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Minimal Bactericidal Dietary Concentration of Minocycline for *Mycobacterium leprae*-infected Mice is Very Low and Similar to its Minimal Inhibitory Dietary Concentration

TO THE EDITOR:

Previously it was found, by both the kinetic and proportional bactericidal method, that minocycline was consistently bactericidal for *Mycobacterium leprae* in infected mice ([2, 4]). This activity was obtained at levels which were exceeded several fold by standard doses in man ([2]). Furthermore, we found that for seven different *M. leprae* isolates continuous administration of 0.01% and 0.04% w/w minocycline in the mouse diet consistently inhibited the growth of *M. leprae* (serum levels 0.11 μg/ml and 0.31 μg/ml, respectively), while 0.004% inhibited 5 of 7 strains and 0.001% was consistently inactive (serum levels ≤ 0.08 μm/ml) ([3]). These present studies were initiated to determine by the proportional bactericidal method ([1]) minocycline's minimal bactericidal dietary concentration for a single strain of *M. leprae.*

In this study, groups of BALB/c female mice (Jackson Laboratories, Bar Harbor, Maine, U.S.A.) were infected in both hind foot pads with 10^1, 10^2, or 10^3 *M. leprae* and fed diets for 60 days thereafter containing various concentrations of minocycline (0.004%, 0.01%, 0.04%, 0.06%, 0.1%); controls = 0%. One year after the discontinuation of therapy, foot pads of 8 or more mice (generally 10) from each group of mice were harvested, and the number of *M. leprae* counted microscopically ([6]). If ≥ 10^5 *M. leprae* were found, multiplication was considered to have occurred ([6]). From these results the percentage of bacteria killed was quantitated by the method of Spearman and Kärber ([5]).

The results of these studies are summarized in The Table. Minocycline 0.004% was found to have had no significant bactericidal activity for *M. leprae* (p = 0.14). On the

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

Minocycline dietary concentration	No. *M. leprae* inoculated			% Killed ± S.E.	Probability that killing occurred
	10^1	10^2	10^3		
	No. foot pads in which *M. leprae* grew/No. foot pads inoculated				
0.0% (Control)	3/10	3/10	8/10		
0.004%	2/10	6/14	5/18	68 ± 25	0.14
0.01%	0/14	4/14	3/10	85 ± 11	0.01
0.04%	0/10	0/10	12/18	82 ± 12	0.01
0.06%	1/10	1/10	3/8	85 ± 12	0.02
0.1%	0/10	1/10	3/10	90 ± 7	0.001

other hand, all other dietary levels of minocycline tested (0.01%, 0.04%, 0.06%, and 0.1%) were bactericidal for *M. leprae* ($p \leq 0.02$).

Unfortunately, in this study the control inoculum itself had low viability. This may account for why the percentage of *M. leprae* killed was less than had been found in previous studies ([2, 4]). Nonetheless, it would appear that concentrations of minocycline required to inhibit and kill *M. leprae* are similar ([2, 3]) and easily attainable in man.

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Improved Method for Purification of *Mycobacterium leprae* from Armadillo Tissues

To the Editor:

An efficient, rapid, and cheap method for the preparation of pure *Mycobacterium leprae* from armadillo tissues is described. It is especially recommended for application in immunology and molecular genetics.

Because the cultivation of *M. leprae* has not yet been possible, the only source for specified living *M. leprae* are animal models, especially the nine-banded armadillo (*Dasypus novemcintus*). Difficulty in the separation and purification of the organism from armadillo tissues is one of the main problems workers face in this field, and it limits the effective use of *M. leprae* in immunology, vaccine preparation, biochemical studies, etc.

We have developed a rapid, efficient, cheap, and easy method for the preparation of *M. leprae* from armadillo tissues which provides bacteria of high quantity and high quality. The method depends on low speed/high speed centrifugation with the use of Percoll as a gradient separation medium.

Armadillo liver tissue (6 grams) from an armadillo inoculated with *M. leprae* 24 months earlier was homogenized with ultraturax at high speed in 10 ml phosphate buffered saline (PBS), pH 7.0, in a Beckman screw-cap centrifuge tube (Cat. no. 355670), and the homogenate was then centrifuged at 55 × *g* for 6 min at 5°C. The supernatant was carefully aliquoted to two or three 5-ml screw-cap tubes (Greiner, Cat. no. 124261)