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Comparative In Vitro Activities of Ciprofloxacin and Other 4-Quinolones against *Mycobacterium tuberculosis* and *Mycobacterium intracellulare*

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The in vitro activity of ciprofloxacin, ofloxacin, amifloxacin, and norfloxacin against 22 clinical isolates of *Mycobacterium tuberculosis* was evaluated by agar dilution. The MICs for 90% of the isolates of ciprofloxacin and ofloxacin were 0.5 and 1 μg/ml, respectively. Amifloxacin and norfloxacin were less active. The MICs for 90% of the isolates of ciprofloxacin and ofloxacin against 20 clinical isolates of *Mycobacterium intracellulare* were determined by agar dilution to be 2 and 8 μg/ml, respectively.

The 4-quinolones are heterocyclic carbonic acid derivatives structurally similar to nalidixic acid. They have a broad spectrum of activity against aerobic and anaerobic gram-positive and gram-negative organisms. The evaluation of the in vitro activity of 4-quinolones against *Mycobacterium tuberculosis* and *Mycobacterium intracellulare* is part of an ongoing effort by our laboratory to identify new, potentially useful antimycobacterial agents. The purpose of this study was threefold: (i) to compare by agar dilution the activity of four 4-quinolones against clinical isolates of *M. tuberculosis*, (ii) to compare the activity of ciprofloxacin and ofloxacin against clinical isolates of *M. intracellulare*, again by agar dilution, and (iii) to evaluate by both agar dilution and broth dilution the ability of ciprofloxacin to inhibit *M. intracellulare*.

MATERIALS AND METHODS

Isolates. Clinical isolates of *M. tuberculosis* were obtained from patients treated at the State University of New York Upstate Medical Center and the Veterans Administration Medical Center in Syracuse, N.Y., and TMC 303, an isoniazid-resistant mutant, was obtained from the Trudeau Mycobacterial Collection. The isolates other than TMC 303 were susceptible to standard antimycobacterial agents.

Clinical isolates of *M. intracellulare* were obtained from Nancy Warren, Division of Consolidated Laboratory Services, Department of General Services, Richmond, Va.; from Barbara Body, University of Virginia Medical Center, Charlottesville; and from patients with acquired immunodeficiency syndrome cared for at the State University of New York Upstate Medical Center and the Veterans Administration Medical Center. *Pseudomonas aeruginosa* ATCC 27853 (Difco Laboratories, Detroit, Mich.) was used as a control organism in some experiments.

Antibiotics. The following antibiotics were obtained as standard powders: ciprofloxacin (potency, 853 μg/ml; Miles Pharmaceuticals, West Haven, Conn.), ofloxacin (potency, 1,000 μg/ml); Ortho Pharmaceutical Corp., Raritan, N.J.), amifloxacin (potency, 1,000 μg/ml; Sterling-Winthrop Research Institute, Rensselaer, N.Y.), and norfloxacin (potency, 1,000 μg/mg; Merck Sharp & Dohme, Rahway, N.J.).

Stock solutions of each antibiotic were prepared by hydrating a known weight of drug with distilled water. Each solution was then filter sterilized through a GA-6 membrane filter (0.22 μm; Gelman Sciences, Inc., Ann Arbor, Mich.). Fresh solutions of the antibiotics were prepared for each experiment.

Media. Mycobacteria were grown in Middlebrook 7H10 broth (16) with Middlebrook OADC enrichment (Difco) and 0.05% Tween 80 on a rotary shaker at 37°C for either 3 days (*M. intracellulare*) or 5 days (*M. tuberculosis*). The 7H10 agar plates (BBL Microbiology Systems, Cockeysville, Md.) used for susceptibility testing were supplemented with Middlebrook OADC enrichment and 0.05% Tween 80. The mycobacteria were counted from duplicate titers on 7H10 agar plates. The smooth, transparent phenotype was the only morphologic variant of *M. intracellulare* present on the titer plates.

Agar dilution method. Compartmented plates were prepared with serial twofold dilutions of the various 4-quinolones. A Klett-Summerson colorimeter (Klett Manufacturing Co., Inc., New York, N.Y.) was used to standardize the cell culture suspensions with 7H10 broth as the diluent. Ten μl of each cell culture suspension was spotted on each antibiotic-containing plate at 1 and 0.1 Klett units per ml to yield final concentrations of approximately 2×10^4 and 2×10^3 CFU per spot, respectively, for *M. intracellulare* and at 5 and 0.5 Klett units per ml to yield similar final concentrations for *M. tuberculosis*. A control compartment, one having no drug, was included for each isolate. All plates were incubated at 37°C for either 3 weeks (*M. intracellulare*) or 4 weeks (*M. tuberculosis*). The MIC was defined as the lowest concentration of antibiotic which produced a 99% (2-log) inhibition of growth.

Broth dilution method. The ability of ciprofloxacin to inhibit *M. intracellulare* was determined by a broth dilution method, since this is a more rapid method than agar dilution for susceptibility testing of mycobacteria (3). The drug was diluted in 7H10 broth to produce serial twofold dilutions from 8 to 0.062 μg/ml. These tubes and a control tube, one containing no drug, were inoculated with a suspension of bacteria to yield a final concentration of approximately 10^4 CFU/ml (range, 7×10^3 to 7.8×10^4 CFU/ml). The MIC for *P. aeruginosa* was determined by broth and agar dilution by using final inocula of 2.5×10^4 CFU/ml for broth dilution and 2×10^4 CFU per spot for agar dilution. After 4 days of

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TABLE 1. MICs of 4-quinolones for *M. tuberculosis* and *M. intracellulare* determined by agar dilution

Organism (no. of isolates)	4-Quinolone	MIC (μg/ml)		
		50%	90%	Range
M. tuberculosis (22)	Ciprofloxacin	0.25	0.50	0.25–0.50
	Ofloxacin	1	1	0.5–1
	Norfloxacin	4	4	2–4
	Amifloxacin	4	4	2–8
M. intracellulare (20)	Ciprofloxacin	1	2	0.25–2
	Ofloxacin	4	8	0.5–16

incubation (overnight for *P. aeruginosa*) at 37°C on a rotary shaker, the tubes were examined for growth, and the MIC was defined as the lowest concentration of antibiotic resulting in no visual turbidity. To determine the lowest concentration of antibiotic which produced 99% killing, we subcultured 100-μl portions from tubes which were not visually turbid.

RESULTS

The MICs determined by agar dilution of ciprofloxacin, ofloxacin, norfloxacin, and amifloxacin for *M. tuberculosis* are shown in Table 1. *P. aeruginosa* ATCC 27853 was used as an internal control for the susceptibility testing of *M. tuberculosis*. Ciprofloxacin was the most active 4-quinolone, having an MIC for 50% of the isolates (MIC_{50}) and an MIC for 90% of the isolates (MIC_{90}) of 0.25 and 0.50 μg/ml, respectively. Ofloxacin had an MIC_{90} of 1 μg/ml; norfloxacin and amifloxacin were less active, with MIC_{90}s of 4 μg/ml. The ability of ciprofloxacin to produce 99% killing of *M. tuberculosis* was determined for each of 10 isolates by broth dilution to be ≤0.25 μg/ml, the lowest concentration used in that particular experiment.

The MICs of ciprofloxacin and ofloxacin for 20 *M. intracellulare* isolates are shown in Table 1. The MIC_{90} of ciprofloxacin was 2 μg/ml compared with an MIC_{90} of ofloxacin of 8 μg/ml. The MIC_{90} of ciprofloxacin determined by both broth dilution and agar dilution was 4 μg/ml (Table 2). The concentration which produced 99% killing was usually one or two dilutions higher than was the corresponding MIC. The MICs for *P. aeruginosa* were determined to be 0.5 μg/ml by broth dilution and 1 μg/ml by agar dilution.

DISCUSSION

Additional agents for treating mycobacterial diseases are needed. The treatment of multi-drug-resistant *M. tuberculosis* infections is often difficult, since many of the "second-line" agents are not well tolerated. There is a greater need for effective drugs against *M. intracellulare*, an organism for which optimal therapy has yet to be defined (4, 10). The published data on the in vitro activity of the 4-quinolones against *M. tuberculosis* and *M. intracellulare* are limited. Gay et al. (6) reported the activities of ciprofloxacin and norfloxacin against 100 mycobacterial isolates, including 20 isolates each of *M. tuberculosis* and *M. intracellulare*. These investigators found an MIC_{50} and an MIC_{90} of ciprofloxacin for *M. tuberculosis* of 0.5 and 1 μg/ml, respectively, values which are similar to ours. The MIC_{50} and the MIC_{90} for *M. intracellulare* were 2 and 16 μg/ml, respectively, values which were somewhat higher than those we observed. This may have resulted from different methodologies. Gaya and Chadwick (7) found that the MICs of ciprofloxacin were < 4 μg/ml for all *M. tuberculosis* isolates but >16 μg/ml for most *M. intracellulare* isolates. Since their methodology was quite different from ours, a direct comparison with their results is difficult. Tsukamura reported in vitro testing of ofloxacin against *M. tuberculosis* (13, 14) and *Mycobacterium avium* and *M. intracellulare* (13). His results agree with those of the present study in regard to *M. tuberculosis*; however, he found that *M. intracellulare* was inhibited by lower concentrations of ofloxacin than was *M. avium* (33 of 38 *M. intracellulare* isolates were inhibited by 2.5 μg/ml, and 17 of 20 *M. avium* isolates were inhibited by 10 μg/ml). Tsukamura et al. (15) studied in vivo activity of ofloxacin, 300 mg given to patients daily, in the treatment of multi-drug-resistant cavitary pulmonary tuberculosis. These investigators felt that ofloxacin was less effective than rifampin but more effective than kanamycin, ethionamide, or cycloserine.

Ciprofloxacin achieves peak concentrations in serum of 2 to 3 μg/ml after a 500-mg oral dose (2, 5, 9), 3.4 to 4.2 μg/ml after a 750-mg oral dose (8), and 2 μg/ml after a 100-mg intravenous bolus (17). Ofloxacin achieves a peak level in serum of 11 μg/ml after a 600-mg oral dose (11). Peak levels in serum of 4 μg/ml are obtainable after a 1,600-mg oral dose of norfloxacin (12). Pharmacokinetic data for amifloxacin are not available.

Although ciprofloxacin is more active in vitro than ofloxacin against *M. tuberculosis* and *M. intracellulare*, the higher levels achievable in serum with ofloxacin make it difficult to predict which might be more active in vivo. The peak levels in serum for both agents greatly exceed the MIC_{90} for *M. tuberculosis*. Their potential in vivo activity against *M. intracellulare* is less promising.

Ciprofloxacin and ofloxacin have been shown to be active in vitro against clinical isolates of *M. tuberculosis* and *M. intracellulare*. These agents have certain in vivo advantages, including their large volume of distribution, their penetration into blister fluid (17), and their ability to accumulate in serum. Additionally, these drugs are well tolerated, with no

TABLE 2. MICs of ciprofloxacin for *M. intracellulare* determined by broth dilution and agar dilution

Isolate	MIC (μg/ml) determined by[a]:	
	Broth dilution	Agar dilution
SKV	2 (8)	4
2-9	1 (2)	1
2-12	0.5 (1)	0.5
1296	1 (2)	2
2-16	0.5 (1)	0.5
LPR	4 (4)	4
2-23	0.5 (1)	0.5
1023	1 (2)	1
AMT	1 (4)	4
953	4 (8)	4
998	1 (4)	4
74	0.5 (1)	1
92	0.5 (2)	2
109	1 (2)	2
119	0.5 (0.5)	0.5
141	4 (8)	4
2-15	2 (8)	2
SHE	2 (8)	2
LYN	2 (2)	2

[a] The MIC_{50}s of ciprofloxacin for the isolates were determined to be 1 and 2 μg/ml by broth dilution and agar dilution, repectively; the MIC_{90}s determined by both methods were 4 μg/ml. The numbers in parentheses represent the concentrations (in micrograms per milliliter) which produced 99% killing.

serious adverse effects reported (1, 15). Ciprofloxacin and ofloxacin deserve in vivo evaluation against *M. tuberculosis* and *M. intracellulare* infections in an animal model and perhaps against clinical diseases in humans.

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LITERATURE CITED

1. **Brumfitt, W., I. Franklin, D. Grady, J. M. T. Hamilton-Miller, and A. Iliffe.** 1984. Changes in the pharmacokinetics of ciprofloxacin and fecal flora during administration of a 7-day course to human volunteers. Antimicrob. Agents Chemother. **26**:757–761.
2. **Crump, B., R. Wise, and J. Dent.** 1983. Pharmacokinetics and tissue penetration of ciprofloxacin. Antimicrob. Agents Chemother. **24**:784–786.
3. **Cynamon, M. H., and G. S. Palmer.** 1983. In vitro activity of amoxicillin in combination with clavulanic acid against *Mycobacterium tuberculosis*. Antimicrob. Agents Chemother. **24**:429–431.
4. **Davidson, P. T., V. Khanijo, M. Goble, and T. S. Moulding.** 1981. Treatment of disease due to *Mycobacterium intracellulare*. Rev. Infect. Dis. **3**:1052–1059.
5. **Davis, R. L., J. R. Koup, J. Williams-Warren, A. Weber, and A. L. Smith.** 1985. Pharmacokinetics of three oral formulations of ciprofloxacin. Antimicrob. Agents Chemother. **28**:74–77.
6. **Gay, J. D., D. R. DeYoung, and G. D. Roberts.** 1984. In vitro activities of norfloxacin and ciprofloxacin against *Mycobacterium tuberculosis*, *M. avium* complex, *M. chelonei*, *M. fortuitum*, and *M. kansasii*. Antimicrob. Agents Chemother. **26**:94–96.
7. **Gaya, H., and M. V. Chadwick.** 1985. *In vitro* activity of ciprofloxacin against mycobacteria. Eur. J. Clin. Microbiol. **4**:345–347.
8. **Gonzalez, M. A., F. Uribe, S. D. Moisen, A. P. Fuster, A. Selen, P. G. Welling, and B. Painter.** 1984. Multiple-dose pharmacokinetics and safety of ciprofloxacin in normal volunteers. Antimicrob. Agents Chemother. **26**:741–744.
9. **Höffken, G., H. Lode, C. Prinzing, K. Borner, and P. Koeppe.** 1985. Pharmacokinetics of ciprofloxacin after oral and parenteral administration. Antimicrob. Agents Chemother. **27**:375–379.
10. **Horsburgh, C. R., Jr., U. G. Mason III, D. C. Farhi, and M. D. Iseman.** 1985. Disseminated infection with *Mycobacterium avium-intracellulare*. Medicine (Baltimore) **64**:36–48.
11. **Lockley, M. R., R. Wise, and J. Dent.** 1984. The pharmacokinetics and tissue penetration of ofloxacin. J. Antimicrob. Chemother. **14**:647–652.
12. **Swanson, B. N., V. K. Boppana, P. H. Vlasses, H. H. Rotmensch, and R. K. Ferguson.** 1983. Norfloxacin disposition after sequentially increasing oral doses. Antimicrob. Agents Chemother. **23**:284–288.
13. **Tsukamura, M.** 1983. *In vitro* antimycobacterial activity of a new antibacterial substance DL-8280. Differentiation between some species of mycobacteria and related organisms by the DL-8280 susceptibility test. Microbiol. Immunol. **27**:1129–1132.
14. **Tsukamura, M.** 1985. In vitro antituberculosis activity of a new antibacteral substance ofloxacin (DL 8280). Am. Rev. Respir. Dis. **131**:348–351.
15. **Tsukamura, M., E. Nakamura, S. Yoshii, and H. Amano.** 1985. Therapeutic effect of a new antibacterial substance ofloxacin (DL 8280) on pulmonary tuberculosis. Am. Rev. Respir. Dis. **131**:352–356.
16. **Vestal, A. L.** 1969. Procedures for the isolation and identification of mycobacteria. Public Health Service publication no. 1995. Laboratory Division, National Communicable Disease Center, Atlanta.
17. **Wise, R., R. M. Lockley, M. Webberly, and J. Dent.** 1984. Pharmacokinetics of intravenously administered ciprofloxacin. Antimicrob. Agents Chemother. **26**:208–210.