ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Apr. 1991, p. 760–763
0066-4804/91/040760-04$02.00/0

Vol. 35, No. 4

Activities of Various Macrolide Antibiotics against *Mycobacterium leprae* Infection in Mice

ROBERT H. GELBER,* PATRICIA SIU, MABEL TSANG, AND LYDIA P. MURRAY

Kuzell Institute for Arthritis and Infectious Diseases, Medical Research Institute of San Francisco, 2200 Webster Street, San Francisco, California 94115-1896

Received 1 October 1990/Accepted 18 January 1991

We evaluated the activities of several macrolide antibiotics against *M. leprae* infections in mouse footpads. Erythromycin and azithromycin were inactive, while both roxithromycin and clarithromycin were found to be consistently active and, in fact, bactericidal. By both methods, clarithromycin was found to be superior to roxithromycin, a finding which, at least in part, may be a consequence of the higher levels of clarithromycin at the site of infection.

The World Health Organization (23) estimates that there are 10 million patients with leprosy, and yet there remains a compelling need for new antimicrobial agents to treat leprosy (2, 14). At present, only dapsone, rifampin, and clofazimine are available to treat patients with leprosy. Because (i) *Mycobacterium leprae* resistance to dapsone and rifampin is encountered and is of considerable concern (18), (ii) toxicities and unacceptable side effects from each of these agents are not uncommon, and (iii) there is general consensus that lepromatous patients require multidrug therapy (25), other antimicrobial agents are certainly required to treat leprosy (2, 14). A number of studies (9–11, 17, 19) performed with cell-free and macrophage in vitro culture systems demonstrated that many macrolide antibiotics inhibit the metabolic activity of *M. leprae*. However, to date there has been only one study that has confirmed that any macrolide has activity against the actual growth of *M. leprae* in vivo (11). In that study, clarithromycin, but not erythromycin or roxithromycin, was found to be effective against *M. leprae* in infected mice. Here we report results of studies on the activities of several macrolides against *M. leprae*-infected mice and findings that, while erythromycin and azithromycin were inactive, roxithromycin and clarithromycin were consistently active and, indeed, bactericidal.

Unfortunately, *M. leprae* cannot be grown on artificial medium. In 1960, Shepard (20) demonstrated that *M. leprae* multiplies locally when it is injected into the footpads of mice: an inoculum of 5×10^3 *M. leprae* per footpad generally results in a plateau of 1×10^6 *M. leprae* per footpad in 6 months. For the past three decades, this animal model has proved extremely useful for evaluating the efficacy of antimicrobial agents against *M. leprae*; and in general, antimicrobial agents active in the mouse footpad model at clinically achievable levels, including dapsone, rifampin, clofazimine, ethionamide, and streptomycin, have proved to be effective clinically (2, 14).

For the initial studies of antimicrobial agents in this model, we used continuous therapy from the time of infection and did not distinguish between bacteriostatic and bactericidal activity. To avoid this problem in these studies, we used the kinetic method of Shepard et al. (22) and, later, the proportional bactericidal test of Colston et al. (4). By the kinetic method, mice were treated from days 60 to 150 after the footpads were infected with 5,000 *M. leprae*. At day 150 and intervals thereafter, the number of *M. leprae* in four feet (two mice) was assessed microscopically. The activity of a drug was determined by measuring the delay in the appearance of the bacterial plateau in treated mice compared with that in control mice. A delay no longer than the period during which the drug was administered represented bacteriostasis, whereas a longer delay that could not be explained by drug accumulation represented bactericidal activity. In the proportional bactericidal test, hind footpads of mice were inoculated with 10^1, 10^2, and 10^3 *M. leprae*; the mice were treated for the initial 60 days; the footpads were harvested and counted a year later (a time sufficient to detect multiplication of *M. leprae* from any bacilli surviving therapy; the number of *M. leprae* per footpad, $\geq 10^5$); and the percentage of bacteria killed was quantitated by the Spearman and Karber method described by Shepard (21). For these studies, the drugs were initially dissolved in 95% ethanol and evenly distributed in chow by using a Patterson-Kelly twin-drum liquid-solid blender (Patterson-Kelly, East Stroudsburg, Pa.). Diets were made fresh every 2 weeks and stored in a refrigerator.

In our first study by the kinetic technique, there were two groups of BALB/c mice (Jackson Laboratories, Bar Harbor, Maine): those administered erythromycin (0.06%) in the diet and untreated controls. We concluded that in this study, erythromycin was inactive; at 5 months after footpad infection, the number of *M. leprae* in untreated control footpads was 1.3×10^6, and in those mice treated with erythromycin, there were 9.5×10^5 *M. leprae* per footpad.

In our second study using the kinetic technique, other BALB/c mice were treated as controls or received 0.1% dietary azithromycin, roxithromycin, or clarithromycin. The results are presented in Fig. 1. While azithromycin was inactive, roxithromycin delayed *M. leprae* multiplication for 5 months after therapy was discontinued. Clarithromycin appeared to be even more active, entirely preventing multiplication until all the mice had died 11 months after therapy ended.

In the study by the proportional bactericidal technique, the killing of *M. leprae* by a dietary concentration of 0.1% by these three macrolides (azithromycin, roxithromycin, clarithromycin) was determined (Table 1). Azithromycin resulted in no significant killing of *M. leprae* (11% ± 74% bactericidal). On the other hand, roxithromycin and espe-

* Corresponding author.

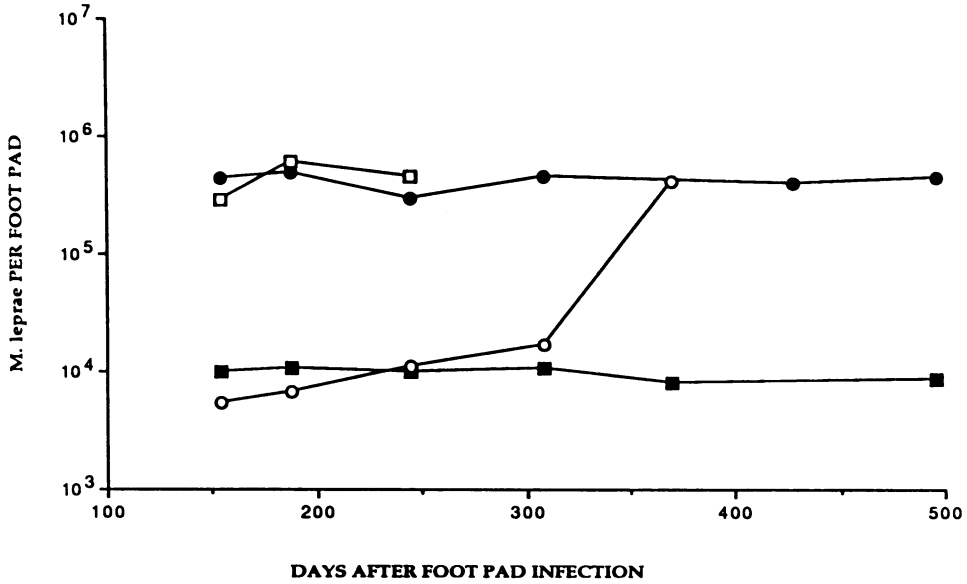

FIG. 1. Effect of 0.1% dietary macrolides on growth of *M. leprae* in the mouse footpad by the kinetic technique. ●, Control; □, azithromycin; ○, roxithromycin; ■, clarithromycin.

cially clarithromycin resulted in significant bacillary killing (82% ± 13% and 96% ± 2% bactericidal, respectively). It is noteworthy that for this same *M. leprae* isolate we found previously (12) that the two antimicrobial agents most commonly used to treat leprosy, dapsone and rifampin, were 87 and 99.9% bactericidal, respectively.

In these studies, the stabilities of macrolides in the diet and drug concentrations in mouse serum and footpads were analyzed just after preparation and at 3, 7, and 14 days subsequently. Azithromycin (Pfizer) (15), roxithromycin (The Clinical Microbiology Institute) (3), and clarithromycin (Abbott) (7) levels were determined by previously published microbiological methods. The levels in serum and footpads were determined on pools obtained from two mice (four hind feet) in the early morning. The concentrations of these macrolides in the diet, sera, and footpads are presented in Table 2. It is noteworthy that all three macrolides appeared to be stable in the diet. Levels in serum were found to be rather similar for the three macrolides, with azithromycin resulting in the lowest levels (0.18 to 0.49 μg/ml), roxithromycin resulting in intermediate levels (0.23 to 0.8 μg/ml), and clarithromycin resulting in the highest levels 0.45 to 1.46 μg/ml. At the site of infection, relative macrolide concentrations followed the same order. It is noteworthy that the footpad concentration of clarithromycin was not only consistently highest but continued to increase significantly over the period of observation.

In these studies, we found that erythromycin and azithromycin are inactive against *M. leprae*, while both roxithromycin and clarithromycin are consistently bactericidal. Because macrolides concentrate intracellularly, they offer a particular advantage for the treatment of infections with *M. leprae*, an obligate intracellular parasite. The newer macrolides appear to have the advantage over erythromycin by virtue of superior pharmacokinetic features (5): (i) acid stability resulting in better absorption and higher levels in serum, (ii) longer serum half-lives, and (iii) superior tissue penetration.

TABLE 1. Bactericidal activities of macrolide antibiotics by the proportional bactericidal test

Therapy	No. of footpads with viable *M. leprae*/total no. of footpads inoculated for the following no. of *M. leprae* inoculated:			% Killed[a]
	10^1	10^2	10^3	
Control	7/14	7/18	9/16	
Azithromycin (0.1%)	5/10	4/10	5/10	11 ± 74
Roxrithromycin (0.1%)	1/10	2/10	4/10	82 ± 13
Clarithromycin (0.1%)	0/10	0/10	0/10	96 ± 2

[a] Values are means ± standard deviations.

TABLE 2. Macrolide levels

Therapy and day postinfection	Macrolide level in:		
	Feed (μg/g)	Serum (μg/ml)	Footpad harvest (μg/g)
Azithromycin			
0	927	0	0
3	846	0.490	1.78
7	894	0.254	0.49
14	874	0.175	0.57
Roxithromycin			
0	1,000	<0.25	<0.4
3	1,300	0.8	5.0
7	1,500	0.23	5.4
14	1,000	0.5	4.6
Clarithromycin			
0	583	<0.5	<0.050
3	535	0.45	3.75
7	555	1.46	7.97
14	563	1.23	9.96

Although, as described in previously published reports (8, 15), azithromycin given to humans results in levels in the kidney, spleen, liver, and lung that are between 8- and 35-fold greater than those in serum, levels in muscle and fat are only 4-fold greater than those in serum. Since footpad tissue is mostly skin (predominantly dermal fat) and muscle, the finding that the footpad levels of azithromycin found in this study were only two to three times those in serum is not surprising. These low footpad levels for azithromycin (0.57 to 1.78 μg/g) may account for its inactivity found in this study, in contrast to the activities of roxithromycin and clarithromycin, which achieved considerably higher levels at the site of infection, 4.6 to 5.4 and 3.75 to 9.96 μg/g, respectively).

This study and those of others (11, 19) have demonstrated that clarithromycin has superior activity against *M. leprae* compared with erythromycin; these activities were also found against *M. avium* (6). Clarithromycin is more lipophilic than erythromycin (6), and perhaps its consistently superior activity against mycobacteria in vitro and in vivo may also be a result of its ability to penetrate the outer mycobacterial lipid-rich cell wall, a situation we found previously (13) to be the likely cause of the unique activity of minocycline among the tetracyclines against *M. leprae*.

In our two studies, clarithromycin appeared to be more active than roxithromycin. These results largely confirm the findings of Franzblau and Hastings (11). They found that all four macrolides, especially roxithromycin and even more profoundly clarithromycin, reduced *M. leprae* oxidation of [1-^{14}C]palmitic acid and phenolic glycolipid synthesis in vitro. They also found that the relative activity in vitro, as determined by reduction of *M. leprae* ATP pools of erythromycin, roxithromycin, and clarithromycin, follows that same order. In their mouse studies, 0.01% dietary erythromycin and roxithromycin were inactive against *M. leprae*, while clarithromycin was found to be active. The fact that we studied roxithromycin at a 10-fold higher dietary concentration almost certainly explains the differences in results between this study and those of Franzblau and Hastings (11). The higher levels in serum found in the previous study (11) and this one and the higher levels of clarithromycin found in footpad tissues in this study compared with those of roxithromycin may, at least partially, account for the superiority in vivo of clarithromycin vis-à-vis roxithromycin. Indeed, Ramasesh et al. (19), using an in vitro assay quantitating the metabolic activity of *M. leprae* within mouse peritoneal macrophages based on the incorporation of radiolabeled palmitic acid into its unique phenolic glycolipid I, found clarithromycin and roxithromycin to be equally active.

It is noteworthy that, in humans, clarithromycin (1) is less protein-bound than roxithromycin is (24) (67 versus 95%, respectively). This advantage of bioavailability in humans for clarithromycin over roxithromycin is negated by the fact that levels of roxithromycin in serum after administration of equivalent oral doses are considerably higher than those of clarithromycin; while 400 mg of clarithromycin results in a peak level in serum of only 1.1 μg/ml and a shorter half-life (3.6 h) (1), 300 mg of roxithromycin yields peak levels in serum of 9 μg/ml and a serum half-life of 7.9 (16). Thus, clarithromycin's superior pharmacokinetics in mice which may contribute to its increased activity against *M. leprae* in that species are not found in humans.

In summary, we found that two new macrolides, clarithromycin and roxithromycin, having pharmacokinetics superior to those of erythromycin, are consistently bactericidal for *M. leprae* in mice and are bactericidal at levels that can be easily obtained in humans. The clinical efficacies of newer macrolides in patients with leprosy are awaited with anticipation.

This work was supported by a contract with the Gillis W. Long Hansen's Disease Center, Carville, La.

REFERENCES

1. **Abbott Laboratories.** Unpublished data.
2. **Baker, R. J.** 1990. The need for new drugs in the treatment and control of leprosy. Int. J. Lepr. **58**:78–97.
3. **Barry, A. L., and R. R. Packer.** 1986. Roxithromycin bioassay procedures for human plasma, urine and milk specimens. Eur. J. Clin. Microbiol. **5**:536–539.
4. **Colston, M. J., G. R. Hilson, and D. K. Banerjee.** 1978. The "proportional bactericidal test": a method for assessing bactericidal activity in drugs against *Mycobacterium leprae* in mice. Lepr. Rev. **49**:7–15.
5. **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.
6. **Fernandes, P. B., D. J. Hardy, D. McDaniel, C. W. Hanson, and R. N. Swanson.** In vitro and in vivo activities of clarithromycin against *Mycobacterium avium*. Antimicrob. Agents Chemother. **33**:1531–1534.
7. **Fernandes, P. B., N. Ramer, R. A. Rode, and L. Freiberg.** 1988. Bioassay for A-56268 (TE-031) and identification of its major metabolite, 14-hydroxy-6-*O*-methyl erythromycin. Eur. J. Clin. Microbiol. Infect. Dis. **7**:73–76.
8. **Foulds, G., R. M. Shepard, and R. B. Johnson.** 1990. The pharmacokinetics of azithromycin in human serum and tissues. J. Antimicrob. Chemother. **25**(Suppl. A):73–82.
9. **Franzblau, S. G.** 1988. Oxidation of palmitic acid by *Mycobacterium leprae* in an axenic medium. J. Clin. Microbiol. **26**:18–21.
10. **Franzblau, S. G., and R. C. Hastings.** 1987. Rapid in vitro metabolic screen for antileprosy compounds. Antimicrob. Agents Chemother. **31**:780–783.
11. **Franzblau, S. G., and R. C. Hastings.** 1988. In vitro and in vivo activities of macrolides against *Mycobacterium leprae*. Antimicrob. Agents Chemother. **32**:1758–1762.
12. **Gelber, R. H.** 1986. The killing of *Mycobacterium leprae* in mice by various dietary concentrations of dapsone and rifampicin. Lepr. Rev. **57**:347–464.
13. **Gelber, R. H.** 1987. Activity of minocycline in *Mycobacterium leprae*-infected mice. J. Infect. Dis. **156**:236–239.
14. **Gelber, R. H.** 1990. Progress in the chemotherapy of leprosy: status, issues and prospects. Prog. Drug Res. (Basel) **34**:421–445.
15. **Girard, A. E., D. Girard, A. R. English, T. D. Gootz, C. R. Cimochowski, J. A. Faiella, S. L. Haskell, and J. A. Retsema.** 1987. Pharmacokinetic and in vivo studies with azithromycin (CP-62,993), a new macrolide with an extended half-life and excellent tissue distribution. Antimicrob. Agents Chemother. **31**:1948–1954.
16. **Halstenson, C. E., J. A. Opsahl, M. H. Shwenk, J. M. Kovarik, S. K. Puri, I. Ho, and G. R. Matzke.** 1990. Disposition of roxithromycin in patients with normal and severely impaired renal function. Antimicrob. Agents Chemother. **34**:385–389.
17. **Harris, E. B., S. G. Franzblau, and R. C. Hastings.** 1988. Inhibition of phenolic glycolipid-I synthesis in extracellular *Mycobacterium leprae* as an indicator of antimicrobial activity. Int. J. Lepr. **56**:588–591.
18. **Ji, B.** 1985. Drug resistance in leprosy—a review. Lepr. Rev. **56**:265–278.
19. **Ramasesh, N., J. L. Krahenbuhl, and R. C. Hastings.** 1989. In vitro effects of antimicrobial agents on *Mycobacterium leprae* in mouse peritoneal macrophages. Antimicrob. Agents Chemother. **33**:657–662.
20. **Shepard, C. C.** 1960. The experimental disease that follows the injection of human leprosy bacilli into foot pads of mice. J. Exp.

Med. **112**:445.
21. **Shepard, C. C.** 1982. Statistical analysis of results obtained by two methods for testing drug activity against *Mycobacterium leprae*. Int. J. Lepr. **50**:96–101.
22. **Shepard, C. C., L. L. Walker, R. M. Van Landingham, and M. A. Redus.** 1971. Kinetic testing of drugs against *Mycobacterium leprae* in mice. Activity of cephaloridine, rifampin, streptovaricin, vadrine, and viomycin. Am. J. Trop. Med. Hyg. **20**:616–620.
23. **United Nations Development Programme/World Bank/World Health Organization Special Programme for Research and Training in Tropical Diseases.** 1985. Seventh programme report. World Health Organization, Geneva.
24. **Wise, R., B. Kirkpatrick, J. Ashby, and J. M. Andrews.** 1987. Pharmacokinetics and tissue penetration of roxithromycin after multiple dosing. Antimicrob. Agents Chemother. **31**:1051–1053.
25. **World Health Organization Study Group.** 1982. Chemotherapy of leprosy for control programmes. WHO Tech. Rep. Ser. 675. World Health Organization, Geneva.