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In Vitro Activities of Three of the Newer Quinolones against Anaerobic Bacteria

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The antimicrobial activities of three new quinolone compounds, sparfloxacin, temafloxacin, and WIN 57273, against anaerobic bacteria were determined in three separate studies. The Wadsworth agar dilution technique using brucella-laked blood agar was used throughout. The activities of other antimicrobial agents, including ciprofloxacin, imipenem, chloramphenicol, metronidazole, cefotetan, cefoxitin, and amoxicillin-clavulanic acid, were also determined. The breakpoints of the new quinolones were 2 μg/ml for sparfloxacin and WIN 57273 and 4 μg/ml for temafloxacin. WIN 57273 displayed very good activity against anaerobes, inhibiting all strains of *Bacteroides fragilis* group species at 2 μg/ml. Only two strains of *Fusobacterium* species were resistant (MIC, 4 μg/ml). Sparfloxacin inhibited 78% of *B. fragilis* strains and 44% of other *B. fragilis* group isolates at 2 μg/ml. At 2 μg/ml, the percentages of other anaerobic species susceptible were as follows: *B. gracilis*, 70%; other *Bacteroides* species, 61%; *Clostridium* species, 50%; *Fusobacterium* species, 70%; *Peptostreptococcus* species, 91%; non-spore-forming gram-positive rods, 71%. Temafloxacin inhibited 91% of *B. fragilis* strains and 87% of other *B. fragilis* group species at 4 μg/ml. All strains of other *Bacteroides* species, 78% of *Fusobacterium* species, 80% of *Clostridium* species, and 90% of *Peptostreptococcus* species were inhibited at 4 μg of temafloxacin per ml.

Bacteroides fragilis group organisms have been tested against a variety of quinolone agents, including lomefloxacin, ciprofloxacin, norfloxacin, pefloxacin, nalidixic acid, enoxacin, cinoxacin, difloxacin, ofloxacin, A-56620, and A-56619, and are generally considered resistant to these agents (1, 2, 4, 6, 7, 10, 11). This report is a summary of three studies that tested the efficacies of three new quinolones, WIN 57273, sparfloxacin, and temafloxacin, against anaerobes. The data are presented in a combined table; however, since the studies were done separately, each of the studies is presented separately to avoid direct comparisons. The sets of strains used in each of the studies had some overlap but were not identical. The other comparative agents tested in the studies included cefotetan, cefoxitin, chloramphenicol, imipenem, metronidazole, and amoxicillin-clavulanic acid.

All bacteria were randomly selected recent clinical isolates from the Veterans Administration Wadsworth Medical Center, Los Angeles, Calif. Bacteria were identified by established procedures (5, 9). MICs were determined by an agar dilution technique described previously (9), using an inoculum of 10^5 CFU and brucella base-laked blood agar. Plates were incubated in GasPak jars (BBL, Cockeysville, Md.) or in an anaerobic chamber (Anaerobe Systems, San Jose, Calif.) for 48 h at 37°C. MICs were defined as the lowest concentration of antimicrobial agent permitting no growth, one discrete colony, a barely visible haze, or any marked change from the growth control (8). Reference strains of *B. fragilis* (ATCC 25285) and *B. thetaiotaomicron* (ATCC 29741) were used as controls in each test. For sparfloxacin, temafloxacin, and WIN 57273, the following quality control values were applied: for *B. fragilis* (ATCC 25285), 1 to 4 μg/ml for sparfloxacin, 0.5 to 2 μg/ml for temafloxacin, and 0.25 to 1 μg/ml for WIN 57273; for *B. thetaiotaomicron* (ATCC 29741), 2 to 8 μg/ml for sparfloxacin, 2 to 8 μg/ml for temafloxacin, and 0.5 to 2 μg/ml for WIN 57273. Antimicrobial agents were obtained as powders from their respective manufacturers: chloramphenicol and sparfloxacin, Parke-Davis (Morris Plains, N.J.); ciprofloxacin, Miles Laboratories (Elkhart, Ind.); cefoxitin and imipenem, Merck Sharp & Dohme (Rahway, N.J.); metronidazole, Searle Laboratories (Chicago, Ill.); cefotetan, Stuart Pharmaceuticals (Wilmington, Del.); WIN 57273, Sterling Winthrop (Rensselaer, N.Y.); temafloxacin, Abbott Laboratories (North Chicago, Ill.).

B. fragilis is much more susceptible to many antimicrobial agents than are the other members of the *B. fragilis* group. It is often difficult to compare data from different laboratories if the same species of the group are not included to the same extent. To facilitate such comparisons, we report the results for *B. fragilis* alone and those for other species of the *B. fragilis* group. The currently used susceptibility testing techniques are considered to be accurate within ±1 twofold dilution. Also, MICs for organisms in the *B. fragilis* group and other anaerobes often cluster at or near breakpoint levels and excessive importance should not be attributed to differences of a few percentage points at a specific (e.g., breakpoint) concentration. Therefore, the percentage of strains susceptible is reported at the breakpoint (middle value) and at concentrations 1 twofold dilution above and below the breakpoint, as indicated in Table 1. The National Committee for Clinical Laboratory Standards-approved breakpoints are listed in boldface type in Table 1, footnote *c*. There are no approved breakpoints for sparfloxacin, WIN 57273, or temafloxacin; for comparative purposes, we assumed the same breakpoint as the other quinolone, ciprofloxacin (i.e., 2 μg/ml), except for temafloxacin (which has a Food and Drug Administration-approved breakpoint of 4 μg/ml).

Results of these studies are listed in Table 1. When enough

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TABLE 1. Activities of antimicrobial agents against various organisms

Organism(s), study (no. of strains tested)	Antimicrobial agent	MIC range (μg/ml)	MIC_{50}[a]	MIC_{90}[b]	% of strains susceptible[c]
Bacteroides fragilis					
A (73)	Sparfloxacin	0.5–4	2	4	27, 78, 100
	Amoxicillin-clavulanic acid	1–8	2	4	92, 100, 100
	Ciprofloxacin	2–>32	8	16	0, 1, 30
B (24)	WIN 57273	0.5–2	0.5	2	88, 100, 100
	Ciprofloxacin	4–64	8	32	0, 0, 25
C (22)	Temafloxacin	1–16	2	4	68, 91, 95
	Ciprofloxacin	4–32	4	16	0, 0, 59
Other *B. fragilis* group species					
A (81)	Sparfloxacin	1–16	4	4	6, 44, 91
	Amoxicillin-clavulanic acid	1–32	2	8	84, 93, 98
	Ciprofloxacin	1–>32	16	32	1, 2, 7
B (45)	WIN 57273	0.125–2	0.5	1	96, 100, 100
	Ciprofloxacin	1–64	16	32	7, 9, 18
C (31)	Temafloxacin	0.25–32	4	8	39, 87, 90
	Ciprofloxacin	4–128	16	32	0, 0, 6
Bacteroides gracilis, A (10)	Sparfloxacin	0.125–>32	0.25	32	70, 70, 70
	Amoxicillin-clavulanic acid	1–>128	16	>128	30, 40, 60
	Ciprofloxacin	0.125–>32	1	16	70, 70, 70
	Imipenem	0.5–>128	2	>128	50, 50, 60
	Metronidazole	1–32	2	32	80, 80, 100
Other *Bacteroides* species					
A (17)[d]	Sparfloxacin	0.5–4	2	2	35, 94, 100
	Amoxicillin-clavulanic acid	1–4	1	4	100, 100, 100
	Ciprofloxacin	0.25–16	4	8	41, 41, 88
C (7)[e]	Temafloxacin	0.0625–4	0.5	NA[f]	71, 100, 100
	Cefotetan	0.125–64	8	NA	86, 86, 100
	Cefoxitin	1–256	4	NA	86, 86, 86
	Ciprofloxacin	0.125–16	0.5	NA	57, 71, 71
Prevotella species, A (24)[g]	Sparfloxacin	2–16	4	8	0, 38, 75
	Amoxicillin-clavulanic acid	1–8	1	4	96, 100, 100
	Ciprofloxacin	1–32	2	16	25, 63, 75
Fusobacterium nucleatum					
A (19)	Sparfloxacin	1–16	2	4	26, 89, 95
	Amoxicillin-clavulanic acid	1–2	1	1	100, 100, 100
	Ciprofloxacin	1–>32	4	4	5, 26, 95
B (8)	WIN 57273	0.0625–1	0.125	NA	100, 100, 100
	Ciprofloxacin	1–16	4	NA	13, 38, 88
Fusobacterium mortiferum-F. varium group					
A (7)	Sparfloxacin	2–16	16	NA	0, 29, 29
	Amoxicillin-clavulanic acid	2–16	4	NA	71, 86, 100
	Ciprofloxacin	2–16	8	NA	0, 14, 29
B (6)	WIN 57273	0.25–4	2	NA	17, 67, 100
	Ciprofloxacin	4–16	8	NA	0, 0, 17
C (9)	Temafloxacin	1–16	2	NA	78, 78, 89
	Cefotetan	2–64	4	NA	89, 89, 100
	Cefoxitin	1–64	4	NA	89, 89, 100
	Ciprofloxacin	2–32	4	NA	0, 44, 78
Other *Fusobacterium* species					
A (7)[h]	Sparfloxacin	1–8	2	NA	14, 57, 86
	Amoxicillin-clavulanic acid	1–16	1	NA	86, 86, 100
	Ciprofloxacin	2–4	4	NA	0, 29, 100
B (5)[i]	WIN 57273	0.0625–0.5	0.125	NA	100, 100, 100
	Ciprofloxacin	4–4	4	NA	0, 0, 100
Clostridium difficile, A (8)	Sparfloxacin	1–8	8	NA	11, 11, 11
	Chloramphenicol	1–32	8	NA	67, 67, 100
	Ciprofloxacin	2–16	8	NA	0, 11, 11
Clostridium perfringens, A (11)	Sparfloxacin	0.125–8	1	2	64, 91, 91

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TABLE 1.—*Continued*

Organism(s), study (no. of strains tested)	Antimicrobial agent	MIC range (μg/ml)	MIC_{50}[a]	MIC_{90}[b]	% of strains susceptible[c]
	Ciprofloxacin	1–16	2	4	18, 64, 91
	Imipenem	0.5–16	0.5	1	91, 91,100
Clostridium ramosum, A (5)	Sparfloxacin	4–8	8	NA	0, 0, 20
	Ciprofloxacin	8–16	8	NA	0, 0, 0
Clostridium species					
A (9)[j]	Sparfloxacin	0.25–4	0.5	NA	67, 67,100
	Ciprofloxacin	0.125–8	0.5	NA	67, 78, 89
B (21)[k]	WIN 57273	0.0625–1	0.5	1	100,100,100
	Chloramphenicol	1–16	4	16	73,100,100
	Ciprofloxacin	0.25–128	8	64	29, 33, 43
C (10)[l]	Temafloxacin	0.25–8	2	8	50, 80,100
	Cefotetan	2–256	8	256	70, 70, 80
	Cefoxitin	2–128	8	128	50, 60, 70
	Ciprofloxacin	0.25–16	2	16	40, 50, 50
Peptostreptococcus species					
A (22)[m]	Sparfloxacin	0.25–8	1	2	74, 91, 96
	Amoxicillin-clavulanic acid	1–32	1	16	83, 88, 96
	Ciprofloxacin	0.5–8	1	4	54, 79, 96
B (10)[n]	Temafloxacin	0.25–8	1	1	90, 90,100
	Ciprofloxacin	0.5–4	1	2	70, 90,100
	Metronidazole	0.5–4	1	1	100,100,100
Gram-positive rods (non-spore forming)					
A (24)[o]	Sparfloxacin	0.25–32	1	8	54, 71, 83
	Amoxicillin-clavulanic acid	1–1	1	1	100,100,100
	Ciprofloxacin	0.25–32	2	16	32, 80, 84
B (18)[p]	WIN 57273	0.0625–1	0.125	0.5	100,100,100
	Ciprofloxacin	0.25–32	2	16	22, 67, 83
C (10)[q]	Temafloxacin	0.25–2	0.5	2	100,100,100
	Cefotetan	1–128	16	128	50, 50, 70
	Cefoxitin	0.125–8	2	8	100,100,100
	Ciprofloxacin	0.5–4	1	2	60, 90,100

[a] MIC_{50}, concentration at which 50% of the strains tested were susceptible (in micrograms per milliliter).
[b] MIC_{90}, concentration at which 90% of the strains tested were susceptible (in micrograms per milliliter).
[c] At concentrations 1 dilution below the breakpoint (first value), at the breakpoint (middle value), and 1 dilution above the breakpoint (last value). The breakpoints used were as follows (National Committee for Clinical Laboratory Standards-approved breakpoints are in boldface): amoxicillin-clavulanic acid, **8/4**; cefotetan, **32**; cefoxitin, **32**; ciprofloxacin, **2**; imipenem, **8**; chloramphenicol, **16**; metronidazole, **16**; sparfloxacin, 2; temafloxacin, 4; WIN 57273, 2 μg/ml.
[d] Includes 11 *B. splanchnicus* and 6 *B. ureolyticus* strains.
[e] Includes three *B. ureolyticus*, one *B. pneumosintes*, one *Porphyromonas asaccharolytica*, and two *Prevotella bivia* strains.
[f] NA, not applicable. When fewer than 10 strains were tested, no MIC_{90} was calculated.
[g] Includes seven *P. bivia*, nine *P. intermedia*, and three *P. oralis* strains and 1 strain each of *P. buccae*, *P. corporis*, *P. denticola*, *P. loescheii*, and *P. melaninogenica*.
[h] Includes one *F. gonidiaformans*, three *F. necrophorum*, and three *Fusobacterium* species strains.
[i] Includes one *F. necrophorum*, one *F. gonidiaformans*, and three *Fusobacterium* species strains.
[j] Includes six *C. sporogenes* and three *C. sordellii* strains.
[k] Includes three *C. ramosum*, one *C. perfringens*, one *C. butyricum*, one *C. cadaveris*, one *C. clostridiiforme*, four *C. innocuum*, one *C. leptum*, one *C. tertium*, three *C. sporogenes*, and five *Clostridium* species strains.
[l] Includes two *C. difficile*, one *C. innocuum*, one *C. cadaveris*, two *C. septicum*, one *C. sporogenes*, one *C. ramosum*, one *C. perfringens*, and one *Clostridium* species strains.
[m] Includes four *P. anaerobius*, one *P. asaccharolyticus*, seven *P. magnus*, one *P. prevotii*, eight *P. micros*, and one *Peptostreptococcus* species strains.
[n] Includes four *P. magnus*, two *P. prevotii*, one *P. tetradius*, one *P. anaerobius*, one *P. asaccharolyticus*, and one *Peptostreptococcus* species strains.
[o] Includes four *Actinomyces israelii*, three *Eubacterium lentum*, six *Eubacterium* species, six *Lactobacillus* species, and five *Propionibacterium acnes* strains.
[p] Includes two *Actinomyces israelii*, four *Eubacterium lentum*, one *E. limosum*, two *Eubacterium* species, three *Lactobacillus* species, five *Propionibacterium acnes*, and one *Propionibacterium* species strains.
[q] Includes nine *Eubacterium* species and one *Lactobacillus* species strains.

strains of one species were tested to give meaningful results, those data are listed separately. The particular species tested for each genus are listed in footnotes. In a few cases, even if fewer than 10 strains were tested, the results were computed separately if they were very different from the results of the rest of the group. Activities of other antimicrobial agents, including amoxicillin-clavulanic acid (study A), chloramphenicol (studies A and B), ciprofloxacin (studies A, B, and C), imipenem (studies A and B), metronidazole (studies A, B, and C), cefotetan (study C), and cefoxitin (study C), were also determined. In cases in which these agents were active against all of the strains tested, consistent with other published reports (3, 13), the data are not included in Table 1 but are discussed in the text. The values for ciprofloxacin

derived from each study are listed separately so that the investigational quinolones may be directly compared with this agent.

Sparfloxacin at 2 μg/ml inhibited 78% of *B. fragilis* strains and 44% of other *B. fragilis* group isolates. At 2 μg/ml, the percentages of other anaerobic gram-negative rods that were susceptible were as follows: *B. gracilis*, 70%; other *Bacteroides* species, 61%; *Fusobacterium* species, 70%. WIN 57273 displayed very good activity against anaerobes, inhibiting all strains of *B. fragilis* group species at 2 μg/ml. Only two strains of the *Fusobacterium mortiferum-F. varium* group were resistant (MIC, 4 μg/ml). Temafloxacin inhibited 91% of the *B. fragilis* strains and 87% of the other *B. fragilis* group species at 4 μg/ml. All strains of the other *Bacteroides* species and 78% of the *Fusobacterium* species were inhibited at 4 μg of temofloxacin per ml. The strains of the *F. mortiferum-F. varium* group were notably more resistant to all of the quinolone agents tested than was either *F. nucleatum* or the other *Fusobacterium* species and are thus reported separately, even when fewer than 10 strains were tested. The activities of the other comparative agents against gram-negative anaerobic bacteria were consistent with previous reports (12). Imipenem and metronidazole inhibited all strains of the *B. fragilis* group, and chloramphenicol inhibited all but one strain (MIC, 32 μg/ml). Cefoxitin and cefotetan both inhibited 95 to 100% of the *B. fragilis* strains; cefoxitin inhibited 87% of the other *B. fragilis* group species, while cefotetan inhibited only 39%. Of the other *Bacteroides* species, both cefoxitin and cefotetan inhibited 86%. Of the 10 strains of *B. gracilis* tested, imipenem inhibited 50% and metronidazole inhibited 80%. All strains of the other *Bacteroides* species (except *B. gracilis*), *Prevotella* species, and *Fusobacterium* species were inhibited by imipenem, metronidazole, and chloramphenicol.

Sparfloxacin inhibited 91% of the *Peptostreptococcus* sp. strains, 50% of the *Clostridium* species strains, and 71% of the non-spore-forming gram-positive rods at 2 μg/ml. Temafloxacin inhibited 90% of the *Peptostreptococcus* sp. strains at 4 μg/ml. The five strains of *Clostridium ramosum* tested were all resistant to both sparfloxacin and ciprofloxacin, while the other *Clostridium* species tested were 67 and 78% susceptible, respectively, to the two agents. Because of the notably different results obtained with *C. ramosum*, the data are reported separately. The three strains of *C. ramosum* tested with WIN 57273 (data not shown separately) were susceptible to ≤1 μg of the agent per ml. The comparative agents behaved as expected from previous studies (12, 13). Imipenem and metronidazole were active against nearly all of the strains of *Clostridium* and *Peptostreptococcus* species tested: for one strain of *C. perfringens*, the MIC of imipenem was 16 μg/ml, and one strain of *C. leptum* was resistant to metronidazole. Amoxicillin-clavulanic acid inhibited all of the strains of *Clostridium* species tested. Chloramphenicol inhibited all strains of *Peptostreptococcus* sp., non-spore-forming gram-positive rods, and *Clostridium* species other than *C. difficile* (67% of the strains of *C. difficile* were inhibited at 16 μg/ml). Imipenem inhibited all strains of non-spore-forming gram-positive rods, while metronidazole inhibited ~50% at breakpoint concentrations. Cefoxitin and cefotetan both inhibited all of the strains of *Peptostreptococcus* spp. tested.

WIN 57273 was the most active overall of the three investigational quinolones, inhibiting 99% of anaerobic bacteria at 2 μg/ml (compared with 89% for temafloxacin at 4 μg/ml and 63% for sparfloxacin at 2 μg/ml.) Chloramphenicol and imipenem both inhibited 98 to 100% of all anaerobes at breakpoint concentrations, while metronidazole inhibited 93 to 98% of the strains tested. Cefoxitin and cefotetan inhibited 91 and 71%, respectively, of the strains tested. Ciprofloxacin exhibited poor activity against anaerobes, inhibiting 20 to 33% of the strains at breakpoint levels.

B. fragilis group organisms have been tested with a variety of quinolone agents and are generally considered resistant (1, 2, 4, 6, 7, 10, 11). Although one study reported that all of the isolates of *B. fragilis* tested were susceptible to ciprofloxacin and ofloxacin at 16 μg/ml and 68 and 81% of isolates were susceptible to less than 4 μg/ml (3), the current breakpoint for most of these agents is 2 μg/ml. Activity against the other *Bacteroides* spp., such as the *B. melaninogenicus-B. oralis* group and *B. ureolyticus*, is generally more variable (6). One study found good activity of CI-934 against the gram-positive anaerobic cocci and found that ofloxacin, ciprofloxacin, A-56619, and A-56620 had good-to-moderate activity against all species of anaerobes except the *B. fragilis* group (7). Synergy of ciprofloxacin with clindamycin and cefotaxime has been noted (14).

The improved activity of some of the newer quinolone agents against anaerobic bacteria raises the possibility of using these compounds for therapy of mixed infections involving anaerobes. Clinical trials will be required to define their role.

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