

Comparative Activities of Eight Quinolones against Members of the *Bacteroides fragilis* Group

MARÍA VICTORIA BOROBIO,* MARÍA DEL CARMEN CONEJO, ENCARNACIÓN RAMIREZ,
ANA ISABEL SUAREZ, AND EVELIO J. PEREA

Department of Microbiology, University of Seville, Apdo 914, 41080-Sevilla, Spain

Received 22 October 1993/Returned for modification 11 January 1994/Accepted 18 March 1994

The in vitro activities of five new quinolones (clinafloxacin [CI-960 or PD-127391], BAY Y 3118, E-4868, E-5065, and E-5068) against 100 *Bacteroides fragilis* group bacterial isolates were compared with those of ciprofloxacin, ofloxacin, and sparfloxacin. Overall, E-5068 was the most active in vitro (MIC for 90% of isolates tested [MIC₉₀], 0.25 µg/ml); this was followed by clinafloxacin and BAY Y 3118 (MIC₉₀, 0.5 µg/ml). Ciprofloxacin, sparfloxacin, and ofloxacin were the least active (MIC₉₀s, 64, 16, and 16 µg/ml, respectively). *B. fragilis* and *Bacteroides caccae* were more susceptible than the other members of the *B. fragilis* group to all of the quinolones tested.

The most important and most frequently isolated anaerobes from clinical infections are those belonging to the *Bacteroides fragilis* group. In the past few years, several reports have emphasized the increasing incidence of resistance of *Bacteroides* species to the currently available antimicrobial agents (1, 7, 9). Consequently, a need has arisen for new, safe, and effective antimicrobial agents that can be used in the treatment of infections caused by anaerobes.

The recent resurgence in the clinical use of quinolones in the treatment of bacterial infections stems from the development of new fluorinated compounds which exhibit both greater and broader ranges of antibacterial activity. These two characteristics allow the use of a single agent in the treatment of infections when a mixed bacterial population is present.

In the work described here, a comparison was made between the in vitro activities of ciprofloxacin and ofloxacin and those of sparfloxacin (a difluoroquinolone with a 7 dimethyl-piperazinyl substituted group), BAY Y 3118 and clinafloxacin (monofluoroquinolones with new substituents at positions 7 and 8), and three mono- or difluorinated naphthyridine derivatives (E-4868, E-5065, and E-5068), which are characterized chemically by the presence at position 7 of an azetidin ring with different C'-3 substituents (Fig. 1), against 100 *B. fragilis* group strains in order to assess their antianaerobic activities.

The 100 anaerobic bacteria studied were isolated from the same number of patients at Seville University Hospital during 1992 and 1993 and were identified by standard criteria (14). These included 31 *B. fragilis*, 12 *Bacteroides caccae*, 16 *Bacteroides distasonis*, 13 *Bacteroides ovatus*, 12 *Bacteroides thetaioamicron*, and 16 *Bacteroides vulgatus*.

The following antimicrobial agents were provided by the indicated manufacturers: ciprofloxacin and BAY Y 3118, Quimica Farmaceutica Bayer S.A., Barcelona, Spain; ofloxacin, Hoechst Iberica S.A., Barcelona, Spain; sparfloxacin, Rhône Poulenc Farma, Madrid, Spain; clinafloxacin, Parke Davis S.A., Barcelona, Spain; E-4868, E-5065, and E-5068, Laboratorios Esteve SA, Barcelona, Spain.

The susceptibilities of the isolates to the test drugs were determined in Wilkins-Chalgren agar by the reference dilution method in accordance with the guidelines of the National

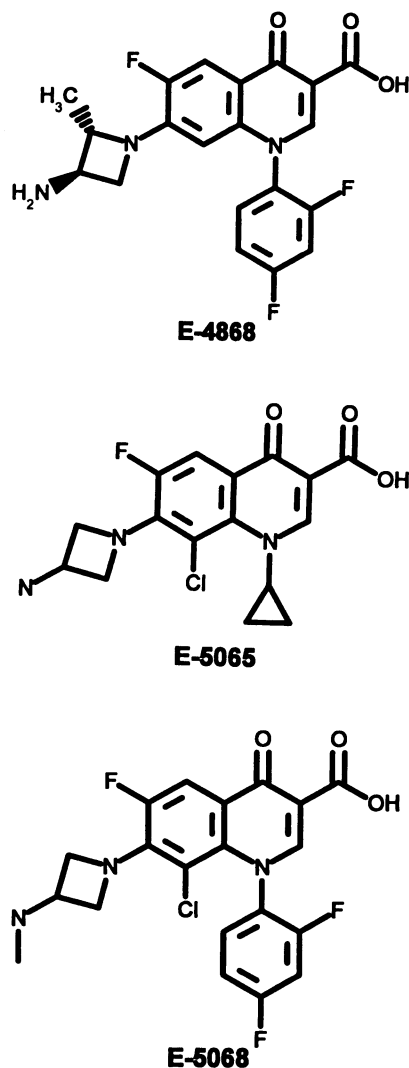


FIG. 1. Chemical structures of compounds E-4868, E-5065, and E-5068.

* Corresponding author.

TABLE 1. Comparative in vitro activities of various fluoroquinolones against members of the *B. fragilis* group

Organism	No. of isolates	Antimicrobial agent	MIC ($\mu\text{g/ml}$) ^a		
			Range	50%	90%
<i>B. fragilis</i>	31	Ciprofloxacin	1-128	4	16
		Ofloxacin	2-64	1	4
		Sparfloxacin	1-32	2	4
		Clinafloxacin	$\leq 0.03-0.5$	0.06	0.25
		BAY Y 3118	0.06-2	0.12	0.25
		E-4868	1-16	4	8
		E-5065	0.25-2	1	1
		E-5068	$\leq 0.015-0.5$	≤ 0.015	0.12
<i>B. caccae</i>	12	Ciprofloxacin	1-128	4	32
		Ofloxacin	0.25-16	2	8
		Sparfloxacin	0.06-16	1	2
		Clinafloxacin	$\leq 0.03-4$	0.06	0.12
		BAY Y 3118	0.06-0.5	0.25	0.5
		E-4868	0.12-16	4	4
		E-5065	0.03-4	0.5	1
		E-5068	0.06-2	≤ 0.03	0.12
<i>B. thetaiotaomicron</i>	12	Ciprofloxacin	4-64	4	64
		Ofloxacin	4-16	8	16
		Sparfloxacin	1-32	2	32
		Clinafloxacin	0.06-0.5	0.12	0.5
		BAY Y 3118	0.12-0.5	0.5	0.5
		E-4868	4-4	4	4
		E-5065	0.5-0.5	0.5	0.5
		E-5068	0.12-0.25	0.12	0.25
<i>B. vulgatus</i>	16	Ciprofloxacin	4-128	4	128
		Ofloxacin	2-64	2	32
		Sparfloxacin	1-32	1	8
		Clinafloxacin	$\leq 0.03-4$	4	4
		BAY Y 3118	0.12-2	0.12	2
		E-4868	4-16	4	16
		E-5065	0.12-4	0.12	4
		E-5068	$\leq 0.03-4$	0.06	4
<i>B. distasonis</i>	16	Ciprofloxacin	8-64	8	64
		Ofloxacin	4-128	4	32
		Sparfloxacin	1-2	1	2
		Clinafloxacin	0.5-1	0.5	1
		BAY Y 3118	0.25-2	0.25	1
		E-4868	2-16	4	16
		E-5065	0.125-4	0.25	4
		E-5068	0.06-0.5	0.06	0.25
<i>B. ovatus</i>	13	Ciprofloxacin	8-64	8	64
		Ofloxacin	4-32	4	32
		Sparfloxacin	1-16	2	16
		Clinafloxacin	0.06-0.5	0.12	0.5
		BAY Y 3118	0.12-2	0.5	1
		E-4868	1-8	2	8
		E-5065	0.25-4	1	2
		E-5068	0.06-0.5	0.06	0.25
Total	100	Ciprofloxacin	0.06-128	0.25	32
		Ofloxacin	0.25-128	2	16
		Sparfloxacin	0.06-32	2	4
		Clinafloxacin	$< 0.03-4$	0.06	0.5
		BAY Y 3118	0.06-2	0.12	0.5
		E-4868	0.12-16	4	8
		E-5065	0.03-4	1	1
		E-5068	$< 0.03-4$	< 0.03	0.25

^a 50% and 90%, MICs required to inhibit 50 and 90% of isolates, respectively.

Committee for Clinical Laboratory Standards (17) and Barry et al. (4). Plates containing serial doubling dilutions of antimicrobial agents ranging from 0.06 to 128 µg/ml were inoculated with a Steer's replicator to give a final inoculum of 10⁵ CFU. They were incubated in an anaerobic chamber (Forma Scientific) for 48 h at 37°C.

The MIC was defined as the lowest concentration of drug that inhibited growth. The appearance of a single colony or a barely visible haze was disregarded (17).

The in vitro activities of the quinolones against members of the *B. fragilis* group are given in Table 1. E-5068 had significantly greater activity than any of the other agents studied against all of the strains tested (MIC for 90% of isolates tested [MIC₉₀], 0.25 µg/ml). The second most active compounds (MIC₉₀s, 0.5 µg/ml) were clinafloxacin and BAY Y 3118; this was followed by E-5065 (MIC₉₀, 2 µg/ml), sparfloxacin (MIC₉₀, 4 µg/ml), E-4858 (MIC₉₀, 8 µg/ml), ofloxacin (MIC₉₀, 16 µg/ml), and ciprofloxacin (MIC₉₀, 64 µg/ml); ofloxacin and ciprofloxacin showed uniformly poor activities against all 100 strains tested. The *B. fragilis* and *B. caccae* species, when considered separately, were generally more susceptible than the other members of the *B. fragilis* group to all of the agents tested.

Clinafloxacin and E-5068 were the most active antimicrobial agents against the *B. fragilis* ATCC 25285 and *Clostridium perfringens* ATCC 131124 control strains (MICs, ≤0.06 µg/ml); this was followed by BAY Y 3118 (MIC, 0.125 µg/ml) and E-5065 (MIC, 0.25 µg/ml). Ofloxacin, sparfloxacin, and E-4868 were more active against *C. perfringens* (MICs, 0.125, 0.125, and 0.5 µg/ml, respectively) than against *B. fragilis* (MICs, 1, 1, and 4 µg/ml, respectively). The MICs of ciprofloxacin were 4 µg/ml for both control strains.

Since the introduction of the fluoroquinolones in 1982 (15), innovative developments have yielded several antimicrobial agents with increased activities and broader antibacterial spectra. However, those in clinical use, such as ciprofloxacin or ofloxacin, have MICs for anaerobic organisms that approach or exceed the achievable levels of those agents in human serum (6, 19).

Because ciprofloxacin is widely used clinically, many recent studies of new fluoroquinolones have used it as a reference agent (3, 12) and, as in the present work, have found its in vitro activity against *B. fragilis* to be very poor.

Ofloxacin possesses an oxazine ring that results in slightly improved activity against anaerobic organisms (8, 12). Some investigators have found that strains of *B. fragilis* were susceptible to ofloxacin (MIC₉₀, 4 µg/ml) but that most other species of the *B. fragilis* group were resistant to this agent. In the current study, it was found that the MIC₉₀ of ofloxacin for *B. fragilis* was 4 µg/ml; the ofloxacin MICs for other species tested in the present study were greater.

Our results, that sparfloxacin is more active than ofloxacin, with overall MIC₉₀s of 4 µg/ml, agree with those of Barry and Fuchs (3) and Goldstein and Citron (12). It should be noted that, in our study, the MIC₉₀ of sparfloxacin for *B. thetaiotaomicron* was 16 times greater (32 µg/ml) than those observed for *B. caccae* and *B. fragilis* (MIC₉₀s, 2 µg/ml).

As reported previously (3, 4, 8), clinafloxacin has very good activity against anaerobes. King et al. (16) and Goldstein and Citron (13) found interspecies differences, as we did, since the MIC₉₀ of clinafloxacin for *B. vulgatus* was 4 µg/ml, or 33 times higher than that for *B. caccae* (0.12 µg/ml).

BAY Y 3118 had the same favorable activity as clinafloxacin against the *B. fragilis* group, with both compounds having overall MIC₉₀s of 0.5 µg/ml, and interspecies differences were observed. These results are in agreement with those of other

investigators (5, 10, 18), even though such interspecies differences were not reported. Clinafloxacin resembles BAY Y 3118 very closely in terms of its potency and range of activity against *B. fragilis* group strains. Both agents also have identical structures except for the moiety at position 7.

The Esteve compounds showed different activities against the members of the *B. fragilis* group. E-4868 was the least active of the three, with an overall MIC₉₀ greater than that of sparfloxacin. E-5065 had an MIC₉₀ of 2 µg/ml, and E-5068 had an MIC₉₀ of 0.25 µg/ml for all 100 strains. Barrett et al. (2) reported an E-4868 MIC₉₀ for the *B. fragilis* group of 4 µg/ml. The activities of these antimicrobial agents against the different species of the *B. fragilis* group also varied in our study. Thus, E-4868 had an MIC₉₀s of 4 µg/ml for *B. caccae* and *B. thetaiotaomicron* and 8 µg/ml for *B. fragilis* and *B. ovatus*. *B. vulgatus* and *B. distasonis* proved to be the most resistant; the MIC₉₀ of E-4868 was 16 µg/ml. E-5065 was most active against *B. thetaiotaomicron* (MIC₉₀, 0.5 µg/ml); this was followed by its activity against *B. fragilis* and *B. caccae* (MIC₉₀s, 1 µg/ml) and *B. vulgatus* and *B. distasonis* (MIC₉₀s, 4 µg/ml). E-5068 was the most active of the three drugs, with MIC₉₀s of between 0.12 and 4 µg/ml. The most striking feature was the difference between the MIC₉₀s of E-5068 for *B. caccae* and *B. fragilis* (0.12 µg/ml) and that for *B. vulgatus* (4 µg/ml). The same phenomenon was observed with clinafloxacin.

Gargallo-Viola et al. (11) studied different azetidin derivatives from the Esteve Laboratories and demonstrated that two of them, E-4502 and E-4500, displayed considerable activity against anaerobes. The differences in the activities of the compounds tested in the present work may be due to the different C'-3 substituents of the azetidin group at position 7 of the molecule.

A comparison of the MIC₉₀s of the eight compounds that we studied shows the enhanced activities that were achieved with E-5068, BAY Y 3118, and clinafloxacin in comparison with those of ciprofloxacin and ofloxacin against members of the *B. fragilis* group. Their very low MICs for these common anaerobic pathogens suggest that they may be useful for the treatment of infections caused by these organisms; anaerobes have previously represented a significant gap in the spectra of the currently available quinolones. The increased levels of activity of these novel compounds suggest that they will have a role to play in the treatment of mixed and anaerobic infections. Their therapeutic usefulness will depend on the results of additional in vitro susceptibility studies involving a larger number of strains, pharmacokinetic and toxicological studies, and clinical trials.

REFERENCES

1. Appelbaum, P. C., S. K. Spangler, and M. R. Jacobs. 1991. Susceptibilities of 394 *Bacteroides fragilis* group, non-*B. fragilis* group *Bacteroides* species, and *Fusobacterium* species to newer antimicrobial agents. *Antimicrob. Agents Chemother.* 35:1214-1218.
2. Barret, M. S., R. N. Jones, and M. E. Erwin. 1993. In vitro activity of E-4868, a new trifluoroquinolone, compared to six similar compounds. *Eur. J. Clin. Microbiol. Infect. Dis.* 12(2):134-141.
3. Barry, A. L., and P. C. Fuchs. 1991. In vitro activities of sparfloxacin, tosufofloxacin, ciprofloxacin, and fleroxacin. *Antimicrob. Agents Chemother.* 35:955-960.
4. Barry, A. L., P. C. Fuchs, D. M. Citron, S. D. Allen, and H. M. Wexler. 1993. Methods for testing the susceptibility of anaerobic bacteria to two fluoroquinolone compounds, PD-131628 and clinafloxacin. *J. Antimicrob. Chemother.* 31:893-900.
5. Bauernfeind, A. 1993. Comparative in vitro activities of the new quinolone, BAY Y 3118 and ciprofloxacin, sparfloxacin, tosufofloxacin, CI-960, and CI-990. *J. Antimicrob. Chemother.* 31:505-522.
6. Borobio, M. V., and E. J. Perea. 1984. Effect of inoculum, pH, and

- medium on the activity of ciprofloxacin against anaerobic bacteria. *Antimicrob. Agents Chemother.* **25**:342-343.
7. **Bourgault, A. M., F. Lamothe, D. Hoban, M. T. Dalton, P. C. Kibsey, G. Harding, J. Smith, D. Low, and H. Gillbert.** 1992. Survey of *Bacteroides fragilis* group susceptibility patterns in Canada. *Antimicrob. Agents Chemother.* **36**:343-347.
 8. **Fuchs, P. C., A. L. Barry, M. A. Pfaller, S. D. Allen, and E. H. Gerlach.** 1991. Multicenter evaluation of the in vitro activities of three new quinolones, sparfloxacin, CI-960 and PD 131.628 compared with the activity of ciprofloxacin against 5,252 clinical bacterial isolates. *Antimicrob. Agents Chemother.* **35**:764-766.
 9. **García-Rodríguez, J. A., and J. E. García-Sánchez.** 1990. Evolution of antimicrobial susceptibility in isolates of the *Bacteroides fragilis* group in Spain. *Rev. Infect. Dis.* **12**(Suppl. 2):S142-S151.
 10. **García-Rodríguez, J. A., J. E. García-Sánchez, M. J. Fresnadillo-Martínez, I. García-García, E. García-Sánchez, and I. Trujillano-Martín.** 1993. BAY Y 3118, a novel 4-quinolone: in vitro activity against anaerobes, abstr. 9. 6th European Congress of Clinical Microbiology and Infectious Diseases, Seville, Spain.
 11. **Gargallo-Viola, D., M. Esteve, M. Moros, R. Coll, M. A. L. Xicota, C. de Andres, A. Rose, and J. Guinea.** 1991. Comparative in vitro and in vivo activities of six new monofluoroquinolone and difluoroquinolone 3-carboxylic acids with a 7-azetidín ring substituent. *Antimicrob. Agents Chemother.* **34**:2318-2326.
 12. **Goldstein, E. J. C., and D. M. Citron.** 1985. Comparative activity of the quinolones against anaerobic bacteria isolated at community hospitals. *Antimicrob. Agents Chemother.* **27**:657-659.
 13. **Goldstein, E. J. C., and D. M. Citron.** 1992. Comparative activity of ciprofloxacin, ofloxacin, sparfloxacin, temafloxacin, CI-960, CI-990, and WIN 57273 against anaerobic bacteria. *Antimicrob. Agents Chemother.* **36**:1158-1162.
 14. **Holdeman, L. V., E. P. Cato, and W. E. C. Moore.** 1977. Anaerobe laboratory manual, 4th ed. Virginia Polytechnic Institute and State University, Blacksburg.
 15. **Ito, A., K. Hirai, M. Inoue, H. Koga, S. Suzue, T. Irikura, and S. Mithunhashi.** 1980. In vitro antibacterial activity of AM-715, a new nalidixic acid analog. *Antimicrob. Agents Chemother.* **17**:103-107.
 16. **King, A., C. Boothman, and I. Phillips.** 1988. The in vitro activity of PD 127,3911, a new quinolone. *J. Antimicrob. Chemother.* **22**:135-141.
 17. **National Committee for Clinical Laboratory Standards.** 1991. Methods for antimicrobial susceptibility testing of anaerobic bacteria. Approved standard M11-A2. National Committee for Clinical Laboratory Standards, Villanova, Pa.
 18. **Nord, C. E., A. Lindmark, and I. Persson.** 1993. In vitro activity of BAY Y 3118 against anaerobic bacteria. *Eur. J. Clin. Microbiol. Infect. Dis.* **12**:640-642.
 19. **Walker, R. C., and A. J. Wright.** 1987. The quinolone. *Mayo Clin. Proc.* **62**:1007-1012.