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Activities of Various Quinolone Antibiotics against *Mycobacterium leprae* in Infected Mice

R. H. GELBER,[1,2]* A. IRANMANESH,[1] L. MURRAY,[1] P. SIU,[1] AND M. TSANG[1]

Medical Research Institute of California Pacific Medical Center, San Francisco, California 94115,[1] and G. W. Long Hansen's Disease Center, Carville, Louisiana 70721[2]

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Previously, pefloxacin and ofloxacin were found to be active against *Mycobacterium leprae* in vitro, in experimental animals, and in clinical trials of lepromatous leprosy patients. In this study, we compared certain more recently developed fluoroquinolones (lomefloxacin, PD 124816, WIN 57273, temafloxacin, and sparfloxacin) with pefloxacin and ofloxacin in *M. leprae*-infected mice at doses of 50, 150, and 300 mg/kg given five times weekly. All seven of the fluoroquinolones studied were active against *M. leprae*; temafloxacin and sparfloxacin were the most active, being fully bactericidal at all three dosage schedules. Additionally, sparfloxacin was found to be fully bactericidal at 15 and 30 mg/kg given five times weekly.

Unfortunately, the presently recommended drugs for general treatment of multibacillary leprosy in humans have been limited to only a few antimicrobial agents: dapsone, rifampin, and clofazimine (38). Because (i) multidrug therapy is generally recommended for the treatment of leprosy (38), (ii) resistance, particularly to dapsone and rifampin, has occurred, resulting in clinical relapse (18), and (iii) significant side effects and toxicities precluding the use of each of these drugs in certain patients occurs, it is imperative that new bactericidal drugs which work in a novel manner be developed and incorporated into the existing therapeutic arsenal to treat this disease. Fluoroquinolones act at a heretofore unexplored locus for the treatment of *Mycobacterium leprae* infections, the DNA gyrase (32). Furthermore, they accumulate severalfold in resident macrophages (3, 25, 37), the obligate site of *M. leprae* infection.

Previous studies of the activities of ciprofloxacin (1, 15), pefloxacin (15, 26), and ofloxacin (13, 26, 31) against *M. leprae*-infected mice found that while ciprofloxacin was ineffective (owing to weak activity in vitro [5] and/or demonstrably poor gastrointestinal absorption in mice [15]), both pefloxacin and ofloxacin were found to be bactericidal. Furthermore, both pefloxacin (14, 24) and ofloxacin (14) have proved extremely promising for treatment of lepromatous leprosy patients. However, more recently developed fluoroquinolones have demonstrated even greater activity against gram-positive organisms; *M. leprae* is gram positive and shares certain similar antimicrobial susceptibilities with gram-positive bacteria. Therefore, we initiated this study to compare the relative activities against *M. leprae* of certain of these newer quinolones with those of pefloxacin and ofloxacin.

We compared the activities of the newer quinolones sparfloxacin, temafloxacin, lomefloxacin, PD 124816, and WIN 57273 with those of pefloxacin and ofloxacin against *M. leprae* in infected mice. We utilized the kinetic method of Shepard et al. (34), wherein groups of BALB/c female mice (Jackson Laboratories, Bar Harbor, Maine) were initially infected in both hind footpads with 5,000 mouse-derived logarithmically multiplying *M. leprae* organisms and subsequently treated from days 60 to 154 after infection with each of the quinolones five times weekly by gavage at doses of 50, 150, and 300 mg/kg. Additionally, because sparfloxacin previously had been found to inhibit the growth of *M. leprae* in nude mice at 15 mg/kg (36), in the present study, sparfloxacin was also evaluated at 15 and 30 mg/kg given five times weekly. At day 154 and at intervals of 2 to 3 months thereafter, generally up to 9 to 12 months, the number of *M. leprae* organisms from pools of four hind feet (from two mice) was determined microscopically until growth of *M. leprae* was considered to have occurred, the number of acid-fast bacilli per footpad equalling or exceeding 10^5 (33). We judged drugs (i) bacteriostatic (34) if at the end of therapy the number of acid-fast bacilli was less than in untreated controls but multiplication commenced immediately thereafter, (ii) partially bactericidal if multiplication was further delayed, and (iii) fully bactericidal if *M. leprae* did not grow even 9 months after therapy was completed.

The results of these studies are detailed in Fig. 1 to 7 and summarized in Table 1. Pefloxacin (Fig. 1) and lomefloxacin (Fig. 2) were found inactive and bacteriostatic, respectively, at 50 mg/kg; bacteriostatic at 150 mg/kg; and partially bactericidal at 300 mg/kg. PD 124816 (Fig. 3) was bacterio-

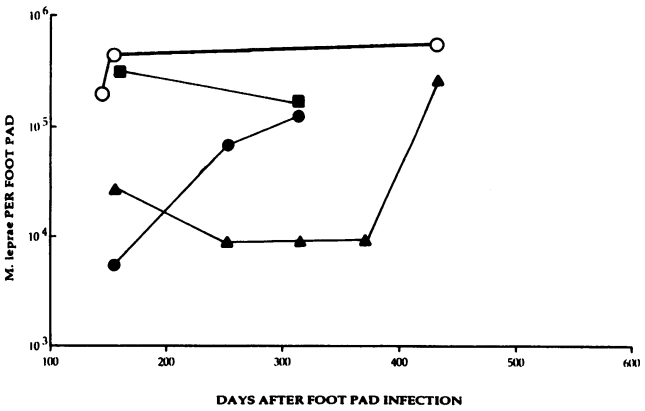

FIG. 1. Activity of pefloxacin for *M. leprae* in mice. Numbers of *M. leprae* organisms in pools of hind feet from two mice (four feet) are shown. Mice were treated five times weekly from days 60 to 154 after *M. leprae* infection. Symbols: ○, control; ■, 50 mg/kg; ●, 150 mg/kg; ▲, 300 mg/kg.

* Corresponding author.

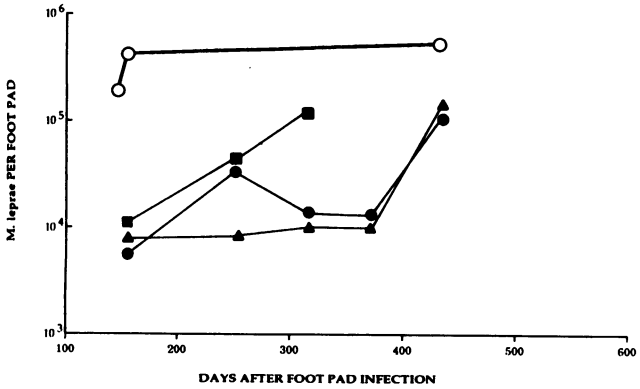

FIG. 2. Activity of lomefloxacin for *M. leprae* in mice. For details, see the legend to Fig. 1.

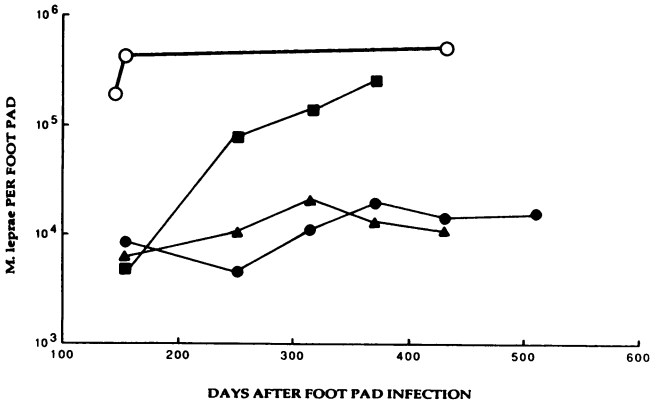

FIG. 4. Activity of ofloxacin for *M. leprae* in mice. For details, see the legend to Fig. 1.

static at 50 and 150 mg/kg and fully bactericidal at 300 mg/kg. Ofloxacin (Fig. 4) was bacteriostatic at 50 mg/kg and fully bactericidal at 150 and 300 mg/kg. WIN 57273 (Fig. 5) was partially bactericidal at 50 mg/kg and fully bactericidal at 150 and 300 mg/kg. Temafloxacin (Fig. 6) was fully bactericidal at all three of the doses tested, while sparfloxacin (Fig. 7) was fully bactericidal at these doses, as well as at 15 and 30 mg/kg.

We found that the seven fluoroquinolones studied all had activity against *M. leprae*. The relatively superior activity we found for ofloxacin compared with pefloxacin is in accord with that found previously by others. While we found pefloxacin to be inactive at 50 mg/kg, bacteriostatic at 150 mg/kg, and partially bactericidal at 300 mg/kg, Guelpa-Lauras et al. (15) found that pefloxacin was bacteriostatic at 50 mg/kg and partially bactericidal at 150 mg/kg; Pattyn (26) found that even 150 mg of pefloxacin per kg was without bactericidal activity. On the other hand, we found ofloxacin at 50 mg/kg to be bacteriostatic, Grosset (13) and Pattyn (26) found it to be bactericidal, and all of us found ofloxacin at 150 mg/kg and higher to be fully bactericidal for *M. leprae*.

Perhaps the most important finding of this study is that a few of the newer quinolones, particularly sparfloxacin and temafloxacin, exhibited greater activity against *M. leprae* than did pefloxacin and ofloxacin. Of the seven fluoroquinolones tested, only sparfloxacin and temafloxacin were found to be fully bactericidal at 50 mg/kg. Additionally, sparfloxacin was found to be fully bactericidal at 15 and 30 mg/kg, while temafloxacin and the other quinolones studied were not evaluated at these dosages. Previously, Franzblau and White (5) found in vitro that sparfloxacin was more active than ofloxacin, and Pattyn (27) found in vivo that temafloxacin was also more active than ofloxacin. Thus, although pefloxacin and ofloxacin are active against *M. leprae* in mice and in clinical trials, we found in this study, as have others, that alternative fluoroquinolones, especially sparfloxacin and temafloxacin, are even more active.

The relatively superior activity of sparfloxacin against *M. leprae* found in this study has precedence for other pathogenic mycobacteria in that sparfloxacin's MIC previously had been found to be lower than that of ofloxacin for both the tubercle bacillus (29) and the *M. avium* complex (30). In fact, although *M. avium* is generally resistant to antimicrobial agents, in one study (30), 7 of 10 strains were inhibited by levels of sparfloxacin below the maximum concentration in serum in humans (1.4 μg/ml) (22), and in another study (35), 90% of *M. avium* strains were inhibited by levels of sparfloxacin which are generally obtained in tissues (4 μg/ml) (22). Furthermore, 4 μg of sparfloxacin per ml was previously found to slow the growth of sparfloxacin susceptible strains of *M. avium* very significantly in human monocyte-derived macrophage culture (28). Sparfloxacin has also been found to be consistently active and generally bactericidal for a broad range of other mycobacteria, both in agar and in macrophage culture (30).

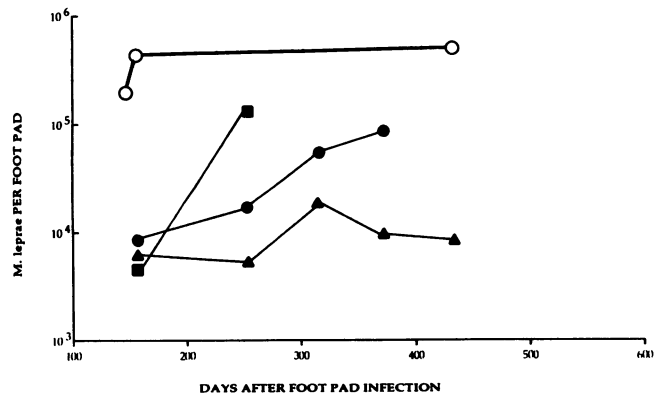

FIG. 3. Activity of PD 124816 for *M. leprae* in mice. For details, see the legend to Fig. 1.

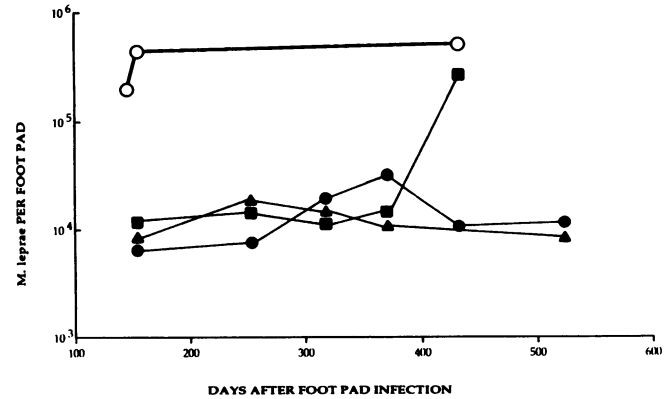

FIG. 5. Activity of WIN 57273 for *M. leprae* in mice. For details, see the legend to Fig. 1.

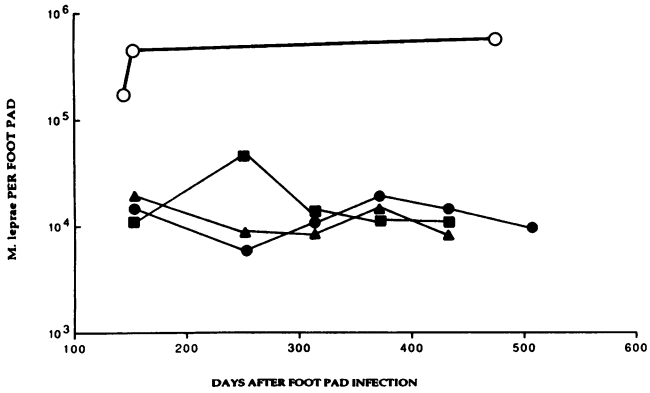

FIG. 6. Activity of temafloxacin for *M. leprae* in mice. For details, see the legend to Fig. 1.

Sparfloxacin, as well as being generally the most effective fluoroquinolone for both gram-positive bacteria (23) and other mycobacteria (28–30), has the following pharmacological advantages that might serve to explain its superior activity for *M. leprae* found in this study: (i) greater hydrophobicity at positions R1 and R4, which was found previously to result in increased activity for a series of 4-quinolones against *M. avium* (16) and would potentially permit greater penetration of the dense, largely lipid outer capsule and cell wall of *M. leprae*; (ii) superior tissue penetration, resulting in levels 2- to 11-fold higher than those obtained in plasma (22); (iii) superior accumulation within macrophages compared with other fluoroquinolones (3); (iv) a significantly longer plasma half-life in mice (5 h [22]) than those of pefloxacin (2 h [15]) and ofloxacin (1 h [12]), which also obtains in humans (2, 20, 39).

Similarly, among the fluoroquinolones, temafloxacin has previously been found to be especially effective for certain mycobacteria. (i) In one study (11), the MIC of temafloxacin in agar against 30 strains of *M. tuberculosis* was found to be the lowest among the six quinolones tested, including pefloxacin and ofloxacin, but not sparfloxacin. (ii) Furthermore, it was also found previously that the MIC of temafloxacin for 90% of 22 strains of the *M. avium* complex was

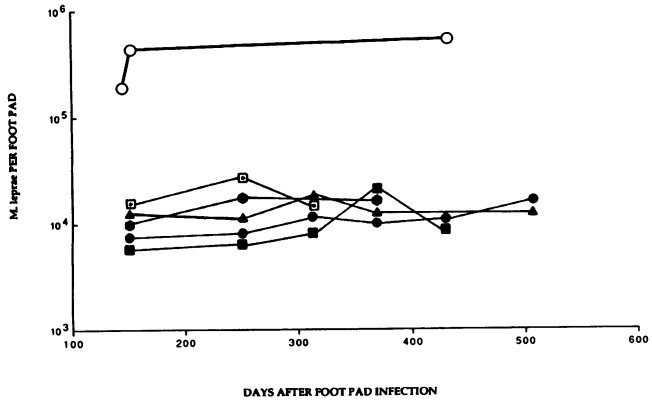

FIG. 7. Activity of sparfloxacin for *M. leprae* in mice. Numbers of *M. leprae* organisms in pools of hind feet from two mice (four feet) are shown. Mice were treated five times weekly from days 60 to 154 after *M. leprae* infection. Symbols: ○, control; ⊡, 15 mg/kg; ⬢, 30 mg/kg; ■, 50 mg/kg; ●, 150 mg/kg; ▲, 300 mg/kg.

TABLE 1. Summary of the activities of fluoroquinolones against *M. leprae* in infected mice

Drug	Activity[a] at a dose[b] (mg/kg) of:				
	15	30	50	150	300
Pefloxacin	—	—	IA	BS	PBC
Lomefloxacin	—	—	BS	BS	PBC
PD 124816	—	—	BS	BS	FBC
Ofloxacin	—	—	BS	FBC	FBC
WIN 57273	—	—	PBC	FBC	FBC
Temafloxacin	—	—	FBC	FBC	FBC
Sparfloxacin	FBC	FBC	FBC	FBC	FBC

[a] IA, inactive; BS, bacteriostatic; PBC, partially bactericidal; FBC, fully bactericidal; —, not done.
[b] Given five times weekly.

less than the peak level in serum attained in humans after a standard oral dose (21) and that temafloxacin inhibited the growth of a susceptible *M. avium* strain intracellularly in an in vitro macrophage culture system (28).

It is further noteworthy that the three fluoroquinolones found to be most active in the present study were all 4-quinolones (sparfloxacin, temafloxacin, and WIN 57273) and were the three of a series of six 4-quinolones previously found to be most active against *M. avium* (16).

It has been found in the past few years that, as well as fluoroquinolones, antibiotics of two other classes, minocycline (6, 8, 17, 19) and clarithromycin (4, 10, 17, 19), have both been discovered to have bactericidal activity for *M. leprae* in experimental animals and in clinical trials. These developments have afforded the addition of three more potent classes of antimicrobial agents for use in combination with rifampin than had been available heretofore. Such combinations present the hope that the prolonged periods (7, 38) still required to treat leprosy patients can be meaningfully shortened. The present studies demonstrate once again that the fluoroquinolones pefloxacin and ofloxacin are bactericidal for *M. leprae*. However, other fluoroquinolones, particularly sparfloxacin and temafloxacin, in this study are even more effective against *M. leprae* in infected mice. Clinical trials now in progress with these agents will ultimately determine whether these will prove more effective in patients.

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