5
Haemophilus influenzae (20)	WIN 57273	0.015–0.12	0.03	0.06
	Ciprofloxacin	0.015–0.06	0.03	0.03
	Cephalexin	8–64	32	64
	Ampicillin	0.5–>64	1	>64
	Imipenem	4	4	4
Klebsiella pneumoniae (30)	WIN 57273	0.5–2	0.5	1
	Ciprofloxacin	0.03–0.25	0.06	0.12
	Cephalexin	4–16	8	8
	Piperacillin	4–>128	8	64
	Imipenem	0.25–1	0.5	1
Legionella spp. (20)[b]	WIN 57273	≤0.003	≤0.003	≤0.003
	Ciprofloxacin	0.03–0.12	0.06	0.06
	Erythromycin	≤0.06–0.25	0.25	0.25
	Imipenem	0.06–0.5	0.12	0.25
Morganella morganii (10)	WIN 57273	0.5–2	1	1
	Ciprofloxacin	0.06–0.12	0.12	0.12
	Cephalexin	≥128	>128	>128
	Ampicillin	16–>128	>128	>128
	Piperacillin	2–128	4	64
	Imipenem	4–8	8	8

Continued

TABLE 1—*Continued*

Organism (no.)	Antimicrobial agent	MIC (μg/ml)[a] Range	50%	90%
Neisseria gonorrhoeae (20) (10 strains β-lactamase positive)	WIN 57273	≤0.003–0.25	0.015	0.06
	Ciprofloxacin	≤0.003–0.25	0.015	0.12
	Cephalexin	1–32	4	8
	Clindamycin	2–32	4	8
	Ampicillin	≤0.06–128	0.5	32
Pasteurella multocida (7)	WIN 57273	0.008–0.03	0.008	
	Ciprofloxacin	≤0.003–0.015	0.008	
	Cephalexin	≤0.06–2	≤0.06	
	Ampicillin	0.12–0.5	0.25	
	Imipenem	≤0.06	≤0.06	
Proteus mirabilis (30)	WIN 57273	1	1	1
	Ciprofloxacin	0.06–0.5	0.25	0.25
	Cephalexin	16–128	16	32
	Ampicillin	1–16	2	4
	Imipenem	0.5–8	4	4
Proteus vulgaris (10)	WIN 57273	0.25–2	0.5	2
	Ciprofloxacin	0.12–0.25	0.12	0.25
	Cephalexin	>128	>128	>128
	Piperacillin	2–16	2	16
	Imipenem	2–8	4	8
Pseudomonas aeruginosa (30)	WIN 57273	1–4	2	4
	Ciprofloxacin	0.12–0.5	0.25	0.5
	Piperacillin	4–128	8	16
	Ceftazidime	1–64	2	4
	Imipenem	4–32	4	16
Pseudomonas cepacia (10)	WIN 57273	0.12–1	0.25	0.25
	Ciprofloxacin	0.06–1	0.25	0.5
	Piperacillin	1–>128	64	>128
	Ceftazidime	2–16	8	16
	Imipenem	0.25–>128	2	32
Pseudomonas (*Xanthomonas*) *maltophilia* (20)	WIN 57273	1–8	2	4
	Ciprofloxacin	1–16	2	8
	Piperacillin	32–>128	>128	>128
	Ceftazidime	2–>128	64	>128
	Imipenem	>128	>128	>128
Serratia marcescens (20)	WIN 57273	0.25–32	2	8
	Ciprofloxacin	0.12–4	0.5	2
	Cephalexin	>128	>128	>128
	Piperacillin	1–>128	8	32
	Imipenem	1–8	2	4
Bacteroides fragilis (20)	WIN 57273	0.12–0.25	0.25	0.25
	Ciprofloxacin	4–16	4	8
	Clindamycin	≤0.06–>128	1	4
	Ampicillin	2–>128	32	>128
	Imipenem	4–16	4	8
Bacteroides melaninogenicus (9)	WIN 57273	0.12–0.5	0.25	0.25
	Ciprofloxacin	0.5–4	0.5	1
	Clindamycin	≤0.06	≤0.06	≤0.06
	Ampicillin	≤0.06–16	4	8
	Imipenem	0.03–0.06	0.06	0.06
Peptococci (10)	WIN 57273	0.015–0.5	0.03	0.12
	Ciprofloxacin	0.25–8	0.5	2
	Clindamycin	≤0.06–>128	1	>128
	Ampicillin	≤0.06–2	0.5	1
	Imipenem	0.008–0.5	0.12	0.25

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TABLE 1—*Continued*

Organism (no.)	Antimicrobial agent	MIC (μg/ml)[a]		
		Range	50%	90%
Mycobacterium avium-M. intracellulare (30)[c]	WIN 57273	0.12–1.0	0.5	1.0
	Ciprofloxacin	0.25–8	1	4

[a] 50% and 90%, MICs for 50 and 90% of strains tested, respectively.
[b] Includes *L. pneumophila* (*n* = 14), *L. longbeachae* (*n* = 2), *L. bozemanii* (*n* = 2), *L. micdadei* (*n* = 1), and *L. dumoffii* (*n* = 1).
[c] Seven-day incubation.

Time-kill studies. The bactericidal activity of WIN 57273 against representative organisms was studied by previously described time-kill techniques (4). Starting inocula of 10^6 to 10^7 CFU/ml were prepared in 10-ml volumes of Mueller-Hinton broth to which the fluoroquinolone was added at multiples of the MIC. Samples of 0.5 ml were removed at 0, 4, 6, and 24 h of incubation for colony counts. Samples were collected on 0.45-μm-pore-size filters (Millipore Corp., Bedford, Mass.) and were washed with 5 ml of physiologic saline to prevent antibiotic carry-over. Organisms collected on filters were suspended in saline and serially diluted for colony counting, which was performed in duplicate.

Selection of resistant organisms. To determine whether resistance to WIN 57273 could be selected by exposure to subinhibitory concentrations of the drug, representative isolates were sequentially plated onto agar containing twofold incremental concentrations of WIN 57273 as described previously (4). Colonies from plates containing the highest drug concentration which permitted growth and colonies that underwent three serial passes on antibiotic-free plates were subjected to MIC determinations directly. To determine the frequencies of spontaneous resistance to WIN 57273, inocula of approximately 5×10^8 to 1×10^9 CFU were plated onto Mueller-Hinton agar containing WIN 57273 at 8× the MIC. Colonies emerging over the first 72 h of incubation were passed three times on antibiotic-free plates and subjected to MIC testing.

RESULTS

Susceptibility studies. Based on comparisons of MICs for 90% of strains tested (MIC_{90}s), WIN 57273 was 16-fold more active than ciprofloxacin against staphylococci and 4- to 128-fold more active than ciprofloxacin against streptococci, *Listeria monocytogenes*, and JK group corynebacteria (Table 1). Although ampicillin, imipenem, and clindamycin demonstrated activity at levels comparable to or even superior to that of WIN 57273 against organisms that were fully susceptible to each class of drugs, virtually all gram-positive organisms resistant to β-lactams or clindamycin retained susceptibility to the new quinolone. Among the gram-positive organisms tested, the only group not inhibited by ≤0.25 μg of WIN 57273 per ml was *Enterococcus faecium* (MIC_{90}, 4 μg/ml).

Among the gram-negative bacteria tested, the following demonstrated exquisite susceptibilities (MIC_{90}s, ≤0.06 μg/ml) to WIN 57273: *Branhamella catarrhalis*, *Haemophilus influenzae*, *Neisseria gonorrhoeae*, *Pasteurella multocida*, and *Legionella* spp. Against these organisms, WIN 57273 was at least as active as ciprofloxacin (MIC_{90}, ≤0.12 μg/ml). Against members of the family *Enterobacteriaceae* (Table 1), WIN 57273 was 4- to 16-fold less active than ciprofloxacin, based on comparisons of MIC_{90}s. The MIC_{90}s of WIN 57273 against each species of the family *Enterobacteriaceae* correlated closely with the MIC_{90}s of ciprofloxacin (r = 0.955, $P < 0.001$). WIN 57273 (MIC_{90}, 4 μg/ml) was less active than ciprofloxacin (MIC_{90}, 0.5 μg/ml) against *Pseudomonas aeruginosa*, but the two drugs demonstrated comparable efficacies against other pseudomonads, *Acinetobacter calcoaceticus* subsp. *anitratus*, and *Campylobacter jejuni*.

TABLE 2. Influence of medium pH on activity of WIN 57273

Organism[a]	Geometric mean MIC (μg/ml) at pH:			
	5.4	6.4	7.4	8.0
Staphylococcus aureus, methicillin susceptible	—[b]	0.004	0.004	0.004
Staphylococcus aureus, methicillin resistant	0.01	0.003	0.01	0.006
Enterococcus faecalis	0.12	0.03	0.04	0.08
Enterococcus faecium	0.14	0.07	0.07	0.14
Streptococcus pyogenes	—	0.06	0.07	0.14
Streptococcus pneumoniae, penicillin resistant	—	0.01	0.04	0.06
Escherichia coli	0.06	0.14	0.25	0.5
Serratia marcescens	0.66	0.76	1.5	5.3
Enterobacter cloacae	0.14	0.22	0.5	2
Citrobacter freundii	0.09	0.16	0.66	1.7
Acinetobacter calcoaceticus subsp. *anitratus*	0.07	0.09	0.37	1.1
Pseudomonas aeruginosa	0.66	0.87	1.7	6.1

[a] Five strains of each species were tested.
[b] —, pH did not support consistent growth of strains on antibiotic-free medium.

The new quinolone was significantly more active than ciprofloxacin against anaerobic bacteria, inhibiting all strains at concentrations of ≤0.5 μg/ml. WIN 57273 inhibited 30 strains of *Mycobacterium avium-M. intracellulare* at concentrations of between 0.12 and 1.0 μg/ml. Based on comparisons of MIC_{90}s, the drug was fourfold more active than ciprofloxacin (MIC_{90}, 4 μg/ml).

Against gram-positive organisms, a variation in the pH of between 5.4 and 8.0 had a minimal effect on WIN 57273 activity (Table 2). Against gram-negative bacteria, activities within this range varied up to 10-fold. The drug was more active at pH 5.4 than at pH 7.4.

Time-kill studies. The bactericidal activity of WIN 57273 was evaluated against two strains each of methicillin-resistant *Staphylococcus aureus*, coagulase-negative staphylococci, *Enterococcus faecalis*, *Escherichia coli*, and *Pseudomonas aeruginosa*. Drug was added at concentrations (0.015 to 2 μg/ml) equal to 4× the MIC against each strain as determined by agar dilution techniques. Against the gram-positive organisms, WIN 57273 demonstrated primarily bacteriostatic activity. Reductions in viable cells relative to the inoculum (in units of $\log_{10}$ CFU per milliliter, rounded to the nearest 0.5 unit) ranged from 0 to 1 (mean, 0.4) at 4 h of incubation, 0.5 to 1.5 (mean, 1.1) at 6 h of incubation, and 1 to 2 (mean, 1.5) at 24 h of incubation. Against four strains of methicillin-resistant *Staphylococcus aureus*, bactericidal activities of WIN 57273 and ciprofloxacin, each at 4× the MIC, were identical (1.5 $\log_{10}$ [two strains] and 2.0 $\log_{10}$ [two strains] CFU/ml reduction in viable cells at 24 h). In contrast, the quinolone demonstrated greater bactericidal activity at early time points against the gram-negative organisms. Reduction in viable cells ($\log_{10}$ CFU per milliliter) ranged from 2.5 to 3.0 (mean, 2.8) at 4 h of incubation and from 2.5 to 4 (mean, 3.4) at 6 h of incubation. By 24 h of incubation,

TABLE 3. Selection of colonies resistant to WIN 57273 by serial passage in incremental concentration of drug

Organism	MIC (μg/ml) for initial isolate	Maximum concn (μg/ml) permitting growth	MIC (μg/ml) after passage[a]
Staphylococcus aureus	0.015	4	1
Enterococcus faecalis	0.12	0.25	0.25
Escherichia coli	0.12	8	4
Pseudomonas aeruginosa	2	>64	16

[a] Colonies from plates containing the maximum concentrations of WIN 57273 which supported growth were passed three times on antibiotic-free plates and subjected to formal agar dilution MIC determinations.

however, each of the four strains demonstrated some regrowth, with the resulting colony counts averaging only 1.3 $\log_{10}$ CFU/ml below the count of the starting inoculum.

Selection of resistant organisms. One strain each of methicillin-resistant *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, and *Pseudomonas aeruginosa* was passed serially on plates containing twofold incremental concentrations of WIN 57273. Significant resistance to the drug could be selected for all strains except *Enterococcus faecalis* (Table 3). Frequencies of spontaneous resistance to the drug at 8× the MIC were $<1.7 \times 10^{-9}$ for *Pseudomonas aeruginosa*, $<1.9 \times 10^{-9}$ for *Escherichia coli*, $<4.2 \times 10^{-9}$ for *Enterococcus faecalis*, and $<3.7 \times 10^{-9}$ for one strain of methicillin-resistant *Staphylococcus aureus*. For a second strain of *Staphylococcus aureus*, a resistance frequency of 1.2×10^{-7} was observed. Resistant colonies subjected to formal testing demonstrated an MIC of 2 μg/ml (versus 0.015 μg/ml for the initial isolate). The frequencies of resistance to WIN 57273 and ciprofloxacin, each at 8× the MIC, were compared against five additional strains of methicillin-resistant *Staphylococcus aureus*. Resistance to WIN 57273 was found in one isolate (4.6×10^{-9}), and resistance to ciprofloxacin was found in three isolates (2.3×10^{-8}, 4.8×10^{-9}, 7.5×10^{-9}). By using a concentration of 2 μg of either drug per ml in selection plates, no strains resistant to WIN 57273 were detected among the seven isolates (resistance frequency, $<5.9 \times 10^{-9}$), whereas three strains yielded colonies resistant to ciprofloxacin at this concentration (1.2×10^{-8}, 1.2×10^{-8}, 1.0×10^{-9}).

DISCUSSION

Among the new fluoroquinolones, those agents which have been approved for use in humans or which have progressed to advanced clinical trials have generally demonstrated excellent activity against most members of the family *Enterobacteriaceae* and fastidious gram-negative bacteria but have shown lower levels of activity against anaerobic organisms and most gram-positive bacteria (3). Subsequent to the introduction of fluoroquinolones into clinical use, significant rates of resistance among methicillin-resistant staphylococci have been noted at some institutions (8, 15). Also, enterococcal colonization and superinfection have occurred during therapy with ciprofloxacin, which is the most active of the currently available agents against these organisms (19). Thus, there appears to be a role for new quinolone antimicrobial agents with improved activity against gram-positive organisms. That synthesis of such quinolone analogs is feasible was demonstrated several years ago with the development of CI-934, a drug which was two- to eightfold more potent than ciprofloxacin against various gram-positive bacteria (5). Recently, several agents of even greater potency have been described, including T-3262 (A-60969) (6, 7, 17) and its tosylate salt tosufloxacin (A-61827) (2), PD 117,558 (13), PD117,596 (16), PD 127,391 (10), and WIN 57273 (9).

This study demonstrated that WIN 57273 is substantially more active than ciprofloxacin against a variety of gram-positive organisms. Although the new drug was four- to eightfold less active than ciprofloxacin against many gram-negative isolates, it retained excellent potency against the more fastidious organisms. Also notable was the superior activity of the new drug over ciprofloxacin against *Legionella* spp. and *Mycobacterium avium-M. intracellulare* complex organisms. Studies examining the activity of WIN 57273 against intracellular organisms would be required to assess this observation further.

The constant or even improved activity of WIN 57273 at decreased pH levels stands in contrast to the response of the currently available quinolones, most of which demonstrate reduced activity at acid pH (3). This characteristic might be advantageous in the treatment of infections of the urinary tract, abscesses, or other sites at which conditions of reduced pH prevail.

Despite increased activity against gram-positive organisms, as with other fluoroquinolones we have tested (4), WIN 57273 was more slowly bactericidal against staphylococcal and enterococcal strains than against gram-negative organisms. Whether such observations are clinically relevant is unclear. Finally, while the frequencies of spontaneous resistance to the drug were generally low, resistant colonies of *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* could be readily selected by serial passage of strains through incremental concentrations of the drug.

Subject to further testing, WIN 57273 might offer advantages over ciprofloxacin against gram-positive bacteria and anaerobes and, possibly, over other agents against *Mycobacterium avium-M. intracellulare* and *Legionella* spp.

ACKNOWLEDGMENT

This study was supported by a grant from the Sterling-Winthrop Research Institute, Rensselaer, N.Y.

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