Minimal Bactericidal Concentration of Clarithromycin Against *M. leprae*

TO THE EDITOR:

The new macrolide antibiotic, clarithromycin, was discovered by Franzblau and Hastings ([3]) to inhibit the metabolism of *Mycobacterium leprae in vitro* and in *M. leprae*-infected mice to demonstrate bactericidal-type activity when administered at 0.01% in the diet. The bactericidal activity (96%) of dietary 0.1% clarithromycin for *M. leprae* was quantitated by the authors ([8]), as

THE TABLE. *Killing of* M. leprae *by various dietary concentrations of clarithromycin.*

Clarithromycin dietary concentration	No. *M. leprae* inoculated			% Killed ± S.E.	Probability that killing occurred
	5×10^1	5×10^2	5×10^3		
	No. positive foot pads/no. foot pads counted				
0.0% (control)	1/6	2/8	4/8		
0.01%	0/10	2/10	5/10	52 ± 39	0.56
0.05%	1/13	0/10	0/10	88 ± 8	0.002
0.1%	0/10	0/10	0/10	88 ± 8	0.002

well as by Ji, *et al.* ([10]), (87%–95%), wherein mice were administered 12.5–50 mg/kg clarithromycin by gavage. Because levels *in vivo* of clarithromycin that were inactive against *M. leprae* had not been studied to date, we ([6]) established for a single *M. leprae* isolate that clarithromycin fed continuously in dietary concentrations of 0.001%, 0.005%, 0.01%, 0.05%, and 0.1% consistently inhibited the growth of *M. leprae,* while 0.0001% did not, establishing a minimal inhibitory mouse serum concentration of no more than 25 ng/ml and likely as little as 2.5 ng/ml.

Because actual bactericidal activity against *M. leprae* is critical to its use in patients, this current study was designed to establish clarithromycin's minimal bactericidal dietary concentration against the same *M. leprae* isolate used by us previously to establish its minimal inhibitory concentration. For these purposes, groups of generally 10 BALB/c female mice were infected in both hindfoot pads with 50, 500, and 5000 *M. leprae* and treated for the first 2 months ([1]) with various dietary concentrations of clarithromycin [0% (control), 0.01%, 0.05%, and 0.1%]. For these studies, clarithromycin was initially dissolved in 95% ethanol and evenly distributed in mouse chow by using a Patterson-Kelly twin-drum liquid-solid blender (Patterson-Kelly, East Stroudsburg, Pennsylvania, U.S.A.). Diets were made fresh every 2 weeks and stored in a refrigerator. One year after therapy had been discontinued, a time sufficient to detect multiplication of *M. leprae* from any bacilli surviving therapy, the number of foot pads wherein *M. leprae* survived (number of *M. leprae*/foot pad $\geq 10^5$) was assessed. ([1]) From these results the percentage of bacteria killed was quantitated by the method of Spearman and Kärber, described previously by Shepard ([12]), and the probability that actual killing occurred determined.

Both 0.05% and 0.1% clarithromycin were found to be bactericidal (88% ± 8%) for *M. leprae* (The Table). Furthermore, we observed that the probability that killing occurred at these two dietary concentrations was highly significant ($p \leq 0.002$). Unfortunately, in this study the control inoculum itself had low viability, perhaps accounting for why these high dietary concentrations were not found to be as bactericidal as previously demonstrated for other isolates. In these studies 0.01% clarithromycin resulted in no significant killing of *M. leprae.*

It is noteworthy in this study that the growth of the *M. leprae* isolate used herein had been found to be consistently inhibited by the continuous dietary administration of clarithromycin of 0.001% and higher ([6]). Thus in this study we found a 50-fold greater concentration of clarithromycin was required to kill *M. leprae* than had been found previously to inhibit the same isolate's growth. It is further noteworthy that Walker and Shinnick ([14]) also found that for several *M. leprae* isolates, including "pan-susceptible," dapsone-resistant, and rifampin-resistant, a discrepancy between clarithromycin's minimal inhibitory dietary concentration and minimal bactericidal dietary concentration was generally demonstrable. Such discrepancies between the amount of drug necessary to inhibit and kill *M. leprae* have been demonstrated previously for active antimicrobials, dapsone ([4]), clofazimine ([5]), and ethionamide ([5]). On the other hand, the amount of minocycline required to inhibit and kill *M. leprae* was found previously to be remarkably similar ([9]). The relative activity of antimicrobials against *M. leprae* after standard doses ([2]) frequently has been compared with regard to the time in patients that inhibitory levels of the drug are maintained. Because of our general recognition now that bactericidal activity is

critical to efficacious treatment of lepromatous patients and inhibitory bactericidal levels for several antimicrobials are discordant, we believe that the actual period of time that levels above those required for bactericide would be best substituted to such considerations.

It has been found previously for dapsone ([13]) and minocycline ([7]) that sensitive *M. leprae* isolates are inhibited consistently by a very narrow range of antimicrobial concentrations. On the other hand, we previously reviewed for rifampin that there is a broad range of published results of both its minimal inhibitory concentration (MIC) and its minimal bactericidal concentration (MBC), suggesting a broader range of susceptibility among isolates ([4]). Franzblau and Hastings ([3]) found for an *M. leprae* isolate that 0.01% dietary clarithromycin inhibited its growth and was fully bactericidal. While we ([6]) found that 0.001% inhibited an isolate, it did not result in significant killing of that same isolate herein, this requiring 0.05% or greater. On the other hand, for another isolate Walker and Shinnick ([14]) found that 0.01% clarithromycin neither inhibited nor killed, killing itself for several *M. leprae* isolates being accomplished consistently only by increasing the dietary concentration to 0.1%. Thus, there appears a significant range of susceptibility among *M. leprae* isolates to the activity of clarithromycin. We ([8]) found previously that 0.1% clarithromycin was bactericidal and that this level of dietary clarithromycin results in mouse serum levels one third of that of the peak obtained in man by a very small (400 mg) dose. It is encouraging that the found range of susceptibility of several *M. leprae* isolates does not limit clarithromycin's general applicability to the treatment of leprosy patients, although the exact time/concentration level for optimal clarithromycin therapy remains to be determined. Such considerations may be especially critical to the application of clarithromycin to certain intermittent schedules, particularly once monthly ([11]), that are now being seriously entertained.

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