Clarithromycin at Very Low Levels and on Intermittent Administration Inhibits the Growth of *M. leprae* in Mice

TO THE EDITOR:

Clarithromycin was found previously to inhibit the metabolic activity of *Mycobacterium leprae* in cell-free and tissue culture systems ([2]). Furthermore, it had been determined that when *M. leprae*-infected mice were fed a diet containing clarithromycin [0.01% ([4]) and 0.1% ([5]) and 25 mg/kg by gavage five times weekly ([7])], bactericidal activity for *M. leprae* was consistently observed. In none of these studies was clarithromycin administered at a dietary concentration which was found ineffective and, therefore, we initiated these current studies to define clarithromycin's minimal inhibitory dietary, serum, and tissue concentrations for *M. leprae* in infected mice. The World Health Organization ([14]) has for the past decade advocated monthly rifampin in the treatment of leprosy. Since a companion antimicrobial agent which has proven activity on intermittent administration might offer particular utility in the practical delivery of effective therapy to leprosy patients in endemic countries, in these studies we also determined the efficacy of intermittent clarithromycin administration in this mouse model system.

Eight-week-old, female, BALB/c weanling mice (Jackson Laboratories, Bar Harbor, Maine, U.S.A.) were infected in both hind foot pads with 5000 *M. leprae*. The *M. leprae* isolate utilized in this study originally had been obtained from a lepromatous leprosy patient, maintained in mouse passage in our laboratory for the previous 10 years, and was harvested from the foot pads of mice near the time of its peak multiplication. From the time of infection, groups of mice were continuously fed diet containing various concentrations of clarithromycin [0% (control), 0.0001%, 0.001%, 0.005%, 0.01%, 0.05%, 0.1%]. Also, groups of mice were treated with 0.1% in diet 3 days weekly (M, W, F), 1 day weekly, and 1 day monthly. At 7, 9, and 11 months after infection, the number of *M. leprae* from the foot pad pools of two mice (four feet) from each group were determined by standard microscopic procedures ([12]). When the number of *M. leprae* per foot pad was found to be $\geq 10^5$, bacillary growth was considered to have occurred ([13]).

At 7 months, *M. leprae* grew to 2×10^5/foot pad in the untreated control mice, and remained at approximately that level at the 9- and 11-month harvest intervals (The Table). Clarithromycin 0.0001% in the diet did not inhibit *M. leprae* multiplication (The Table). However, clarithromycin 0.001%, 0.005%, 0.01%, 0.05%, and 0.1% each prevented *M. leprae* multiplication at all three harvest intervals (The Table). Clarithromycin 0.1% in the diet 3 days weekly and even 1 day weekly, also, consistently inhibited the growth of *M. leprae,* while multiplication of *M. leprae* in mice treated only once monthly was inhibited both at 7 and 9 months, but not at the 11-month interval (The Table).

Also, in these studies, mice were fed 2 weeks of each of the test diets, and clarithromycin concentrations in the mouse serum (five mice) and foot pads (one mouse) were determined by an agar disk diffusion method employing *Micrococcus luteus* ATCC 93418 ([3]) (Abbott Laboratories, North Chicago, Illinois, U.S.A.). In the untreated con-

THE TABLE. *Activity of clarithromycin against* M. leprae-*infected mice.*

Clarithromycin concentration in diet	Months after foot pad infection		
	No. *M. leprae* per foot pad		
	7	9	11
Control	2.42×10^5	1.13×10^5	1.01×10^5
0.0001%[a]	6.59×10^5	8.36×10^5	1.87×10^5
0.001%[a]	2.63×10^4	$< 8.24 \times 10^3$	7.02×10^3
0.005%[a]	$< 6.6 \times 10^3$	$< 9.79 \times 10^3$	$< 6.69 \times 10^3$
0.01%[a]	$< 9.86 \times 10^3$	$< 6.02 \times 10^3$	$< 6.33 \times 10^3$
0.05%[a]	$< 8.76 \times 10^3$	$< 8.22 \times 10^3$	$< 6\ 69 \times 10^3$
0.1%[a]	$< 6.69 \times 10^3$	$< 8.88 \times 10^3$	$< 6.69 \times 10^3$
Three days per week 0.1%	2.66×10^4	$< 1.15 \times 10^4$	6.33×10^3
One day per week 0.1%	9.34×10^3	$< 2.4 \times 10^4$	1.07×10^4
One day per month 0.1%	6.13×10^4	1.66×10^4	1.07×10^5

[a] Mice fed continuously; numbers of *M. leprae* per foot pad were obtained from a pool of four feet.

trol mice and those treated with 0.0001%, 0.001%, 0.005%, and 0.01%, clarithromycin serum levels were consistently < 0.025 μg per ml and foot pad concentrations < 0.7 μg/g. Clarithromycin 0.05% in the diet resulted in serum levels of 0.16 ± 0.11 μg/ml and a foot pad level of 1.7 μg/g, while clarithromycin 0.1% in diet resulted in serum levels of 0.36 ± 0.23 μg/ml and foot pad level of 3.3 μg/g.

These studies have demonstrated that low dietary, serum, and tissue levels of clarithromycin inhibit the growth of *M. leprae* in mice. Previously, it was found that 0.01% and 0.1% dietary clarithromycin prevented the multiplication of *M. leprae* in mouse foot pads. In this study, 0.001% dietary clarithromycin and all higher tested concentrations (0.005%, 0.01%, 0.05% and 0.1%) consistently prevented the growth of *M. leprae* in infected mice. Furthermore, clarithromycin was found effective on intermittent administration, consistently when administered in the diet 1 day and 3 days weekly and even partially active when administered 1 day monthly. Of the three new candidate antimicrobials that appear promising for the therapy of leprosy, minocycline ([6]) and now clarithromycin, but not fluoroquinolones ([10]), are active against *M. leprae*-infected mice when administered less frequently than three times per week.

Previously we found that against *M. leprae*-infected mice, dietary erythromycin (0.06%) and azithromycin (0.1%) were consistently inactive. On the other hand, clarithromycin (0.1%) and roxithromycin (0.1%) were consistently active and, in fact, bactericidal, clarithromycin being found more active than roxithromycin. Franzblau and Hastings ([4]) found that while clarithromycin (0.01%) was bactericidal for *M. leprae,* erythromycin (0.01%) and roxithromycin (0.01%) were inactive. These current studies demonstrate that a tenfold lower concentration of clarithromycin than had been studied previously, 0.001% clarithromycin, has activity for *M. leprae.* Thus, it would appear that, as had been found previously for both *M. avium* ([2, 9]) and *M. chelonae* ([1]), clarithromycin is superior to other macrolides in its activity for *M. leprae.*

The levels of clarithromycin found in the mouse serum and foot pad with 0.1% administered in the diet were of the same order of magnitude ([4, 5]), albeit somewhat lower than the levels generated by that same diet in previous studies. Because of the lack of sufficient sensitivity of the bioassay, the minimal inhibitory serum concentration against *M. leprae* could not be determined. However, it was certainly remarkably low, < 25 ng/ml. Assuming the same linear relationship between dietary concentration and resultant serum levels obtained between dietary concentrations of 0.01% and 0.001% as was found at 0.1% and 0.05%, the minimal inhibitory serum concentration for clarithromycin against *M. leprae* would be < 2.5 ng/ml, approximately that found previously only for dapsone against *M. leprae,* 3 ng/ml ([8]). Because a small 400-mg human dose of clarithromycin results in a peak serum level of 1.1 μg per ml and in mice the highest dietary concentration tested, 0.1% dietary clarithromycin (100-fold

greater than minimal effective dietary concentration), results in serum levels of approximately threefold less, there is every reason to believe that the pharmacokinetics in man would result in clarithromycin being equally effective in the treatment of human disease.

In conclusion, the very low dietary, serum, and tissue concentrations required to inhibit *M. leprae* found in these studies, and the fact that clarithromycin has been found consistently bactericidal against *M. leprae* in mice, lend optimism to the application of clarithromycin in the treatment of leprosy. Because clarithromycin is active on intermittent administration in mice and also at levels easily obtained in man, there are prospects that clarithromycin might, as with rifampin, be applied in some intermittent schedule for the treatment of lepromatous leprosy patients.

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