ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Feb. 1990, p. 368–370
0066-4804/90/020368-03$02.00/0
Copyright © 1990, American Society for Microbiology
Vol. 34, No. 2

In Vitro Activities of Tosufloxacin, Temafloxacin, and A-56620 against Pathogens of Diarrhea

JOE P. BRYAN,[1]* CATHERINE WATERS,[2] JAQUELINE SHEFFIELD,[2] RICHARD E. KRIEG,[1] PETER L. PERINE,[1] AND KENNETH WAGNER[2]

Department of Preventive Medicine and Biometrics, Uniformed Services University of the Health Sciences, 4301 Jones Bridge Road,[1] and Division of Infectious Diseases, National Naval Medical Center,[2] Bethesda, Maryland 20814-4799

Received 26 September 1989/Accepted 13 November 1989

Tosufloxacin (A-60969 HCl), a new quinolone with broad activity against gram-positive and anaerobic organisms, was compared in vitro with other quinolones against bacterial pathogens of diarrhea. Tosufloxacin was the most active agent against *Salmonella* spp., *Shigella* spp., *Campylobacter* spp., *Aeromonas hydrophila*, and *Vibrio* spp. Temafloxacin (A-62254) also demonstrated good activity against these organisms.

Early therapy of bacterial enteritis may hasten recovery from diarrheal illness (3, 12). For presumptive therapy, the quinolone class of antimicrobial agents offers the advantage of marked bactericidal activity against pathogens of bacterial enteritis, including *Campylobacter* spp., enterotoxigenic *Escherichia coli*, and *Vibrio* spp. (7, 14). Tosufloxacin (T-3262; A-60969 HCl; A-61827 tosylate) has improved activity compared with other quinolones against gram-positive cocci and anaerobic organisms (4, 6), as well as *Listeria monocytogenes* (4). A-56620 is of interest because it is poorly absorbed from the gastrointestinal tract and therefore has less potential for systemic toxicity than better absorbed compounds. In this study, we compared the in vitro activities of several newer quinolones, tosufloxacin, temafloxacin (A62254; A63004 HCl), A-56620, and difloxacin (A-56619), with those of ciprofloxacin and norfloxacin. In addition, the in vitro activity of a newer macrolide, clarithromycin (A-56268; TE-031), was compared with that of erythromycin against *Campylobacter* spp., the leading cause of bacterial diarrhea in some studies (1).

Antimicrobial agents difloxacin, A-56620, tosufloxacin, temafloxacin, and clarithromycin were obtained from Abbott Pharmaceuticals, Abbott Park, Ill. Ciprofloxacin was provided by Miles Pharmaceuticals, West Haven, Conn.; norfloxacin was provided by Merck Sharpe & Dohme, West Point, Pa.; and erythromycin and trimethoprim-sulfamethoxazole were from Sigma Chemical Co., St. Louis, Mo. *Vibrio* spp. were provided by J. Glenn Morris, University of Maryland School of Medicine, Baltimore. *Campylobacter* spp. and enterotoxigenic *E. coli* were provided by L. Bourgeois of the Naval Medical Research Institute, Bethesda, Md.

Antimicrobial agents were prepared in microtiter plates by twofold dilutions in concentrations as follows: all quinolones, 0.015 to 1.0 μg/ml; erythromycin and clarithromycin, 0.06 to 8.0 μg/ml; and trimethoprim-sulfamethoxazole, 0.25 to 32 μg of the trimethoprim component per ml. Unique clinical isolates from patients with enteritis in various locations around the world were collected and identified by standard methods (10). We tested 39 *Salmonella enteritidis* isolates, including 18 serogroup B, 4 serogroup C_1, 1 serogroup C_2, and 16 serogroup D isolates. *Shigella* spp. included four *S. boydii*, four *S. flexneri*, two *S. dysenteriae*, and seven *S. sonnei* isolates. *Campylobacter* spp. included 48 *C. jejuni* and 2 *C. coli* isolates. Heat-labile exotoxin production by *E. coli* was determined by Y1 adrenal cell assay, and heat-stable toxin production was determined by inoculation of suckling mice. All *E. coli* produced heat-labile toxin, and most produced heat-stable toxin. *Vibrio* spp. included 5 non-serogroup O1 *V. cholerae*, 5 *V. cholerae* O1, and 10 *V. parahaemolyticus* isolates. Isolates were stored at −70°C and were subcultured twice before testing.

Antimicrobial susceptibility testing was performed by standard microdilution methods (11). Cation-supplemented Mueller-Hinton broth with 5% lysed horse blood was used for *Campylobacter* spp. *Campylobacter* spp. were incubated in a microaerophilic environment using a CampyPak (BBL Microbiology Systems, Cockeysville, Md.) without catalyst for 48 h, while other organisms were incubated for 24 h at 37°C in a non-CO_2 environment. The MIC was read as the lowest concentration of antimicrobial agent preventing visible growth after incubation. The MBC was determined as the lowest concentration killing 99.9% of the inoculum, i.e., ≤3 CFU/0.01 ml.

Table 1 shows the in vitro activities of tosufloxacin and temafloxacin compared with those of other quinolones and two macrolides. Overall, with the exception of a small number of *Campylobacter* spp., all isolates were highly susceptible to the quinolones. Tosufloxacin had inhibitory and bactericidal activities against *Salmonella* spp., *Shigella* spp., enterotoxigenic *E. coli*, and *Aeromonas* spp. equal to or better than those of ciprofloxacin and the other quinolones. Against *Campylobacter* spp., tosufloxacin had the best bactericidal activity, followed by temafloxacin and norfloxacin. Difloxacin, A-56620, and ciprofloxacin had relatively less bactericidal activity, with an MBC of 1.0 μg/ml for 90% of isolates tested. The inhibitory activities of the compounds, however, were similar.

The macrolide clarithromycin had slightly more bactericidal activity (MBC for 90% of isolates, 4.0 μg/ml) against *Campylobacter* spp. than erythromycin (MBC for 90% of isolates, >8.0 μg/ml). The bacteriostatic nature of trimethoprim-sulfamethoxazole was demonstrated against all genera tested, with less activity against *Campylobacter* spp., *Aeromonas hydrophila*, and *E. coli*.

* Corresponding author.

TABLE 1. Comparative in vitro activities of tosufloxacin, temafloxacin, and other agents

Organism (no. of isolates)	Agent	MIC (μg/ml)[a]			MBC (μg/ml)[a]		
		Range	50%	90%	Range	50%	90%
Salmonella enteritidis (39)	A-56620	≤0.015–0.06	0.03	0.06	≤0.015–0.25	0.06	0.12
	Tosufloxacin	≤0.015–0.06	≤0.015	0.03	≤0.015–0.125	≤0.015	0.06
	Temafloxacin	≤0.015–0.25	0.015	0.12	0.06–0.5	0.12	0.25
	Norfloxacin	≤0.015–0.125	0.03	0.06	≤0.015–0.25	0.03	0.125
	Ciprofloxacin	≤0.015	≤0.015	≤0.015	≤0.015–0.125	0.03	0.06
	TMP-SMX[b]	≤0.25–>32.0	0.5	>32.0	0.5–>32.0	>32.0	>32.0
Shigella spp. (17)	A-56620	≤0.015–0.06	0.03	0.06	≤0.015–0.12	0.03	0.06
	Tosufloxacin	≤0.015	≤0.015	≤0.015	≤0.015–0.06	≤0.015	0.03
	Temafloxacin	≤0.015–0.25	0.06	0.12	0.03–0.25	0.06	0.25
	Norfloxacin	≤0.015–0.06	0.03	0.06	≤0.015–0.06	0.03	0.06
	Ciprofloxacin	≤0.015–0.06	≤0.015	0.03	≤0.015–0.06	≤0.015	0.06
	TMP-SMX	≤0.25–32.0	0.5	32.0	1.0–32.0	32.0	32.0
Campylobacter spp. (50)	Difloxacin	≤0.015–>2.0	0.06	0.25	≤0.015–>2.0	0.125	0.5
	A-56620	≤0.015–>2.0	0.06	0.25	≤0.015–>2.0	≤0.125	1.0
	Tosufloxacin	≤0.015–0.06	≤0.015	0.06	≤0.015–0.12	≤0.015	0.06
	Temafloxacin	≤0.015–>2.0	0.03	0.125	≤0.015–>2.0	0.06	0.25
	Norfloxacin	≤0.015–>2.0	0.06	0.25	≤0.015–>2.0	0.25	0.5
	Ciprofloxacin	≤0.015–>2.0	0.03	0.25	≤0.015–>2.0	0.06	1.0
	TMP-SMX	0.25–32.0	1.0	4.0	1.0–>32.0	16.0	>32.0
	Clarithromycin	≤0.06–>8.0	0.25	1.0	0.06–>8.0	1.0	4.0
	Erythromycin	≤0.06–>8.0	0.25	2.0	≤0.06–>8.0	1.0	>8.0
Escherichia coli, enterotoxigenic (12)	A-56620	≤0.015–0.06	0.03	0.03	≤0.015–0.25	0.03	0.12
	Tosufloxacin	≤0.015–0.03	≤0.015	≤0.015	≤0.015–0.06	≤0.015	0.06
	Temafloxacin	≤0.015–0.03	≤0.015	≤0.015	≤0.015–0.03	0.03	0.03
	Norfloxacin	≤0.015–0.03	≤0.015	0.03	≤0.015–0.125	≤0.15	0.03
	Ciprofloxacin	≤0.015	≤0.015	≤0.015	≤0.015–0.03	≤0.015	≤0.015
	TMP-SMX	≤0.015–>32.0	32.0	>32.0	0.5–>32.0	>32.0	>32.0
Aeromonas hydrophila (18)	A-56620	≤0.015–0.03	≤0.015	≤0.015	≤0.015–0.03	≤0.015	0.03
	Tosufloxacin	≤0.015–0.06	≤0.015	0.03	≤0.015–0.06	0.03	0.03
	Temafloxacin	≤0.015–0.25	≤0.015	0.03	≤0.015–0.25	0.03	0.06
	Norfloxacin	≤0.015–0.03	≤0.015	0.03	≤0.015–0.06	≤0.015	0.06
	Ciprofloxacin	≤0.015–0.03	≤0.015	≤0.015	≤0.015–0.12	≤0.015	0.03
	TMP-SMX	≤0.25–16.0	≤0.25	16.0	4.0–>32.0	>32.0	>32.0
Vibrio spp. (20)	A-56620	≤0.015–0.25	0.06	0.25	≤0.015–0.5	0.12	0.25
	Tosufloxacin	≤0.015	≤0.015	≤0.015	≤0.015	≤0.015	≤0.015
	Temafloxacin	≤0.015–0.06	0.03	0.06	≤0.015–0.12	0.03	0.06
	Norfloxacin	≤0.015–0.12	0.03	0.12	≤0.015–0.12	0.03	0.12
	Ciprofloxacin	≤0.015–0.12	0.015	0.06	≤0.015–0.12	0.03	0.12
	TMP-SMX	≤0.25–0.5	≤0.25	0.25	≤0.015–>32.0	8.0	16.0
Yersinia enterocolitica (7)	A-56620	≤0.015–0.06			0.06–0.12		
	Tosufloxacin	≤0.015–0.03			≤0.015–0.03		
	Temafloxacin	≤0.015–0.12			0.06–0.25		
	Norfloxacin	≤0.015–0.12			0.03–0.5		
	Ciprofloxacin	≤0.015			≤0.015–0.06		
	TMP-SMX	≤0.25			1.0–8.0		

[a] 50% and 90%, MIC or MBC for 50 and 90% of isolates tested, respectively.
[b] TMP-SMX, Trimethoprim-sulfamethoxazole. Concentrations shown are for the trimethoprim component.

Ciprofloxacin, norfloxacin, and ofloxacin have been used successfully in the prevention and treatment of traveler's diarrhea (3, 9, 12) and in certain problems in salmonellosis, such as salmonella osteomyelitis and arthritis (2), enteric fever (13), and the chronic carriage state (5, 8). Newer quinolones, such as difloxacin, temafloxacin, and tosufloxacin, with good in vitro activity and favorable pharmacokinetics may also be useful in these instances. With improved activity against the vegetative form of sporeforming anaerobes such as *Clostridium difficile* and *Clostridium perfringens* (4, 6), tosufloxacin may also be useful for the pseudomembranous colitis or enteritis necroticans syndromes caused by these organisms. A-56620, a poorly absorbed quinolone with bactericidal activity approaching that of ciprofloxacin, may be of use in treating infection localized to the intestine, for bowel decontamination or prophylaxis in neutropenic hosts. It would not be the agent of choice for bacteria that regularly cause bacteremia, such as *Salmonella typhi*, *Salmonella choleraesuis*, or *Campylobacter fetus*. The quinolones, especially tosufloxacin, have much greater activity against *Campylobacter* spp. than either macrolide tested. Resistance by *Campylobacter* spp. to erythromycin, the usual therapy for *Campylobacter* enteritis, has been noted in Thailand (15).

LITERATURE CITED

1. **Blaser, M. M., J. G. Wells, R. A. Feldman, R. A. Pollard, J. R. Allen, and the Collaborative Diarrheal Disease Study Group.** 1983. *Campylobacter* enteritis in the United States: a multicenter study. Ann. Intern. Med. **98**:360–365.
2. **Bryan, J. P., H. Rocha, and W. M. Scheld.** 1986. Problems in salmonellosis: rationale for clinical trials with newer beta-lactam agents and quinolones. Rev. Infect. Dis. **8**:189–206.
3. **Ericsson, C. D., P. C. Johnson, H. L. Dupont, D. R. Morgan, H. M. Bitsura, and F. Javier De La Cabada.** 1987. Ciprofloxacin or trimethoprim/sulfamethoxazole as initial therapy for traveler's diarrhea. Ann. Intern. Med. **106**:216–220.
4. **Espinoza, A. M., N.-X. Chin, A. Novelli, and H. Neu.** 1988. Comparative in vitro activity of a new fluorinated 4-quinolone, T-3262 (A-60969). Antimicrob. Agents Chemother. **32**:663–670.
5. **Ferreccio, C., J. G. Morris, Jr., C. Valdivieso, I. Prenzel, V. Sotomayor, G. L. Drusano, and M. M. Levine.** 1988. Efficacy of ciprofloxacin in the treatment of chronic typhoid carriers. J. Infect. Dis. **157**:1235–1238.
6. **Fujimaki, K., T. Noumi, I. Saikawa, M. Inoue, and S. Mitsuhashi.** 1988. In vitro and in vivo antibacterial activities of T-3262, a new fluoroquinolone. Antimicrob. Agents Chemother. **32**:827–833.
7. **Goossens, H., P. De Mol, H. Coignau, J. Levy, O. Grados, G. Ghysels, H. Innocent, and J.-P. Butzler.** 1985. Comparative in vitro activities of aztreonam, ciprofloxacin, norfloxacin, ofloxacin, HR 810 (a new cephalosporin), RU28965 (a new macrolide), and other agents against enteropathogens. Antimicrob. Agents Chemother. **27**:388–392.
8. **Gotuzzo, E., J. G. Guerra, L. Benavente, J. C. Palomino, C. Carillo, J. Lopera, F. Delgado, D. R. Nalin, and J. Sabbaj.** 1988. Use of norfloxacin to treat chronic typhoid carriers. J. Infect. Dis. **157**:1221–1225.
9. **Keusch, G. T.** 1988. Antimicrobial therapy for enteric infections and typhoid fever: state of the art. Rev. Infect. Dis. **10**: S199–S205.
10. **Lennette, E. H., A. Balows, W. J. Hausler, Jr., and H. J. Shadomy (ed.).** 1985. Manual of clinical microbiology, 4th ed., p. 263–277. American Society for Microbiology, Washington, D.C.
11. **National Committee for Clinical Laboratory Standards.** 1985. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard M7-A. National Committee for Clinical Laboratory Standards, Villanova, Pa.
12. **Pichler, H. E. T., G. Diridl, K. Stickler, and D. Wolf.** 1987. Clinical efficacy of ciprofloxacin compared with placebo in bacterial diarrhea. Am. J. Med. **82**:329–332.
13. **Ramirez, C. A., J. L. Bran, C. R. Mejia, and J. F. Garcia.** 1985. Open, prospective study of the clinical efficacy of ciprofloxacin. Antimicrob. Agents Chemother. **28**:128–132.
14. **Segreti, J., J. A. Nelson, L. J. Goodman, R. L. Kaplan, and G. M. Trenholme.** 1989. In vitro activities of lomefloxacin and temafloxacin against pathogens causing diarrhea. Antimicrob. Agents Chemother. **33**:1385–1387.
15. **Taylor, D. T., M. J. Blaser, P. Echeverria, C. Pitarangsi, L. Bodhidatta, and W.-L. L. Wang.** 1987. Erythromycin-resistant *Campylobacter* infections in Thailand. Antimicrob. Agents Chemother. **31**:438–442.