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WIN 57273, a New Fluoroquinolone with Enhanced In Vitro Activity versus Gram-Positive Pathogens

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WIN 57273 is a new fluoroquinolone with excellent in vitro activity versus gram-positive pathogens, including methicillin-susceptible and -resistant *Staphylococcus aureus* and *Staphylococcus epidermidis* and gentamicin-susceptible and -resistant *Enterococcus faecalis*. We compared the microdilution MICs and MBCs of this compound to those of other antimicrobial agents for more than 30 clinical isolates of each of these groups of organisms and found that with few exceptions, it was at least 10 times more active than all other drugs tested. Selection for resistance to ciprofloxacin (≥5 μg/ml) or WIN 57273 (≥0.16 μg/ml) by the gradient plate method produced mutants with diminished susceptibility to the other fluoroquinolone; however, the MICs and MBCs of WIN 57273 for such strains were still quite low and remained below the preliminary susceptibility breakpoint (≤2 μg/ml). Spontaneous mutations conferring resistance to two and five times the WIN 57273 MIC were detectable at low frequencies for *S. aureus* and *S. epidermidis*; such mutations were virtually undetectable for *E. faecalis*. Further testing is necessary to establish if the effectiveness of WIN 57273 is maintained in vivo, first in animals and then in humans with infections caused by methicillin-susceptible and -resistant strains of *S. aureus* and *S. epidermidis* or gentamicin-susceptible and -resistant strains of *E. faecalis*.

Fluoroquinolones are potent antimicrobial agents that are active against many gram-positive and gram-negative bacteria (12). Recently, new compounds of this class that have improved activity against gram-positive organisms compared with the activities of currently available fluoroquinolones have been developed (2, 3, 10, 11). With the appearance of multiple-drug-resistant staphylococci and enterococci (including strains resistant to glycopeptides [7, 9]), the development of alternative agents with activity against such organisms is desirable.

WIN 57273 is a new fluoroquinolone with the structural formula 1-cyclopropyl-7-(2,6-dimethyl-4-pyridinyl)-6-fluoro-1,4-dihydro-4-oxo-3-quinolonecarboxylic acid. This compound is similar in structure to ciprofloxacin, differing only in the presence of a 2,6-dimethylated pyridinyl moiety rather than a piperazinyl moiety at position 7 of the quinolone nucleus. Two recently published studies demonstrated WIN 57273 to have remarkable activity against many gram-positive pathogens, including methicillin-susceptible and -resistant *Staphylococcus aureus* and *Staphylococcus epidermidis* and *Enterococcus faecalis* (4, 8). However, the bactericidal activity of the drug and its activity against fluoroquinolone-resistant gram-positive species were not extensively studied.

In an effort to better define the competency of WIN 57273 against gram-positive pathogens, we compared its inhibitory and bactericidal properties with those of several other antimicrobial agents against multiple clinical isolates of methicillin-susceptible and -resistant *S. aureus* and *S. epidermidis* and gentamicin-susceptible and -resistant *E. faecalis* (including β-lactamase-producing strains). We also examined the activity of WIN 57273 against organisms from each of the above groups selected for fluoroquinolone resistance.

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MATERIALS AND METHODS

Antimicrobial agents. WIN 57273 was supplied by Sterling-Winthrop Research Institute (Rensselaer, N.Y.). All other antimicrobial agents were obtained from their manufacturers and included ampicillin, nafcillin, imipenem, cefazolin, daptomycin, vancomycin, gentamicin, rifampin, ciprofloxacin, and ofloxacin.

Bacterial strains. The organisms used in this study were clinical isolates obtained mainly from the Detroit, Mich., area. Some strains of *E. faecalis* originated in New Haven, Conn. The methicillin susceptibility of staphylococci was determined by the oxacillin disk method (6). The gentamicin susceptibility or resistance of strains of *E. faecalis* was established by the method of Zervos et al. (13). Organisms used for quality assurance included *S. aureus* ATCC 29213 and *E. faecalis* ATCC 29212.

Determination of MICs and MBCs. MICs for an inoculum of 5×10^5 CFU/ml were determined by a microdilution method with Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.) cation-adjusted with Ca^{2+} and Mg^{2+} (5). The MIC was defined as the lowest concentration of antimicrobial agent inhibiting visible growth after 20 h of incubation at 35°C. MBCs, defined as the lowest concentrations of antimicrobial agents that killed ≥99.9% of the original inoculum, were determined by subculturing 0.01 ml from clear wells onto tryptic soy agar (Difco) plates.

MICs for use in the determination of spontaneous mutation frequencies (see below) were determined on Mueller-Hinton agar (Difco) with a spot inoculum of 10^4 CFU (5).

Time-kill studies. To determine the kinetics and the extent of the bactericidal effect of WIN 57273 against *E. faecalis*, time-kill studies were done with three gentamicin-susceptible and three gentamicin-resistant strains. Organisms were grown overnight in cation-adjusted Mueller-Hinton broth, followed by dilution to ~10^6 CFU/ml with fresh broth prewarmed to 35°C. WIN 57273 was added to a final con-

TABLE 1. Activity of WIN 57273 compared with activities of other agents against gram-positive pathogens

Organism (no. of isolates)	Agent	MIC (μg/ml)[a]			MBC (μg/ml)[a]		
		50%	90%	Range	50%	90%	Range
Staphylococcus aureus, methicillin susceptible (34)	Nafcillin	0.39	0.78	≤0.19–1.56	0.78	1.56	0.39–12.5
	Cefazolin	0.78	1.56	0.39–6.25	1.56	6.25	0.78–25
	Daptomycin	0.39	0.78	≤0.19–0.78	0.39	0.78	≤0.19–1.56
	Vancomycin	0.78	1.56	0.78–1.56	1.56	3.13	0.78–6.25
	Rifampin	≤0.002	≤0.002	≤0.002	0.63	1.25	0.031–1.25
	Ciprofloxacin	0.78	1.56	≤0.19–1.56	0.78	1.56	≤0.19–3.13
	Ofloxacin	0.39	0.78	≤0.19–1.56	0.78	1.56	0.39–3.13
	WIN 57273	0.008	0.016	≤0.002–0.031	0.016	0.063	0.004–0.063
Staphylococcus aureus, methicillin resistant (33)	Nafcillin	50	100	6.25–100	100	>100	25–>100
	Cefazolin	100	>100	6.25–>100	>100	>100	50–>100
	Daptomycin	0.39	0.78	≤0.19–0.78	0.39	0.78	≤0.19–1.56
	Vancomycin	0.78	1.56	0.78–1.56	1.56	3.13	0.78–6.25
	Rifampin	≤0.002	≤0.002	≤0.002–0.08	0.31	1.25	0.063–1.25
	Ciprofloxacin	0.78	1.56	≤0.19–1.56	1.56	3.13	≤0.19–6.25
	Ofloxacin	0.39	0.78	≤0.19–3.13	1.56	1.56	0.78–3.13
	WIN 57273	0.004	0.008	≤0.002–0.008	0.008	0.016	0.004–0.031
Staphylococcus epidermidis, methicillin susceptible (34)	Nafcillin	≤0.19	0.78	≤0.19–1.56	0.39	3.13	≤0.19–3.13
	Cefazolin	0.39	0.78	≤0.19–3.13	0.78	3.13	≤0.19–12.5
	Daptomycin	≤0.19	0.39	≤0.19–0.39	0.39	1.56	≤0.19–12.5
	Vancomycin	1.56	1.56	0.39–3.13	3.13	6.25	0.78–12.5
	Rifampin	≤0.002	0.004	≤0.002–0.016	0.31	0.63	0.031–1.25
	Ciprofloxacin	0.39	0.78	≤0.19–12.5	0.78	3.13	≤0.19–50
	Ofloxacin	0.78	0.78	≤0.19–12.5	0.78	1.56	0.39–50
	WIN 57273	0.016	0.031	0.004–1.25	0.031	0.063	0.004–1.25
Staphylococcus epidermidis, methicillin resistant (33)	Nafcillin	3.13	50	1.56–>100	50	100	3.13–>100
	Cefazolin	3.13	25	1.56–100	50	100	3.13–>100
	Daptomycin	≤0.19	0.39	≤0.19–0.78	0.39	1.56	≤0.19–1.56
	Vancomycin	1.56	3.13	0.39–6.25	3.13	6.25	0.78–12.5
	Rifampin	≤0.002	0.008	≤0.002–>1.25	0.31	1.25	0.016–>1.25
	Ciprofloxacin	0.39	0.78	≤0.19–0.78	0.78	3.13	≤0.19–3.13
	Ofloxacin	0.39	0.78	≤0.19–1.56	1.56	3.13	0.39–12.5
	WIN 57273	0.008	0.016	≤0.002–0.031	0.016	0.13	≤0.002–0.25
Enterococcus faecalis, gentamicin susceptible (33)	Ampicillin	0.78	1.56	0.39–1.56	1.56	12.5	0.78–100
	Imipenem	0.78	1.56	0.39–1.56	1.56	3.13	0.39–50
	Daptomycin	0.78	1.56	≤0.19–1.56	6.25	12.5	3.13–12.5
	Vancomycin	1.56	3.13	0.39–3.13	50	100	12.5–100
	Gentamicin	15.6	31.3	7.8–31.3	31.3	31.3	15.6–62.5
	Ciprofloxacin	1.56	3.13	0.78–3.13	3.13	25	1.56–100
	Ofloxacin	3.13	6.25	1.56–6.25	12.5	25	3.13–100
	WIN 57273	0.08	0.16	0.04–0.16	0.16	1.25	0.04–2.5
Enterococcus faecalis, gentamicin resistant (31)	Ampicillin	1.56	1.56	0.78–3.13	3.13	6.25	0.78–100
	Imipenem	0.78	1.56	0.39–3.13	3.13	25	1.56–100
	Daptomycin	0.78	0.78	≤0.19–0.78	6.25	12.5	0.78–25
	Vancomycin	0.78	1.56	0.78–3.13	50	100	25–100
	Gentamicin	>1,000	>1,000	>1,000	>1,000	>1,000	>1,000
	Ciprofloxacin	0.78	1.56	0.39–1.56	6.25	50	1.56–100
	Ofloxacin	3.13	6.25	1.56–6.25	50	100	12.5–100
	WIN 57273	0.04	0.08	≤0.02–0.08	0.16	1.25	0.04–2.5

[a] 50% and 90%, MIC or MBC for 50 and 90% of isolates, respectively.

centration of 2 μg/ml, and the cultures were incubated with agitation at 35°C. Parallel cultures containing no antimicrobial agent served as controls. Colony counts were determined on a frequent basis by serial dilution and plating techniques. Antibiotic carry-over was eliminated by use of a minimum dilution factor of at least 100.

Selection of fluoroquinolone-resistant isolates. Randomly selected isolates of methicillin-susceptible and -resistant *S. aureus* and *S. epidermidis* were selected for resistance to ≥5 μg of ciprofloxacin per ml or ≥0.16 μg of WIN 57273 per ml by the gradient plate method (1). Gentamicin-susceptible and -resistant strains of *E. faecalis* were selected for resistance to ciprofloxacin only by the same method. After selection, the appropriate panels of MICs and MBCs were repeated and the results were expressed as geometric means.

Determination of spontaneous mutation frequencies. Five randomly selected strains from each group of organisms were studied. Organisms were grown in cation-adjusted Mueller-Hinton broth until mid-exponential growth phase was achieved. The strains were then concentrated by centrifugation, and ~10^{10} CFU were plated onto Mueller-Hinton agar plates containing ciprofloxacin or WIN 57273 at

TABLE 2. Comparative in vitro activities of WIN 57273 and other agents against gram-positive pathogens before and after selection for ciprofloxacin resistance

Organism (no. of isolates)	Agent	Preselection susceptibility[a]		Postselection susceptibility[a]			
		MIC	MBC	MIC	Rise[b]	MBC	Rise
Staphylococcus aureus, methicillin susceptible (10)	Ciprofloxacin	0.84	1.03	23.33	28	68.99	67
	Ofloxacin	0.59	0.78	12.5	21	17.68	23
	WIN 57273	0.006	0.015	0.17	28	0.41	27
Staphylococcus aureus, methicillin resistant (10)	Ciprofloxacin	0.63	1.18	28.72	46	73.94	63
	Ofloxacin	0.45	1.27	13.4	30	17.68	14
	WIN 57273	0.004	0.01	0.10	24	0.15	14
Staphylococcus epidermidis, methicillin susceptible (8)	Ciprofloxacin	0.33	0.43	16.21	49	42.04	98
	Ofloxacin	0.60	1.02	12.5	21	33.34	33
	WIN 57273	0.011	0.017	0.16	15	0.46	27
Staphylococcus epidermidis, methicillin resistant (10)	Ciprofloxacin	0.33	0.72	10.88	33	43.53	60
	Ofloxacin	0.63	1.53	10.15	16	26.79	18
	WIN 57273	0.009	0.022	0.07	8	0.20	9
Enterococcus faecalis, gentamicin susceptible (10)	Ciprofloxacin	1.18	6.26	13.4	11	50	8
	Ofloxacin	4.13	11.66	13.4	4	50	4
	WIN 57273	0.068	0.21	0.25	4	1.09	5
Enterococcus faecalis, gentamicin resistant (10)	Ciprofloxacin	0.78	6.70	17.68	23	65.98	10
	Ofloxacin	3.13	35.36	25	8	81.23	2
	WIN 57273	0.039	0.18	0.41	11	0.95	5

[a] Geometric mean in micrograms per milliliter.
[b] Multiple of preselection MIC or MBC.

two or five times the appropriate agar dilution MIC. Colonies were counted after 48 h of incubation at 35°C, and results were expressed as geometric means. Resistance to the selecting agent was verified by determining the MICs for several colonies from each plate.

RESULTS

Inhibitory and bactericidal activity of WIN 57273. The activity of WIN 57273 versus those of other antimicrobial agents against more than 60 strains each of *S. aureus*, *S. epidermidis*, and *E. faecalis* is shown in Table 1. Not included in the table are four gentamicin-resistant and one gentamicin-susceptible β-lactamase-producing strains of *E. faecalis*; for these strains, the MICs and MBCs of all drugs tested were similar to those shown in Table 1 for non-β-lactamase producing strains. With the exception of rifampin, WIN 57273 was 10 to 20 times more active than all other drugs tested (including ciprofloxacin and ofloxacin) against *S. aureus* and *S. epidermidis*, regardless of methicillin susceptibility. The new fluoroquinolone generally was 10 times more active than comparison drugs against all tested strains of *E. faecalis*, with equal activity against gentamicin-susceptible and -resistant strains.

Time-kill studies. In time-kill analyses, ≥99.9% of the original inocula of two of three gentamicin-susceptible and one of three gentamicin-resistant strains of *E. faecalis* were killed after 8 h of exposure to 2 μg of WIN 57273 per ml (data not shown). All isolates but one (a gentamicin-resistant strain) were killed to this degree by 24 h.

Activity of WIN 57273 against fluoroquinolone-resistant strains. MIC and MBC determinations for organisms before and after selection for resistance to ≥5 μg of ciprofloxacin per ml are shown in Table 2. Such organisms demonstrated no changes in MICs or MBCs of nonfluoroquinolone agents but did show diminished susceptibility to ofloxacin and WIN 57273. However, the MICs and MBCs of WIN 57273 were still quite low and remained below the preliminary susceptibility breakpoint of 2 μg/ml. Of interest was the fact that the rises in MICs and MBCs of ofloxacin and WIN 57273 were proportionally lower than those of ciprofloxacin.

Table 3 shows pre- and postselection MICs and MBCs for strains of *S. aureus* and *S. epidermidis* when WIN 57273 was used as the selecting agent. Postselection strains were less susceptible to ciprofloxacin, but rises in MICs and MBCs were proportionally lower than those seen for WIN 57273. As was true for strains selected for resistance to ciprofloxacin, strains selected for resistance to WIN 57273 were still inhibited and killed by less than 2 μg of the drug per ml.

Spontaneous mutation frequencies. The geometric mean frequencies of mutations conferring resistance to two and five times the ciprofloxacin MIC were 3.5×10^{-9} and $<10^{-10}$ for methicillin-susceptible strains of *S. aureus*. The corresponding results for WIN 57273 were 1.0×10^{-7} and 6.4×10^{-9}. Similar frequencies were found at two and five times the ciprofloxacin and WIN 57273 MICs for methicillin-resistant isolates (ciprofloxacin, 5.0×10^{-9} and $<10^{-10}$; WIN 57273, 4.9×10^{-8} and 6.0×10^{-9}).

For *S. epidermidis*, the frequencies of mutation to ciprofloxacin resistance for methicillin-susceptible isolates were 2.8×10^{-6} and 2.0×10^{-10} at two and five times the MIC, respectively. The corresponding frequencies for WIN 57273 were 3.9×10^{-8} and 7.0×10^{-10}. The mutation frequencies for both drugs were somewhat lower at both concentrations for methicillin-resistant strains (ciprofloxacin, 7.1×10^{-9} and $<10^{-10}$; WIN 57273, 3.0×10^{-9} and $<10^{-10}$).

Mutations to ciprofloxacin resistance were undetectable for *E. faecalis*. Mutation to WIN 57273 resistance was

TABLE 3. Activities of WIN 57273 and ciprofloxacin against *Staphylococcus* spp. before and after selection for WIN 57273 resistance

Organism (no. of isolates)	Agent	Preselection susceptibility[a]		Postselection susceptibility[a]			
		MIC	MBC	MIC	Rise[b]	MBC	Rise
Staphylococcus aureus, methicillin susceptible (5)	Ciprofloxacin	0.90	1.03	7.18	8	18.95	18
	WIN 57273	0.007	0.007	0.47	67	1.44	206
Staphylococcus aureus, methicillin resistant (5)	Ciprofloxacin	0.78	1.56	5.44	7	8.25	5
	WIN 57273	0.004	0.004	0.54	135	0.95	238
Staphylococcus epidermidis, methicillin susceptible (5)	Ciprofloxacin	0.39	0.59	1.36	3	3.59	6
	WIN 57273	0.016	0.027	0.41	26	1.09	40
Staphylococcus epidermidis, methicillin resistant (5)	Ciprofloxacin	0.34	0.68	0.94	3	1.79	3
	WIN 57273	0.009	0.016	0.24	27	0.47	29

[a] Geometric mean in micrograms per milliliter.
[b] Multiple of preselection MIC or MBC.

detectable in only one gentamicin-resistant strain of *E. faecalis* at two times the MIC (mutation frequency, 5.3×10^{-10}).

DISCUSSION

WIN 57273 is a new fluoroquinolone differing from other members of this class of antimicrobial agents by its enhanced activity versus *S. aureus*, *S. epidermidis*, and *E. faecalis*. Our results corroborate and extend those of earlier studies and establish the fact that compared with many, if not all, currently available fluoroquinolones (both marketed and investigational), WIN 57273 has equivalent, and in most cases greater, activity against these organisms, including strains resistant to multiple antibiotics (2–4, 8, 10, 11). However, as is true for all currently available antimicrobial agents, some tolerance to the bactericidal effect of WIN 57273 was demonstrated by selected strains of *E. faecalis*. Time-kill studies established that, for the strains evaluated in this study, the drug generally does kill *E. faecalis* at a relatively low concentration, but the rate of killing is variable and strain dependent and can be quite slow.

WIN 57273 maintains good activity against strains of *S. aureus*, *S. epidermidis*, and *E. faecalis* selected for diminished susceptibility to fluoroquinolones. An interesting finding was that the rises in MICs and MBCs in such strains were proportionally higher for the agent used in the selection process. This suggests that the change(s) that occurred in resistant strains was somewhat drug specific. The mechanism of this phenomenon deserves further study.

Spontaneous, and probably single-step, in vitro mutations conferring diminished susceptibility to WIN 57273 occurred with a variable frequency among strains of *S. aureus* and *S. epidermidis* and occurred rarely among strains of *E. faecalis*. For *S. aureus*, mutation frequencies tended to be somewhat higher for WIN 57273 than for ciprofloxacin, but only a small number of strains were tested. However, it is clear that a concentration five times the MIC for most strains of *S. aureus* and *S. epidermidis* is significantly below the preliminary susceptibility breakpoint of 2 μg/ml. On the basis of our results, it appears unlikely that spontaneous mutants resistant to ≥2 μg/ml will occur in vivo.

WIN 57273 has been shown to be superior to ciprofloxacin and ofloxacin with a murine infection model in which 50% protective doses were determined for selected strains of *S. aureus*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, and *Listeria monocytogenes* (8). Additional work along these lines is necessary to define further the in vivo activity of WIN 57273. The potency of this antimicrobial agent against fluoroquinolone-susceptible and -resistant strains of *S. aureus*, *S. epidermidis*, and *E. faecalis* suggests that it may have clinical utility in infections in which such organisms are etiologic. This possibility requires in vivo testing in experimental infections with these organisms. If the in vitro effectiveness of WIN 57273 is maintained in vivo and if its toxicity profile and achievable levels in serum are favorable, this fluoroquinolone holds much promise for therapy of infections caused by certain gram-positive organisms and human trials with the drug would be in order.

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