ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Jan. 1999, p. 194–195 Vol. 43, No. 1
0066-4804/99/$04.00+0

In Vitro Activities of Six New Fluoroquinolones against *Brucella melitensis*

IGNACIO TRUJILLANO-MARTÍN,[1] ENRIQUE GARCÍA-SÁNCHEZ,[1] ISAÍAS MONTES MARTÍNEZ,[2] MARÍA JOSÉ FRESNADILLO,[1] JOSÉ ELÍAS GARCÍA-SÁNCHEZ,[1] AND JOSÉ ÁNGEL GARCÍA-RODRÍGUEZ[1]*

Departamento de Microbiología y Parasitología, Hospital Universitario, Salamanca,[1] and Unidad de Microbiología, Hospital Virgen del Puerto, Plasencia, Cáceres,[2] Spain

Received 24 June 1998/Returned for modification 13 August 1998/Accepted 23 October 1998

We have tested the in vitro activities of eight fluoroquinolones against 160 *Brucella melitensis* strains. The most active was sitafloxacin (MIC at which 90% of the isolates are inhibited [MIC_{90}], 0.12 μg/ml). In decreasing order, the activities (MIC_{90}s) of the rest of the tested fluoroquinolones were as follows: levofloxacin, 0.5 μg/ml; ciprofloxacin, trovafloxacin, and moxifloxacin, 1 μg/ml; and ofloxacin, grepafloxacin, and gatifloxacin, 2 μg/ml.

Although brucellosis is primarily a disease of domestic animals, it remains a public health problem worldwide, mainly in the Mediterranean and in some developing countries (15). Current treatment of acute brucellosis requires combined regimens of antibiotics and is conditioned by the fact that brucellae are facultative intracellular parasites; thus, it is important to treat patients with drugs that penetrate macrophages. This fact seems to be responsible for the long duration of the disease and the high incidence of relapses. The most effective antibiotic treatment is the combination of doxycycline with streptomycin or rifampin. Each of these regimens has disadvantages: the relapse rates and the toxicity and side effects, especially in children and pregnant women. In addition, twice-daily long-term dosing of oral doxycycline plus intramuscular administration of streptomycin often leads to poor patient compliance (1, 14). The necessity of combined treatment, the length of treatment, and the proportion of therapeutic failures with some regimens oblige us to look for new drugs for the treatment of brucellosis. Fluoroquinolones, as a class, exhibit a broad spectrum of antibacterial activity. Their oral bioavailability, high tissue concentrations, evidence of intracellular penetration (fluoroquinolones appear to achieve intracellular concentrations in phagocytic cells significantly in excess of extracellular concentrations) (4, 6), and in vitro activity against *Brucella* spp. (2, 7, 9, 12, 17–19) make these antimicrobial agents attractive as candidates for use against infections caused by intracellular bacteria, such as *Brucella* spp. However, the marketed fluoroquinolones, such as ciprofloxacin, ofloxacin, fleroxacin, and sparfloxacin, have moderate activity and lack effective bactericidal activity under intracellular conditions (8), and therapeutic failures caused by the development of resistance by *B. melitensis* have also been reported (15, 17). We have determined the in vitro activities of six newer fluoroquinolones (levofloxacin, trovafloxacin, sitafloxacin, moxifloxacin, grepafloxacin, and gatifloxacin) in comparison with those of ciprofloxacin, ofloxacin, and established agents against 160 strains of *Brucella melitensis*, including the type strain, *B. melitensis* ATCC 23456, because, in general, these new antimicrobial agents have improved pharmacokinetic characteristics (4) and they may have better pharmacokinetic and pharmacodynamic parameters (5).

The following antibiotics were kindly provided by their respective manufacturers: ciprofloxacin and moxifloxacin (BAY 12-8039) (Bayer, Química Farmacéutica Bayer, Barcelona, Spain); ofloxacin, levofloxacin, and rifampin (Hoechst Marrion Roussel, S. A., Barcelona, Spain); trovafloxacin (CP-99,219) and doxycycline (Pfizer, S. A., Madrid, Spain); sitafloxacin (DU-6859a) (Daiichi Pharmaceutical, Tokyo, Japan); grepafloxacin (OPC-17116) (Glaxo Wellcome, S. A., Madrid, Spain); gatifloxacin (AM-1155, CG5501, BMS-206584) (Grünenthal GmbH, Aachen, Germany); and streptomycin (CEPA, Madrid, Spain). The 160 strains of *B. melitensis*, except for the type strain, ATCC 23456 biotype 1, were clinical isolates (biotype undetermined); all of them were obtained from blood cultures at the Hospital Virgen del Puerto of Plasencia and the Hospital Universitario of Salamanca, Spain, during 1997. The organisms were identified by standard methods (16), stored in skim milk at −70°C, and subcultured twice before the study was started. The MICs of the antimicrobial agents tested were determined by agar dilution, according to previously described methods (8) using Mueller-Hinton agar (Oxoid Ltd., Basingstoke, Hampshire, England), supplemented with 1% hemoglobin (bioMerieux, Charbonnieres les Bains, France) and 1% PoliViteX

TABLE 1. In vitro activities of six new fluoroquinolones and other antimicrobial agents against 160 *B. melitensis* strains

Antimicrobial agent	MIC (μg/ml)[a]		
	Range	50%	90%
Ciprofloxacin	0.25–1	1	1
Ofloxacin	1–2	2	2
Levofloxacin	0.5	0.5	0.5
Trovafloxacin	0.5–1	1	1
Sitafloxacin	0.06	0.06	0.06
Moxifloxacin	1	1	1
Grepafloxacin	1–4	1	2
Gatifloxacin	1–2	1	2
Rifampin	0.5–1	1	1
Doxycycline	0.12–0.25	0.25	0.25
Streptomycin	4–16	8	8

[a] 50% and 90%, MIC at which 50 and 90% of the isolates are inhibited, respectively.

* Corresponding author. Mailing address: Department of Microbiology, Hospital Universitario, Paseo de San Vicente 108, 37007 Salamanca, Spain. Phone: 34-23-264825. Fax: 34-23-262261. E-mail: jagarrod@gugu.usal.es.

(bioMerieux), and an inoculum of 10^4 CFU per spot. The MIC was defined as the lowest concentration of an antimicrobial agent that completely inhibits growth, disregarding a single colony or a faint haze caused by the inoculum.

The MICs of the various antimicrobial agents by the agar dilution method are listed in Table 1. The most active fluoroquinolone was sitafloxacin, whose activity was 8 to more than 32-fold greater than those of the rest of the fluoroquinolones tested. The MIC range and the MICs at which 50 and 90% of the isolates were inhibited (MIC_{50}s and MIC_{90}s, respectively) were as follows (in micrograms per milliliter): sitafloxacin, 0.06 and 0.06; ciprofloxacin, 0.25 to 1, 1, and 1; ofloxacin, 1 to 2, 2, and 2; levofloxacin, 0.5 and 0.5; trovafloxacin, 0.5 to 1, 1, and 1; moxifloxacin, 1 and 1; grepafloxacin, 1 to 4, 1, and 2; and gatifloxacin, 1 to 2, 1, and 2. The MIC_{90} of sitafloxacin was 4-fold lower than that of doxycycline (0.25 μg/ml), 16-fold lower than that of rifampin (1 μg/ml), and even farther below that of streptomycin (8 μg/ml).

Fluoroquinolones, such as ciprofloxacin, initially had good promise, since preliminary studies usually showed MIC_{90}s of $\leq$1 μg/ml (9, 14–16) and they penetrate leukocytes and macrophages (6). However, a lack of bactericidal activity against *Brucella* spp. (8), development of resistance in *B. melitensis* during ciprofloxacin therapy, and cross-resistance to other fluoroquinolones have been described (2, 3). Moreover, treatment with ciprofloxacin alone, although effective for acute brucellosis, is associated with an appreciable rate of relapse (2, 3, 6, 13, 15, 17, 18). A 6-week course of a combination of ciprofloxacin and doxycycline is effective and well tolerated (13), but this antibiotic regimen is a two-antimicrobial-agent therapy similar to that previously recommended. In this study, sitafloxacin, the most active fluoroquinolone tested, showed excellent in vitro activity against all strains of *B. melitensis*. These results, probably due to the 8-chloro substituent, as in clinafloxacin (10, 19) and BAY y 3118 (11), make it a good candidate for the treatment of human brucellosis. Other fluoroquinolones studied, such as levofloxacin, trovafloxacin, and moxifloxacin, have activities similar to or slightly greater than that of ciprofloxacin. However, due to their pharmacokinetic properties (4), these antimicrobial agents may warrant study for the treatment of human brucellosis, depending on the pharmacokinetic and pharmacodynamic criteria (5). Also, it is necessary to determine the effect of a pH of 5 (the pH of phagolysosomes) on the activity of these fluoroquinolones, considering the lower in vitro activity against *B. melitensis* of other quinolones at that pH (8).

REFERENCES

1. **Acocella, G., A. Bertrand, J. Beytout, J. B. Durrande, J. A. García-Rodríguez, J. Kosmidis, M. Micoud, M. Rey, M. Rodríguez Zapata, J. Roux, and J. P. Stahl.** 1990. Comparison of three different regimens in the treatment of acute brucellosis: a multicenter multinational study. J. Antimicrob. Chemother. **23:**433–439.
2. **Al-Sibai, M. B., and S. M. H. Qadri.** 1990. Development of ciprofloxacin resistance in *Brucella melitensis*. J. Antimicrob. Chemother. **25:**302–303.
3. **Al-Sibai, M. B., M. A. Halim, M. M. El-Shaker, B. A. Khan, and S. M. H. Qadri.** 1992. Efficacy of ciprofloxacin for treatment of *Brucella melitensis* infections. Antimicrob. Agents Chemother. **36:**150–152.
4. **Bergan, T.** 1998. Pharmacokinetics of the fluoroquinolones, p. 143–182. *In* V. T. Andriole (ed.), The quinolones, 2nd ed. Academic Press, San Diego, Calif.
5. **Craig, W. A.** 1998. Pharmacokinetic/pharmacodynamic parameters: rationale for antibacterial dosing of mice and men. Clin. Infect. Dis. **26:**1–12.
6. **Easmon, C. S. F., J. P. Crane, and A. Blowers.** 1986. Effect of ciprofloxacin on intracellular organisms. In-vitro and in-vivo studies. J. Antimicrob. Chemother. **18**(Suppl. D)**:**43–48.
7. **García-Rodríguez, J. A., J. E. García-Sánchez, J. L. Muñoz Bellido, E. García-Sánchez, and I. Trujillano.** 1989. Kinetics of antimicrobial activity of ciprofloxacin against *Brucella melitensis*. Rev. Esp. Quimioter. **2:**61–63.
8. **García-Rodríguez, J. A., J. E. García-Sánchez, and I. Trujillano.** 1991. Lack of effective bactericidal activity of new quinolones against *Brucella* spp. Antimicrob. Agents Chemother. **35:**756–759.
9. **García-Rodríguez, J. A., J. E. García-Sánchez, I. Trujillano, and J. L. Muñoz Bellido.** 1989. *In-vitro* activity of new quinolones against *Brucella melitensis*. Rev. Infect. Dis. **11**(Suppl. 5)**:**S992–S993.
10. **García-Rodríguez, J. A., J. E. García Sánchez, I. Trujillano, E. García Sánchez, M. I. García García, and M. J. Fresnadillo.** 1995. Susceptibilities of *Brucella melitensis* isolates to clinafloxacin and four other new fluoroquinolones. Antimicrob. Agents Chemother. **39:**1194–1195.
11. **García-Rodríguez, J. A., J. E. García-Sánchez, I. Trujillano-Martín, E. García-Sánchez, M. I. García-García, and M. J. Fresnadillo-Marínez.** 1995. *In-vitro* activity of BAY y 3118, a novel 4-quinolone, against *Brucella melitensis*. J. Chemother. **7:**189–196.
12. **Gobernado, M., E. Cantón, and M. Santos.** 1984. In-vitro activity of ciprofloxacin against *Brucella melitensis*. Eur. J. Clin. Microbiol. **3:**371.
13. **Kalo, T., S. Novi, A. Nushi, and S. Dedja.** 1996. Ciprofloxacin plus doxycycline versus rifampicin plus doxycycline in the treatment of acute brucellosis. Méd. Maladies Infect. **26:**587–589.
14. **Kosmidis, J., A. Karagounis, J. Tslentis, and G. K. Daikos.** 1982. The combination rifampin-doxycycline in brucellosis is better than the WHO regimen. Chemioterapia **1**(Suppl. 4)**:**107.
15. **Lang, R., M. Banai, M. Lishner, and E. Rubinstein.** 1995. Brucellosis. Int. J. Antimicrob. Agents **5:**203–208.
16. **Moyer, N. P., and L. A. Holcomb.** 1995. Brucella, p. 549–555. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 6th ed. ASM Press, Washington, D.C.
17. **Qadri, S. M., M. Akhtar, Y. Ueno, and M. B. Al-Sibai.** 1989. Susceptibility of *Brucella melitensis* to fluoroquinolones. Drugs Exp. Clin. Res. **15:**483–485.
18. **Qadri, S. M., S. Al-Sedairy, and Y. Ueno.** 1990. Antibacterial activity of lomefloxacin against *Brucella melitensis*. Diagn. Microbiol. Infect. Dis. **13:**277–279.
19. **Qadri, S. M., and Y. Ueno.** 1993. Susceptibility of *Brucella melitensis* to new fluoroquinolone PD 131628: comparison with other drugs. Microbiology **39:**128–131.