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NOTES

Time-Kill Studies of the Antianaerobe Activity of Garenoxacin Compared with Those of Nine Other Agents

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The activities of garenoxacin, ciprofloxacin, levofloxacin, moxifloxacin, trovafloxacin, amoxicillin-clavulanate, piperacillin-tazobactam, imipenem, clindamycin, and metronidazole against 20 anaerobes were tested. At two times the MIC, garenoxacin was bactericidal against 19 of 20 strains after 48 h and against 17 of 20 after 24 h. Other drugs, except clindamycin (which gave lower killing rates), gave killing rates similar to those for garenoxacin.

Although β-lactamase production, and concomitant resistance to β-lactams, is the norm for the *Bacteroides fragilis* group, other anaerobic gram-negative bacilli have become increasingly β-lactamase positive. β-Lactamase production in clostridia has also been described. Resistance to metronidazole in organisms other than non-spore-forming gram-positive bacilli has been described, as has resistance to clindamycin in anaerobic gram-negative bacilli (2–4, 14).

Currently available quinolones with significant antianaerobe activity include gatifloxacin (6) and moxifloxacin (13). Quinolones with increased antianaerobic activity whose use was discontinued or decreased due to toxicity include Bay y3118, clinafloxacin, sitafloxacin, and trovafloxacin (1). Because most anaerobic infections are a mixture of aerobic and anaerobic infections, empirical coverage of these infections must include aerobes and anaerobes.

Garenoxacin (BMS 284756) is a novel des-F(6)-quinolone with a broad spectrum of activity against gram-positive and -negative organisms (5, 7–11, 19). A previous study has documented excellent activity of garenoxacin against 357 anaerobes, with an MIC at which 50% of the isolates tested were inhibited of 0.5 μg/ml and an MIC at which 90% of the isolates tested were inhibited of 2.0 μg/ml. (13). The present study aims to shed more light upon the antianaerobic activity of garenoxacin by using agar dilution and time-kill studies to compare the antianaerobic activity of garenoxacin to those of ciprofloxacin, levofloxacin, moxifloxacin, trovafloxacin, amoxicillin-clavulanate, piperacillin-tazobactam, imipenem, clindamycin, and metronidazole.

Twenty anaerobes recently isolated from clinical specimens (Table 1) and chosen to represent organisms commonly encountered in human infections were used. Strains were identified by standardized methodology (18). Garenoxacin powder was obtained from Bristol Myers Squibb Laboratories, Wallingford, Conn., and other drugs were obtained from their respective companies.

MICs were determined by agar dilution according to NCCLS specifications (15). All of the plates were incubated in an anaerobic chamber (Sheldon Manufacturers, Cornelius, Oreg.) in an atmosphere of 90% N_2–5% H_2–5% CO_2. Clavulanate was added to amoxicillin in a 1:2 ratio, and tazobactam was added to piperacillin at a fixed concentration of 4.0 μg/ml. Oxyrase (Oxyrase, Inc., Mansfield, Ohio) cannot reliably be tested by microdilution (J. Copeland, personal communication), and therefore MICs determined by agar dilution were used as standards. In our studies, agar dilution and microdilution have consistently yielded MICs within one dilution of each other (16, 17).

Time-kill testing was performed as described previously (16, 17). Inocula were prepared inside the anaerobic chamber: a suspension equal to a McFarland standard of 1 was made by suspending five colonies from brucella agar plates in a tube containing 5 ml of prereduced brucella broth (Difco), and then the suspension was vortexed. A 100-μl aliquot of this suspension was delivered by syringe (to avoid the introduction of air) into each vial, which contained 2.9 ml of prereduced brucella broth (2.7 ml when 0.2 ml of Oxyrase was added) supplemented with additives (5% laked horse blood cells, 5 μg of hemin per ml, and 1 μg of vitamin K per ml) and 1 ml of antibiotic dilution prepared in prereduced brucella broth. The contents of the vials were mixed thoroughly. The final inoculum was 10^6 to 10^7 CFU/ml. All preparations and dilutions were made in the anaerobic chamber. The tubes were removed from the chamber and incubated for 48 h in a shaking water bath at 35°C. For all drugs except β-lactams, 200 μl of Oxyrase solution was added (16, 17). Oxyrase, an enzymatic component of the *Escherichia coli* cell membrane, contains a penicillin binding protein that may inactivate β-lactams and therefore cannot be used when the latter drugs are being tested (15, 16; J. Copeland, personal communication). Previous studies (16, 17) have shown that the addition of Oxyrase does not alter the MICs of non-β-lactam drugs tested in this study. Kill kinetics

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TABLE 1. MICs of drugs tested

Strain	MIC (μg/ml)									
	Garenoxacin	Ciprofloxacin	Levofloxacin	Moxifloxacin	Trovafloxacin	Amoxicillin-clavulanate	Piperacillin-tazobactam	Imipenem	Clindamycin	Metronidazole
Bacteroides fragilis	0.125	4.0	2.0	0.25	0.125	0.5	0.125	0.06	1.0	1.0
	0.25	8.0	1.0	0.25	0.5	0.5	0.25	0.25	0.25	2.0
Bacteroides thetaiotaomicron	0.5	8.0	4.0	1.0	1.0	0.5	4.0	0.25	0.5	1.0
	0.5	8.0	4.0	1.0	0.5	1.0	8.0	0.5	2.0	2.0
Bacteroides distasonis	0.5	4.0	2.0	0.5	0.5	8.0	16.0	1.0	4.0	0.5
	0.25	4.0	1.0	0.25	0.5	1.0	2.0	0.5	2.0	0.5
Prevotella bivia	2.0	16.0	8.0	4.0	4.0	0.03	≤0.016	0.03	0.016	0.5
	4.0	32.0	8.0	4.0	8.0	0.5	≤0.016	0.06	≤0.016	1.0
Prevotella disiens	0.25	1.0	1.0	0.5	2.0	0.5	≤0.016	0.06	≤0.016	1.0
	0.25	1.0	1.0	0.5	2.0	0.125	≤0.016	0.03	≤0.016	2.0
Prevotella intermedia	0.25	1.0	1.0	0.5	1.0	0.5	≤0.016	0.06	≤0.016	1.0
	0.25	1.0	1.0	1.0	2.0	0.5	≤0.016	0.06	≤0.016	1.0
Fusobacterium nucleatum	0.5	2.0	1.0	0.125	0.5	≤0.016	≤0.016	0.016	0.06	0.06
	1.0	2.0	1.0	0.25	0.5	0.03	≤0.016	0.06	0.016	0.5
Fusobacterium necrophorum	0.125	0.5	1.0	0.5	0.125	0.016	≤0.016	0.016	0.03	0.25
	0.125	1.0	1.0	1.0	0.5	0.016	≤0.016	≤0.016	0.03	0.5
Peptostreptococcus asaccharolyticus	0.25	0.5	0.5	0.125	0.25	0.25	0.125	0.06	0.06	2.0
Peptostreptococcus anaerobius	0.125	1.0	0.5	0.25	0.25	0.125	0.125	0.06	0.06	0.5
Clostridium perfringens	2.0	4.0	1.0	0.5	0.5	0.25	1.0	0.25	1.0	8.0
Clostridium difficile	2.0	16.0	8.0	2.0	4.0	0.5	8.0	2.0	4.0	0.5

data were analyzed by the Fisher exact test for all MICs and time periods.

One antibiotic-free growth control was used in each experiment. Suspensions were incubated in air at 35°C in a shaking water bath, and viability counts were performed at 0, 6, 12, 24, and 48 h while plates were incubated for 48 h inside the chamber. Data were analyzed by expressing growth as the $\Delta\log_{10}$ CFU per milliliter from that of the original inoculum at 0 h. Drug carryover was minimized by dilution as described previously (16, 17).

The ranges of drug MICs for the tested strains (Table 1) were as follows (in micrograms per milliliter): garenoxacin, 0.125 to 4.0; ciprofloxacin, 0.5 to 32.0; levofloxacin, 0.5 to 8.0; moxifloxacin, 0.125 to 4.0; trovafloxacin, 0.125 to 8.0; amoxicillin-clavulanate, ≤0.016 to 8.0; piperacillin-tazobactam, ≤0.016 to 16.0; imipenem, ≤0.016 to 2.0; clindamycin, ≤0.016 to 4.0; and metronidazole, 0.06 to 8.0.

Garenoxacin, at two times the MIC, was bactericidal against 19 of 20 strains after 48 h and against 17 of 20 strains after 24 h. Significant killing rates were also seen at earlier times. The kill kinetics of other quinolones, β-lactams, and metronidazole were similar to those of garenoxacin relative to their respective MICs. However, clindamycin at two times the MIC killed strains more slowly, with bactericidal activity against 15 of 20 strains after 48 h and at lower rates at earlier times. Clindamycin killed strains more slowly ($P < 0.05$) than did garenoxacin at 6 and 12 h; no other statistically significant differences were found (Table 2). When two strains of the same species were compared, their kill kinetics, as defined in the present study, were similar.

The results of this study confirm that garenoxacin has excellent antianaerobic activity based on MIC and time-kill. At two times the MIC, garenoxacin was bactericidal against all strains, except for one *Clostridium difficile* strain, after 48 h. No quinolone was bactericidal against this strain at comparable MICs and times. The kill kinetics of garenoxacin for anaerobes were similar to those of the other quinolones tested (e.g., ciprofloxacin) but relative to higher MICs of the other compounds. The MICs and kill kinetics of other quinolone and nonquinolone compounds reflect those of previous reports (6, 12, 13, 16, 17). Compared to the rates of killing for other agents, those for clindamycin have been reported to be lower, especially at earlier times (12).

Our sample size was small, complicating the determination of accurate comparisons (16). However, this is the largest time-kill study of anaerobes of which we are currently aware, and the results confirm previous findings (see above). In view of the known clinical efficacy of clindamycin, its lower killing rates may be important only in serious systemic infections, especially in an immunocompromised host.

Other workers have recently documented the rapid killing of *Enterobacteriaceae* by garenoxacin, i.e., within 2 h rather than ≥6 h by β-lactams. Gram-positive cocci were killed more slowly, with >6 h required for optimal bactericidal activity (11).

Because of the broad spectrum of garenoxacin's activity

TABLE 2. Time-kill analysis of 20 strains

Drug and MIC[a]	No. of strains killed at[b]:											
	6 h			12 h			24 h			48 h		
	−1	−2	−3	−1	−2	−3	−1	−2	−3	−1	−2	−3
Garenoxacin												
4× MIC	20[b]	12	3	20	18	10	20	20	18	20	20	19
2× MIC	15	10	4	20	17	8	20	20	17	20	20	19
MIC	13	6	3	16	12	7	18	16	14	19	17	13
Ciprofloxacin												
4× MIC	17	5	1	20	17	4	20	20	16	20	20	19
2× MIC	15	4	2	20	16	6	20	20	16	20	20	18
MIC	13	4	0	17	14	4	19	16	13	17	16	13
Levofloxacin												
4× MIC	20	10	1	20	19	8	20	20	18	20	20	19
2× MIC	16	10	1	20	17	7	20	20	16	20	19	18
MIC	15	6	1	19	15	7	20	19	14	19	16	14
Moxifloxacin												
4× MIC	20	13	4	20	20	11	20	20	18	20	20	19
2× MIC	19	12	3	20	18	9	20	20	18	20	20	19
MIC	16	6	3	18	14	6	19	19	14	19	15	12
Trovafloxacin												
4× MIC	20	12	1	20	18	12	20	20	18	20	20	19
2× MIC	19	7	0	20	16	7	20	20	18	20	20	18
MIC	14	3	0	18	11	3	20	20	14	18	14	11
Amoxicillin-clavulanate												
4× MIC	19	17	11	19	19	16	19	19	19	20	19	19
2× MIC	18	17	9	19	19	16	19	19	19	20	19	19
MIC	16	12	3	19	16	12	19	19	16	20	19	16
Piperacillin-tazobactam												
4× MIC	16	9	6	19	17	12	20	19	18	20	20	19
2× MIC	16	8	5	18	17	11	20	19	17	19	18	18
MIC	12	6	5	18	15	8	19	18	13	18	17	15
Imipenem												
4× MIC	20	16	10	20	19	15	20	20	18	20	20	20
2× MIC	20	14	8	20	19	14	20	20	19	20	20	19
MIC	17	12	5	20	18	14	20	20	18	19	14	14
Clindamycin												
4× MIC	11	0[c]	0	16	6[c]	1[c]	19	14	11	20	18	15
2× MIC	7	0[c]	0	14	6	1[c]	16	13	7	20	18	15
MIC	5	0[c]	0	14	2[c]	0[c]	16	11	5	18	12	9
Metronidazole												
4× MIC	20	20	8	20	20	19	20	20	20	20	20	20
2× MIC	20	20	8	20	20	18	20	20	20	20	20	18
MIC	20	16	5	20	20	14	20	20	15	19	13	12

[a] 4 × MIC, four times the MIC; 2 × MIC, two times the MIC.
[b] −1, 90% killing; −2, 99% killing; −3, 99.9% killing.
[c] The proportions of strains at different extents of killing were significantly less ($P < 0.05$) than those observed with garenoxacin.

against both aerobes and anaerobes (5, 7–11, 13, 19), this compound may have a place in the empirical treatment of mixed infections caused by these bacterial groups. Clinical studies will be necessary to validate this hypothesis.

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