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Comparative In Vitro Activities of Clinafloxacin and Trovafloxacin against 1,000 Isolates of *Bacteroides fragilis* Group: Effect of the Medium on Test Results

DAVID R. SNYDMAN,* NILDA V. JACOBUS, LAURA A. MCDERMOTT, AND STACEY E. SUPRAN

Departments of Medicine and Pathology and the Division of Clinical Care Research, New England Medical Center and Tufts University School of Medicine, Boston, Massachusetts

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The in vitro antibacterial activities of clinafloxacin, trovafloxacin, ciprofloxacin, and cefoxitin against 1,000 clinical isolates of *Bacteroides fragilis* group were compared by agar dilution in brucella blood agar (BBA) and Wilkins Chalgren agar (WCA). Significantly higher geometric mean MICs for the three quinolones and cefoxitin ($P < 0.001$) were obtained in BBA than in WCA. Regardless of medium, clinafloxacin was slightly more active than trovafloxacin. The activity of clinafloxacin and trovafloxacin was greater than that of cefoxitin against *B. distasonis*, *B. ovatus*, and *B. thetaiotaomicron* but lower against *B. vulgatus*. High cross resistance between trovafloxacin and clinafloxacin was observed.

The use of quinolones as monotherapy for anaerobic infections has been limited by their lack of activity against this group of pathogens. The major quinolones in clinical use today have very limited activity against anaerobic bacteria (3, 11). Ciprofloxacin has been shown to be useful for the therapy of intra-abdominal sepsis, but only when combined with an active anti-anaerobic agent such as metronidazole (6). Clinafloxacin and trovafloxacin are newer quinolones with enhanced activity against anaerobic bacteria (1–3, 5, 7, 8, 11). Given the increasing resistance of isolates from the *Bacteroides fragilis* group to β-lactam antibiotics and other antianaerobic agents, these newer quinolones with activity against a broad range of anaerobic and aerobic bacteria could be ideal agents for potential use as monotherapy against anaerobic infections, especially those involved in intra-abdominal sepsis.

(This study was presented in part at the Second Anaerobe World Congress, Nice, France, October 1998.)

This study compares the antibacterial activity of clinafloxacin and trovafloxacin against a wide range of *B. fragilis* group isolates. Because of the controversy regarding the media used for susceptibility testing of anaerobes, we used the two media evaluated in a multicenter study by the anaerobe working group of the NCCLS Subcommittee on Antimicrobial Susceptibility Testing: supplemented brucella agar with Wilkins Chalgren agar (WCA) (9). Cefoxitin and ciprofloxacin were used as reference antibiotics.

One thousand nonduplicated clinical isolates from the *Bacteroides fragilis* group were tested. The isolates were referred (from 1995 through 1996) by eight medical centers representing diverse geographical areas in the United States. Cefoxitin-resistant strains were included in the study.

Standard powders of the following antibiotics were provided by the companies indicated: clinafloxacin, Parke-Davis, Morris Plains, N.J.; trovafloxacin, Pfizer, Inc., New York, N.Y.; ciprofloxacin, Bayer Corporation, West Haven, Conn.; and cefoxitin, Merck, Sharp, and Dohme, Rahway, N.J. Stock solutions of the antibiotics were prepared at ten times the test concentration, and kept frozen at −70°C until the day of the test.

MICs were determined by agar dilution following NCCLS recommendations in two different media: brucella agar supplemented with laked sheep red blood cell, vitamin K, and hemin (BBA) and WCA (9). The plates were prepared on the day of the test. Isolates were grown in supplemented brain heart infusion broth to logarithmic phase, and their turbidity adjusted to a 0.5 McFarland standard. The inocula were delivered to the surface of the agar with a Steers replicator (~10^5 cfu/spot). The plates were incubated in an anaerobic chamber at 37°C for 48 h. MICs were read as the lowest concentration of antibiotic that resulted in no visible growth. *B. fragilis* ATCC 25285 and *B. thetaiotaomicron* ATCC 29741 were included in each test.

Statistical analyses were performed using SAS statistical software, version 6.12. Geometric mean MICs were compared using paired *t* tests, and resistance percentages were compared using McNemar's tests. Alpha was set at 0.05.

The susceptibilities of the 1,000 *B. fragilis* group isolates as determined in the two media are shown in Table 1. The results are expressed as the geometric mean MIC, the MIC at which 50% of strains tested are inhibited (MIC_{50}), the MIC_{90}, the range of MICs, and the percentage of strains resistant at the specified breakpoint. Table 1 also shows the *P* values from the analysis comparing the geometric mean MICs and the percentage of strains resistant between the two media. Independent of the test medium, both clinafloxacin and trovafloxacin showed excellent activity against the 1,000 *B. fragilis* group isolates. With the exception of *B. vulgatus*, the resistance of all the other species to both quinolones was ≤12%; the resistance of *B. vulgatus* to both clinafloxacin and trovafloxacin was 23.2% using BBA. The activity of cefoxitin was somewhat lower than that of either quinolone (~14% of all species combined were resistant). The poor activity of ciprofloxacin (>98% of strains resistant) against the *B. fragilis* group was confirmed. In all instances the geometric mean MICs were lower for clinafloxacin than for trovafloxacin. In addition, 9 of 12 of the MIC_{50}s and 5 of 12 of the MIC_{90}s were also lower for clinafloxacin than for trovafloxacin. However, because of the difference in breakpoints, lower resistance percentages were demonstrated for trovafloxacin than for clinafloxacin. The comparison of the geometric mean MICs obtained from results in BBA versus WCA showed that, for all the species combined, the BBA

* Corresponding author. Mailing address: Box 238, New England Medical Center, 750 Washington St., Boston, MA 02111. Phone: (617) 636-5788. Fax: (617) 636-8525. E-mail: dsnydman@lifespan.org.

TABLE 1. Susceptibilities of *B. fragilis* isolates to various isolates in different media

Species	Antibiotics	Geometric mean MIC (μg/ml)		MIC_{50} (μg/ml)		MIC_{90} (μg/ml)		MIC range (μg/ml)		% Resistant[a]		Significance of difference (*P*) by method	
		Bru-Bld	Wilk-ChaI	Bru-Bld	Wilk-ChaI	Bru-Bld	Wilk-ChaI	Bru-Bld	Wilk-ChaI	BBA	WCA	in G M	in % Res
B. fragilis (*n* = 503)	Clinafloxacin	0.7	0.5	0.5	0.5	4	2	0.125–32	0.03125–32	3.6	2.6	<0.001	0.059
	Trovafloxacin	1.1	0.8	1	0.5	4	4	0.125–64	0.03125–32	8.2	5.0	<0.001	<0.001
	Ciprofloxacin	19	15.1	16	16	64	64	2–128	2–128	99.6	99.8	<0.001	0.564
	Cefoxitin	19	15.2	16	16	64	32	2–256	2–256	10.1	9.7	<0.001	0.746
B. distasonis (*n* = 129)	Clinafloxacin	0.5	0.4	0.5	0.5	2	1	0.125-8	0.0625–8	0.8	1.6	0.002	0.317
	Trovafloxacin	1.0	0.8	1	1	2	2	0.125–8	0.0625–8	3.9	2.3	<0.001	0.317
	Ciprofloxacin	14.6	11.8	16	8	64	32	0.5–128	0.5–128	99.2	99.2	<0.001	1.000
	Cefoxitin	23.7	21	32	16	64	64	4–128	4–128	14.7	12.4	0.017	0.366
B. ovatus (*n* = 94)	Clinafloxacin	0.8	0.6	0.25	0.5	4	4	0.125–16	0.125–32	6.4	4.3	<0.001	0.157
	Trovafloxacin	1.3	1.1	1	1	4	4	0.125–8	0.125–16	7.5	4.3	0.008	0.083
	Ciprofloxacin	23.6	19.7	16	16	>64	64	4–128	4–128	100.0	100.0	<0.001	1
	Cefoxitin	25.1	28.4	32	32	64	64	4–256	4–256	20.2	28.7	0.26	0.011
B. thetaiotaomicron (*n* = 116)	Clinafloxacin	0.8	0.7	0.5	0.5	4	2	0.125–16	0.125–16	4.3	4.3	0.002	1
	Trovafloxacin	1.4	1.2	1	1	4	4	0.125–16	0.125–8	7.8	4.3	<0.001	0.102
	Ciprofloxacin	27.2	25.0	16	16	>64	>64	4–128	4–128	100.0	100.0	0.061	1
	Cefoxitin	29.8	31.1	32	32	64	64	4–256	2–512	28.5	32.8	0.388	0.166
B. vulgatus (*n* = 56)	Clinafloxacin	0.7	0.6	0.25	0.25	8	8	0.125–64	0.0-625–32	12.5	14.3	0.179	0.317
	Trovafloxacin	1.3	0.9	1	0.5	16	8	0.125–16	0.03125–32	23.2	17.9	<0.001	0.083
	Ciprofloxacin	23.5	23.2	16	16	>64	>64	2–128	2–128	98.2	98.2	0.880	1
	Cefoxitin	12.5	10.9	8	8	32	32	2–64	2–64	3.6	3.6	0.117	1
Bacteroides species[b] (*n* = 102)	Clinafloxacin	0.6	0.4	0.5	0.25	2	1	0.0625–16	0.03125–16	2.0	2.0	<0.001	1
	Trovafloxacin	1.1	0.7	1	0.5	2	2	0.125–8	0.125–16	2.9	2.0	<0.001	0.317
	Ciprofloxacin	19.5	16.6	16	16	>64	64	2–128	2–128	99.0	99.0	0.004	1
	Cefoxitin	19.8	18.8	16	16	64	64	4–512	2–512	13.7	16.7	0.471	0.366
All species combined (*n* = 1,000)	Clinafloxacin	0.6	0.5	0.5	0.5	2	2	0.0625–64	0.03125–32	3.9	3.4	<0.001	0.166
	Trovafloxacin	1.1	0.8	1	1	4	4	0.125–64	0.03125–32	7.8	4.9	<0.001	<0.001
	Ciprofloxacin	19.8	16.4	16	16	>64	64	0.5–128	0.5–128	99.5	99.6	<0.001	0.705
	Cefoxitin	20.7	18.3	16	16	64	64	2–512	2–512	13.8	14.9	<0.001	0.233

[a] The following breakpoints for resistance wer used: ciprofloxacin ≥ 4 μg/ml; clinafloxacin ≥8 μg/ml; trovafloxacin ≥ 8 μg/ml; cefoxitin ≥ 64 μg/ml.
[b] Include 52 *B. uniformis*, 45 *B. caccae*, and 5 other species.

geometric mean MIC was significantly higher than the WCA geometric mean MIC for all four agents. This was also true for each individual species, with the exception of cefoxitin tested against *B. ovatus* and *B. thetaiotaomicron*, for which the WCA geometric mean MIC was higher than the BBA geometric mean MIC.

Cross-resistance between clinafloxacin and trovafloxacin was high: 70% of the strains resistant to clinafloxacin were also resistant to trovafloxacin (69 of 98), while 88% of the strains resistant to trovafloxacin were resistant to clinafloxacin (69 of 78). All strains resistant to trovafloxacin or clinafloxacin were also resistant to ciprofloxacin. Of the 138 strains resistant to cefoxitin, 15 (11%) were resistant to clinafloxacin and 12 (9%) were resistant to trovafloxacin (data not shown).

The comparison of results in the two media, BBA and WCA, showed significant differences for all antibiotics; with BBA, higher MICs were determined for all four antibiotics. These differences were not observed for cefoxitin or trovafloxacin in the study performed by the anaerobe working group of NCCLS (9). However, we noticed that growth of the isolates in WCA was considerably less than growth in BBA, an observation also noted in the NCCLS multicenter study that was thus their basis for recommending BBA as the medium for the reference agar dilution method (9). The poorer growth of the isolates in WCA could explain the lower MICs observed on this medium.

High cross-resistance among the quinolones, particularly among those with related structures, is not a new observation (4). The finding that 1/10 of the strains resistant to cefoxitin were also resistant to clinafloxacin and/or trovafloxacin (and multidrug-resistant isolates of the *B. fragilis* group are not uncommon) underscores the importance of continued testing and reporting of these pathogens.

Our data demonstrate that, with the exception of the species *B. vulgatus*, the in vitro activities of clinafloxacin and trovafloxacin against the *B. fragilis* group exceeds that of cefoxitin. Studies by Fuchs et al. and MacGowan et al. on the activity of clinafloxacin showed similar results (7, 8). Reports on the activity of trovafloxacin against the *B. fragilis* group by other investigators (1, 3, 5, 11) showed MIC_{50}s and MIC_{90}s 1 to 3 dilutions lower than ours, that is, MIC_{50}s of 0.25 to 0.5 μg/ml and MIC_{90}s of 1 to 2 μg/ml compared to our results of 0.5 to 1 and 2 to 16 μg/ml, respectively. Explanations for the differences could be methodologic, caused by testing of a larger number of isolates or isolate selection.

Based on the in vitro activities of clinafloxacin and trovafloxacin, both agents could be good alternatives in the anti-anaerobic armamentarium. Ultimately the question as to which agent proves to be more effective in a clinical setting will depend on pharmacokinetic parameters, emergence of resistant strains, and dose-limiting toxicity.

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REFERENCES

1. **Aldridge, K. E., D. Ashcraft, and K. A. Bowman.** 1997. Comparative in vitro activities of trovafloxacin (CP99,219) and other antimicrobials against clinically significant anaerobes. Antimicrob. Agents Chemother. **41:**484–487.
2. **Bowker, K. E., M. Wootton, H. A. Holt, D. S. Reeves, and A. P. MacGowan.** 1996. The in vitro activity of trovafloxacin and other antimicrobials against 413 anaerobic bacteria. J. Antimicrob. Chemother. **38:**271–281.
3. **Cohen, M. A., M. D. Huband, J. W. Gage, S. L. Yoderm, G. E. Roland, and S. J. Gradcheck.** 1997. In vitro activity of clinafloxacin, trovafloxacin and ciprofloxacin. J. Antimicrob. Chemother. **40:**205–211.
4. **Cormican, M. G., S. A. Marshall, and R. N. Jones.** 1995. Cross-resistance analysis for DU-6859a, a new fluoroquinolone, compared to six structurally similar compounds. Diag. Microbiol. Infect. Dis. **21:**51–54.
5. **Ednie, L. M., M. R. Jacobs, and P. C. Appelbaum.** 1998. Activities of gatifloxacin compared to those of seven other agents against anaerobic organisms. Antimicrob. Agents Chemother. **42:**2459–2462.
6. **Eliopolous, G. M., and C. T. Eliopolous.** 1989. Quinolone antimicrobial agents: activity in vitro, p. 45. *In* J. S. Wolfson and D. C. Hooper (ed.), Quinolone antimicrobial agents. American Society for Microbiology, Washington, D.C.
7. **Fuchs, P. C., A. L. Barry, and S. D. Brown.** 1998. In vitro activities of clinafloxacin against contemporary clinical bacterial isolates from 10 North American centers. Antimicrob. Agents Chemother. **42:**1274–1277.
8. **MacGowan, A. P., K. E. Bowker, H. A. Holt, M. Wootton, and D. S. Reeves.** 1997. Bay 12-8039, a new 8-methoxy-quinolone: comparative in vitro activity with nine other antimicrobials against anaerobic bacteria. J. Antimicrob. Chemother. **40:**503–509.
9. **National Committee for Clinical Laboratory Standards.** 1997. Methods for antimicrobial susceptibility of anaerobic bacteria: approved standard, 4th Edition. NCCLS publication M11-A4, vol. 17. National Committee for Clinical Laboratory Standards, Villanova, PA.
10. **Snydman, D. R., and L. McDermott.** 1996. Analysis of the in vitro activity of trovafloxacin (CP-99,219) against Bacteroides species. Infect. Dis. Clin. Pract. **5**(3 Suppl.)**:**S96–S100.
11. **Wexler, H. M., E. Molitoris, D. Molitoris, and S. M. Finegold.** 1996. In vitro activities of trovafloxacin against 557 strains of anaerobic bacteria. Antimicrob. Agents Chemother. **40:**2232–2235.
12. **Wise, R., S. Jones, I. Das, and J. M. Andrews.** 1998. Pharmacokinetics and inflammatory fluid penetration of clinafloxacin. Antimicrob. Agents Chemother. **42:**428–430.