ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Apr. 1989, p. 591–592
0066-4804/89/040591-02$02.00/0

Vol. 33, No. 4

Comparative Antimycobacterial Activities of Difloxacin, Temafloxacin, Enoxacin, Pefloxacin, Reference Fluoroquinolones, and a New Macrolide, Clarithromycin

EUGENE A. GORZYNSKI,[1,2]* STEVEN I. GUTMAN,[1,3] AND WILLIAM ALLEN[1]

Laboratory Service, Veterans Administration Medical Center, Buffalo, New York 14215,[1] and Department of Microbiology[2] and Department of Pathology,[3] School of Medicine and Biomedical Sciences, State University of New York at Buffalo, Buffalo, New York 14214

Received 7 November 1988/Accepted 27 January 1989

The activities of fluoroquinolones and a new macrolide against 30 clinical isolates of *Mycobacterium tuberculosis* were determined in vitro by agar diffusion. In order of relative potencies against *M. tuberculosis*, temafloxacin (MIC for 90% of isolates [MIC_{90}], 2.3 μg/ml) was at least as active as the reference quinolones ofloxacin (MIC_{90}, 2.4 μg/ml) and ciprofloxacin (MIC_{90}, 4.3 μg/ml). Less active were difloxacin (MIC_{90}, 4.7 μg/ml), pefloxacin (MIC_{90}, 6.7 μg/ml), and enoxacin (MIC_{90}, 8.3 μg/ml). The macrolide clarithromycin was more potent than erythromycin but less potent than the fluoroquinolones. Our results suggest that the newer fluoroquinolones and clarithromycin should be included with ciprofloxacin and ofloxacin in pharmacokinetic studies that may lead to trials in human subjects with mycobacterial infections.

The fluoroquinolones, a new class of antimicrobial agents, are structurally related to nalidixic acid and have pharmacologic and microbiologic properties attractive for their use in treating many infectious diseases caused by gram-negative and gram-positive bacteria. Good in vitro potencies against *Mycobacterium tuberculosis* have been reported for the fluoroquinolones ciprofloxacin (2, 3, 5, 6) and ofloxacin (3, 11); less active are norfloxacin (3, 5) and amifloxacin (3). Clarithromycin activity has not been reported.

In this report, we compare with reference antimicrobial agents the in vitro potencies against *M. tuberculosis* of fluoroquinolones, difloxacin, temafloxacin, enoxacin, pefloxacin, and a new macrolide, clarithromycin.

Antimicrobial agents. The following reagent powders were synthesized and supplied by Abbott Laboratories, North Chicago, Ill.: difloxacin (activity, 850 μg/mg), temafloxacin (activity, 1,000 μg/mg), and pefloxacin (activity, 1,000 μg/mg); erythromycin (activity, 763 μg/mg) and clarithromycin (activity, 993 μg/mg) (licensed from Taisho Pharmaceuticals) were also prepared and supplied by Abbott Laboratories. Other reagent powders were received as gifts from their manufacturers: ciprofloxacin (activity, 858 μg/mg; Miles Pharmaceuticals, West Haven, Conn.), ofloxacin (activity, 1,000 μg/mg; Ortho Pharmaceuticals, Raritan, N.J.), and enoxacin (activity, 1,000 μg/mg; Warner Lambert, Ann Arbor, Mich.). Stock solutions (1,000 μg/ml) were prepared according to manufacturer instructions. Subsequent dilutions in sterile distilled water were added to molten media used in assays for the MICs of drugs.

Mycobacteria. A total of 30 clinical isolates of *M. tuberculosis* were recovered from sputum specimens of patients prior to treatment at the Veterans Administration Medical Center, Buffalo, N.Y. After isolation and identification, the microorganisms were stored at 4°C on Lowenstein-Jensen medium (Difco Laboratories, Detroit, Mich.). For antimicrobial susceptibility testing, an indirect method (12) was used. Briefly, the strains were subcultured in Dubos broth base enriched with Dubos medium albumin (Difco) and incubated for 7 days or until growth produced turbidity equal to a McFarland no. 1 standard.

Susceptibility testing. MICs were determined by incorporating doubling dilutions of the investigational agent (10 to 0.3 μg/ml) in Middlebrook 7H10 agar (Difco) prepared as slants in screw-cap test tubes (20 by 150 mm). The inoculum (0.2 ml of the standardized culture diluted 10^{-5}) was spread over the surface of the control slant (drug free) and each antimicrobial agent slant. To eliminate expected confluent growth in control tubes and to allow for enumerating and calculating CFU per milliliter, we seeded an additional control with the inoculum diluted 1:20. The tubes were incubated at 37°C and examined for growth at 3- and 4-week intervals. The extent of growth in drug-containing tubes was compared with that in control tubes. If <1% of a population (i.e., <10 CFU of mycobacteria) failed to grow in the concentration of antimicrobial agent tested, the agent was recorded as effective at that concentration.

The MICs of arylfluoroquinolones and a new macrolide, clarithromycin, for mycobacteria are presented in Table 1. In order of relative potencies against *M. tuberculosis*, temafloxacin was at least as active as the reference quinolones (ofloxacin and ciprofloxacin) and more potent than difloxacin, pefloxacin, or enoxacin; pefloxacin was the least active. Clarithromycin, the 6-*O*-methyl derivative of erythromycin, was more active than the latter macrolide against 12 of the 30 strains of *M. tuberculosis* tested. A total of 1, 3, 7, 12, and 30 strains were inhibited by clarithromycin MICs of 0.3, 2.5, 5.0, 10, and >10 μg/ml, respectively.

In vitro the growth of many *Mycobacterium* species, including *M. tuberculosis*, is suppressed by ciprofloxacin (2, 3, 5, 6) and ofloxacin (3, 11). The current study supports these reports and extends knowledge regarding the potencies against *M. tuberculosis* strains of the fluoroquinolones difloxacin, temafloxacin, enoxacin, and pefloxacin. Our data showed that temafloxacin had MICs for 90% of *M. tuberculosis* isolates similar to those of ciprofloxacin and ofloxacin. Against all strains difloxacin, enoxacin, and pefloxacin were the least potent. Fenlon and Cynamon (3) reported MICs of ciprofloxacin and ofloxacin for 90% of *M. tuberculosis*

* Corresponding author.

TABLE 1. Comparative MICs of difloxacin, temafloxacin, enoxacin, pefloxacin, reference fluoroquinolones, and clarithromycin for *M. tuberculosis*

Antimicrobial agent	MIC (μg/ml)[a]		
	Range	50%	90%
Difloxacin	2.5–10	2.7	4.7
Temafloxacin	1.3–5	1.4	2.3
Enoxacin	2.5–>10	3.0	8.3
Pefloxacin	2.5–>10	3.3	6.7
Ciprofloxacin	0.6–5	2.0	4.3
Ofloxacin	1.3–10	1.3	2.4
Clarithromycin	1.3–10	>10	>10
Erythromycin	>10	>10	>10

[a] Defined by <1% growth of the inoculum. 50% and 90%, MIC for 50 and 90% of isolates tested, respectively.

isolates of 0.5 and 1 μg/ml, respectively, values lower than ours (4.3 and 2.4 μg/ml, respectively). There is an obvious advantage to testing under the same experimental conditions the potencies of a series of antimicrobial agents against the same populations of microbial isolates. Accordingly, whereas our higher values may reflect methodology, which will affect the results obtained (10), our conclusion that at least three of the reagents we tested (temafloxacin, ciprofloxacin, and ofloxacin) had similar potencies and were more active than difloxacin, enoxacin, or pefloxacin against *M. tuberculosis* is tenable.

The fluoroquinolones have pharmacologic and microbiologic properties acceptable for treating systemic infections (1, 9). The use of ciprofloxacin and ofloxacin against *M. tuberculosis* in an animal model and in clinical trials has been proposed (3). The in vitro activity of temafloxacin in the current report and its rapid oral absorption and bioavailability in the mouse model (7) justify assessing its antimycobacterial efficacy in animal studies.

Clarithromycin was more potent than erythromycin in vitro against 12 of the 30 strains of *M. tuberculosis* studied; each strain was refractory to 10 μg of erythromycin per ml. Although the in vitro potency of clarithromycin against *M. tuberculosis* was marginal, study of its intracellular efficacy is justified on the basis of recent reports with animal models and other microorganisms. Fernandes and colleagues (4) reported that clarithromycin administered orally to mice was better absorbed than erythromycin and resulted in a peak level and half-life in serum higher than those of erythromycin. In addition, the latter investigators showed that clarithromycin given intraperitoneally eliminated or significantly reduced the viable population of *Legionella pneumophila* in the lungs and spleens of experimentally infected guinea pigs. More recently, Hastings et al. reported that administration in the feed of mice of clarithromycin, in contrast to erythromycin or roxithromycin, fully suppressed the growth of *Mycobacterium leprae* in the footpad (8). In his commentary on *Mycobacterium avium* infection in patients with acquired immunodeficiency syndrome, Young postulated that combination approaches for delivering medications to intracellular sites of phagocytes may offer the best hope for eventual control of this systemic infection (13).

Because mycobacterial infections have both intra- and extracellular loci, temafloxacin, difloxacin, and clarithromycin alone or in combination with compounds that have in vitro activity should be included in pharmacokinetic studies that may lead to trials in humans.

We thank D. J. Hardy and P. B. Fernandes for their helpful advice.

This research was supported by the Medical Research Service of the Veterans Administration and by a grant from Abbott Laboratories.

LITERATURE CITED

1. **Campoli-Richards, D. M., J. P. Monk, A. Price, P. Benfield, P. A. Todd, and A. Ward.** 1988. Ciprofloxacin. A review of its antibacterial activity, pharmacokinetic properties and therapeutic use. Drugs **35**:373–447.
2. **Collins, C. H., and H. C. Uttley.** 1985. *In vitro* susceptibility of mycobacteria to ciprofloxacin. J. Antimicrob. Chemother. **16**: 575–580.
3. **Fenlon, C. H., and M. H. Cynamon.** 1986. Comparative in vitro activities of ciprofloxacin and other 4-quinolones against *Mycobacterium tuberculosis* and *Mycobacterium intracellulare*. Antimicrob. Agents Chemother. **29**:386–388.
4. **Fernandes, P. B., R. Bailer, R. Swanson, C. W. Hanson, E. McDonald, N. Ramer, D. Hardy, N. Shipkowitz, R. R. Bower, and E. Gade.** 1986. In vitro and in vivo evaluation of A-56268 (TE-031), a new macrolide. Antimicrob. Agents Chemother. **30**:865–873.
5. **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.
6. **Gaya, H., and M. V. Chadwick.** 1985. *In vitro* activity of ciprofloxacin against mycobacteria. Eur. J. Clin. Microbiol. **4**:345–347.
7. **Hardy, D. J., R. N. Swanson, D. M. Hensey, N. R. Ramer, R. R. Bower, C. W. Hanson, D. T. W. Chu, and P. B. Fernandes.** 1987. Comparative antibacterial activities of temafloxacin hydrochloride (A-62254) and two reference fluoroquinolones. Antimicrob. Agents Chemother. **31**:1768–1774.
8. **Hastings, R. C., T. P. Gillis, J. L. Krahenbuhl, and S. G. Franzblau.** 1988. Leprosy. Clin. Microbiol. Rev. **1**:330–348.
9. **Monk, J. P., and D. M. Campoli-Richards.** 1987. Ofloxacin. A review of its antibacterial activity, pharmacokinetic properties and therapeutic use. Drugs **33**:346–391.
10. **Trimble, K. A., R. B. Clark, W. E. Sanders, Jr., J. W. Frankel, R. Caciatore, and H. Valdez.** 1987. Activity of ciprofloxacin against mycobacteria *in vitro*: comparison of BACTEC and macrobroth dilution methods. J. Antimicrob. Chemother. **19**: 617–622.
11. **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.
12. **Vestal, A. L.** 1975. Procedures for the isolation and identification of mycobacteria. U.S. Department of Health, Education, and Welfare publication no. (CDC) 79-8230, p. 97–115. Centers for Disease Control, Atlanta.
13. **Young, L. S.** 1988. *Mycobacterium avium* complex infection. J. Infect. Dis. **157**:863–867.