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In Vitro Activities of Six Fluoroquinolones against 250 Clinical Isolates of *Mycobacterium tuberculosis* Susceptible or Resistant to First-Line Antituberculosis Drugs

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Two hundred fifty isolates of *Mycobacterium tuberculosis* were evaluated for susceptibility to ciprofloxacin, ofloxacin, levofloxacin, grepafloxacin, trovafloxacin, and gemifloxacin (SB-265805). Levofloxacin, ciprofloxacin, and grepafloxacin showed the greatest activity (MIC for 90% of strains tested [MIC_{90}] 1 μg/ml), although ofloxacin also showed good activity, with an MIC_{90} of 2 μg/ml. Trovafloxacin and gemifloxacin showed lower in vitro activity, with MIC_{90}s of 64 and 8 μg/ml, respectively.

The increase in drug-resistant *Mycobacterium tuberculosis* isolates during recent years presents a therapeutic challenge to physicians selecting antimicrobial agents (2, 3, 4, 10, 12). Fluoroquinolones may have a useful role in the treatment of these infections not only because some of their derivatives, e.g., ofloxacin (11), have already been used for the treatment of pulmonary tuberculosis but also because newer derivatives are continually being developed. However, comparative in vitro susceptibility data for classic and new agents of this class against a representative number of *M. tuberculosis* isolates are scarce (5, 13, 14).

In our study, we compared the activities of the fluoroquinolones ciprofloxacin, ofloxacin, levofloxacin, grepafloxacin, trovafloxacin, and the novel compound gemifloxacin (SB-265805) against 250 clinical isolates of *M. tuberculosis* with different levels of susceptibility to first-line antituberculosis drugs.

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The active substances of the assayed antimycobacterial agents were kindly provided as reference powders by Smith-Kline Beecham (Worthing, United Kingdom). Ofloxacin was obtained from Sigma Chemical Co. (St. Louis, Mo.). Agent corrections were made for purity of antimicrobials. Stock solutions of all of the fluoroquinolones were prepared at 10,000 μg/ml in distilled water by adding a 0.1 M NaOH solution for dilution when necessary. Aliquots of the antituberculosis agents were frozen at −70°C until use. *Staphylococcus aureus* strain ATCC 29213 was used for quality control to ensure the potency of the fluoroquinolones tested.

Two hundred fifty clinical isolates of *M. tuberculosis* from 250 tuberculosis patients were selected from our laboratory collection (1988 to 1999). Of the samples tested, 197 were of respiratory origin and 53 were of nonrespiratory origin. Testing of susceptibility to first-line antituberculosis drugs (isoniazid, rifampin, ethambutol, and streptomycin) was performed by the agar proportion method in a reference laboratory. Of the strains tested, 44 (18%) were resistant to at least one first-line antituberculosis drug (R-MTB group; 24 monodrug-resistant and 20 multidrug-resistant strains) while the rest were fully susceptible (S-MTB group). The agar proportion method was performed as recommended by the National Committee for Clinical Laboratory Standards (9). Briefly, 7H10 agar medium (Difco) was prepared from a dehydrated base as recommended by the manufacturer. After the agar was autoclaved, oleic acid-albumin-dextrose-catalase supplement (Becton Dickinson) and fluoroquinolones were added at 50 to 56°C by doubling dilutions to yield final concentrations of each drug of 0.125 to 128 μg/ml. Five milliliters of each concentration of antimycobacterial-containing medium was dispensed into plastic quadrant petri dishes. As a growth control, one quadrant in each plate was filled with 7H10 agar medium with no drug. An inoculum of each isolate was prepared in Middlebrook 7H9 broth, and the absorbance was adjusted until it was equivalent to that of a McFarland no. 1 standard. Final suspensions were performed by adding Middlebrook 7H9 broth to prepare 10^{-2} and 10^{-4} dilutions of the standardized suspensions. Upon solidification of the medium, the plates received 0.1 ml of the dilutions by inoculation of 3 drops at different points on each quadrant of the agar plates. The inoculated plates were then incubated at 37°C for 3 weeks. Blood agar plates were inoculated as contamination controls. The MICs of each isolate-drug pair was the lowest concentration of the antimycobacterial agent that inhibited >99% of the colonies growing on the drug-free control. *M. tuberculosis* ATCC 27294 (H37Rv strain) was used as a control strain.

The MICs at which 50% of the isolates were inhibited (MIC_{50}s), MIC_{90}s, MIC ranges, and geometric mean MICs of the six fluoroquinolones are shown in Table 1. Overall, levofloxacin (MIC_{90}, 1 μg/ml) showed the greatest activity against the *M. tuberculosis* strains tested, with 96.4% of the strains inhibited at 1 μg/ml. Ciprofloxacin (MIC_{90}, 1 μg/ml; 92.0%), grepafloxacin (MIC_{90}, 1 μg/ml; 90.4%), and ofloxacin (MIC_{90}, 2 μg/ml; 88.8%) also showed good activity. Trovafloxacin (MIC_{90}, 64 μg/ml; 0%) and gemifloxacin (MIC_{90}, 8 μg/ml; 6.4%) were inactive against most of the strains tested.

Besides cross-resistance to all of these fluoroquinolones, the MIC ranges for six clinical isolates of *M. tuberculosis* were as follows: ciprofloxacin, 8 to 16 μg/ml; ofloxacin, 8 to 16 μg/ml; levofloxacin, 8 μg/ml; grepafloxacin, 8 to 32 μg/ml; trovafloxacin, 128 to >128 μg/ml; gemifloxacin, 32 to 64 μg/ml. Four of

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TABLE 1. Antimycobacterial activities of six fluoroquinolones against 250 clinical isolates of *M. tuberculosis*

Organism group (no. of strains) and drug	MIC (μg/ml)			
	Range	For 50% of strains	For 90% of strains	Geometric mean
S-MTB (206)				
Ciprofloxacin	≤0.125–16	1	1	0.744
Ofloxacin	≤0.125–16	1	1	0.842
Levofloxacin	≤0.125–8	0.5	1	0.549
Grepafloxacin	0.25–32	1	1	0.782
Trovafloxacin	4–>128	32	64	29.917
Gemifloxacin	≤0.125–64	4	8	4.878
R-MTB (44)				
Ciprofloxacin	0.5–8	1	2	0.882
Ofloxacin	0.5–16	1	2	1.171
Levofloxacin	≤0.125–8	0.5	1	0.741
Grepafloxacin	0.5–32	1	2	1.189
Trovafloxacin	4–>128	32	128	34.623
Gemifloxacin	2–64	8	8	6.417
All isolates (250)				
Ciprofloxacin	≤0.125–16	1	1	0.766
Ofloxacin	≤0.125–16	1	2	0.893
Levofloxacin	≤0.125–8	0.5	1	0.579
Grepafloxacin	0.25–32	1	1	0.842
Trovafloxacin	4–>128	32	64	30.697
Gemifloxacin	≤0.125–64	4	8	5.120

these resistant isolates were S-MTB, and the other two were isoniazid- and rifampin-resistant strains, one of them with added resistance to ethambutol.

In general, fluoroquinolone activity was higher in S-MTB strains than in R-MTB strains, with a twofold difference in the MIC_{90}s of ciprofloxacin, ofloxacin, grepafloxacin, and trovafloxacin. Although there were no differences in the MIC_{90}s of levofloxacin and gemifloxacin for both S-MTB and R-MTB strains, the geometric mean MICs of these agents were higher for R-MTB than for S-MTB strains (levofloxacin, 0.549 versus 0.741 μg/ml; gemifloxacin, 4.878 versus 6.417 μg/ml). When we analyzed R-MTB strains, there was no relationship between the level of resistance to first-line drugs and the activity of fluoroquinolones against the mycobacteria.

Tuberculosis caused by drug-resistant strains of *M. tuberculosis* poses a therapeutic challenge in terms of the selection of appropriate antimicrobial agents. The development of new fluoroquinolones with a broader spectrum has become an alternative in the treatment of drug-resistant *M. tuberculosis* infections.

Ciprofloxacin, ofloxacin, levofloxacin, and grepafloxacin yielded good in vitro potency against *M. tuberculosis*, with geometric mean MIC_{90}s of <1 μg/ml, while trovafloxacin and gemifloxacin showed significantly greater values ($P < 0.0001$, paired-samples t test of $\log_2$ MICs). Naphthyridone structure, such as that of trovafloxacin and gemifloxacin, has been identified as a negative factor in a quantitative structure-activity relationship study of antimycobacterial activity (7), which may explain the poor activity of these fluoroquinolones against *M. tuberculosis*.

Like other authors (14), we found slightly higher fluoroquinolone activity against S-MTB strains than against R-MTB strains. The mechanism of fluoroquinolone resistance is known to involve mutations in the A and B subunits of the mycobacterial DNA gyrase (1). In our study, *M. tuberculosis* showed cross-resistance to all of the fluoroquinolones tested.

Combination therapies with drugs using different mechanisms of action produce better efficacy with less probability of resistance. Like the high in vitro activity ciprofloxacin and ofloxacin, used successfully to treat resistant *M. tuberculosis* infections (6, 8), that of levofloxacin and grepafloxacin makes them promising drugs for use against these infections. Unfortunately, the toxicity of grepafloxacin precludes its use for therapy. The potential role of levofloxacin in the treatment of tuberculosis requires further clinical evaluation.

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REFERENCES

1. **Chen, X., B. N. Kreiswirth, S. Sreevatsan, J. M. Musser, and K. Drlica.** 1996. Fluoroquinolone resistance associated with specific gyrase mutations in clinical isolates of multidrug-resistant *Mycobacterium tuberculosis*. J. Infect. Dis. **174:**1127–1130.
2. **Culliton, B. J.** 1992. Drug-resistant TB may bring epidemic. Nature (London) **356:**472.
3. **Frieden, T. R., T. Sterling, A. Pablos-Mendez, J. O. Kilburn, G. M. Cauthen, and S. W. Dooley.** 1993. The emergence of drug-resistant tuberculosis in New York City. N. Engl. J. Med. **328:**521–526.
4. **Goble, M., M. D. Iseman, L. A. Madsen, D. Waite, L. Ackerson, and C. R. Horsburgh.** 1993. Treatment of 171 patients with pulmonary tuberculosis resistant to isoniazid and rifampin. N. Engl. J. Med. **328:**527–532.
5. **Hoffner, S., L. Gezelius, and L. B. Olsson.** 1997. In vitro activity of fluorinated quinolones and macrolides against *Mycobacterium tuberculosis*. J. Antimicrob. Chemother. **40:**885–888.
6. **Iseman, M. D.** 1993. Treatment of multidrug-resistant tuberculosis. N. Engl. J. Med. **329:**784–791.
7. **Jacobs, M. R.** 1995. Activity of quinolones against mycobacteria. Drugs **49**(Suppl. 2)**:**67–75.
8. **Kennedy, N., R. Fox, G. M. Kisyombe, et al.** 1993. Early bactericidal and sterilizing activities of ciprofloxacin in pulmonary tuberculosis. Am. Rev. Respir. Dis. **148:**1547–1551.
9. **National Committee for Clinical Laboratory Standards.** 1995. Antimycobacterial susceptibility testing for *Mycobacterium tuberculosis*. Proposed standard M24-T. National Committee for Clinical Laboratory Standards, Villanova, Pa.
10. **Sudre, P., H. G. Ten Dam, and A. Kochi.** 1992. Tuberculosis: a global overview of the situation today. Bull. W. H. O. **70:**149–159.
11. **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.
12. **World Health Organization Working Group.** 1991. Tuberculosis research and development. WHO/TB91-162. World Health Organization, Geneva, Switzerland.
13. **Yamane, N., B. Chilima, M. Tosaka, Y. Okazawa, and K. Tanno.** 1996. Determination of antimycobacterial activities of fluoroquinolones against clinical isolates of *Mycobacterium tuberculosis*: comparative determination with egg-based Ogawa and agar-based Middlebrook 7H10 media. Kekkaku **71:**453–458.
14. **Yew, W., L. Piddock, M. Li, D. Lyon, C. Chan, and A. Cheng.** 1994. In vitro activity of quinolones and macrolides against mycobacteria. J. Antimicrob. Chemother. **34:**343–351.