Effectiveness of Clarithromycin and Minocycline Alone and in Combination against Experimental *Mycobacterium leprae* Infection in Mice

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As determined by the proportional bactericidal method, clarithromycin had strong bactericidal activity against *Mycobacterium leprae*. Clarithromycin was administered to mice by gavage as 20 daily doses at dosages of 12.5 to 50 mg/kg of body weight. At a dosage of 25 mg/kg, minocycline was more active than clarithromycin at a dosage of 50 mg/kg. Additive effects were displayed with the combination of clarithromycin (50 mg/kg) and minocycline (25 mg/kg), both of which were administered daily by gavage, and of clarithromycin and minocycline, both of which were administered daily by gavage at dosages of 25 mg/kg each, with rifampin at a single oral dose of 10 mg/kg.

Clarithromycin is a new macrolide antibiotic, and minocycline is a longer-acting tetracycline derivative. Recently, both drugs were reported to exhibit strong activity against *Mycobacterium leprae* both in vitro and in vivo (1–3). However, although the proportional bactericidal method (6) is more precise, the kinetic method was used to study clarithromycin in vivo (2). Moreover, in studies of both clarithromycin (2) and minocycline (3) in vivo, the drugs were incorporated into the mouse diet, a form of administration much different from that used clinically.

Because the search for additional drugs that exhibit bactericidal activity against *M. leprae* is so urgent (5), it was important to attempt to confirm the reported findings. Therefore, we studied the activities of the two drugs in *M. leprae*-infected mice by the proportional bactericidal method, administering the drugs by gavage. In addition, because both the tetracyclines and the macrolides interfere with protein synthesis of microorganisms (the tetracyclines bind to the 30S ribosome [11] and the macrolides bind to the 50S ribosome [12] of susceptible bacteria), we studied the potential effect of a combination of clarithromycin and minocycline. Finally, the combination of clarithromycin and minocycline with rifampin was studied, because leprosy is optimally treated by combinations of antimicrobial agents and rifampin, because of its great potency against *M. leprae*, is the most important component of the combined regimens used for the treatment of both paucibacillary and multibacillary leprosy (13).

An inoculum containing $5 \times 10^5$ *M. leprae* 17547 cells per 0.03 ml was prepared (8), and serial 10-fold dilutions were made, so that the final dilution contained $5 \times 10^{-1}$ organism per 0.3 ml. Each hind footpad of 90 mice was inoculated with 0.03 ml of one of the five dilutions. The 450 mice were divided into nine equal groups, with each group containing 10 mice that were inoculated with each dilution (see Table 1). Beginning from day 3 after inoculation, the various drugs and drug combinations were administered by gavage five times weekly for 30 days (a total of 20 doses). Rifampin was administered as a single dose, however, and control mice were not treated. The mice were then held for 1 year, a period of time theoretically sufficient for one surviving organism to multiply to a level of $\geq 10^{10}$ per footpad, according to the criterion of multiplication (6), after which harvests of *M. leprae* were taken individually from the inoculated footpads.

Harvests of *M. leprae* were taken from all control mice. Among the treated mice, organisms were harvested first from the animals inoculated with $5 \times 10^5$ organisms per footpad. Depending on the results of these harvests, *M. leprae* was harvested from the mice inoculated with at least two additional dilutions, in the attempt to include sets of harvests showing multiplication of *M. leprae* in all footpads harvested and other sets in which no footpad demonstrated organism multiplication. When the organisms were found to have multiplied in all of the footpads inoculated with one dilution, no harvests were taken from mice that were inoculated with lower dilutions.

The proportions of viable organisms remaining at the end of the period of treatment and the significance of differences between groups were calculated by the Spearman and Kärber method (9).

The results of harvests are presented in Table 1. All eight regimens exerted significant bactericidal effects ($P < 0.01$). Clarithromycin was more active at a dosage of 25 or 50 mg/kg of body weight than it was at a dosage of 12.5 mg/kg ($P < 0.05$). Minocycline at 25 mg/kg was more active than clarithromycin at 25 or 50 mg/kg ($P < 0.01$). The combination of clarithromycin and minocycline (each at 25 mg/kg) was no more active than minocycline alone at a dosage of 25 mg/kg ($P > 0.05$); on the other hand, the combination of clarithromycin at 50 mg/kg and minocycline at 25 mg/kg was very significantly more active than either of the components administered alone was ($P < 0.01$). Finally, the combination of three drugs—rifampin at a single dose of 10 mg/kg plus clarithromycin and minocycline, each at dosages of 25 mg/kg—was more active than rifampin administered as a single dose alone ($P < 0.01$) and the combination of clarithromycin and minocycline, with each drug administered at a dosage of 25 mg/kg ($P < 0.05$).

Results of our experiments by the proportional bactericidal method confirmed the strong bactericidal activity of clarithromycin and minocycline against *M. leprae*. Except for rifampin (10) and, possibly, pefloxacin and ofloxacin (4), no

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other compound has demonstrated bactericidal activity of the degree of rapidity and potency shown by clarithromycin and minocycline. Although minocycline was more active than clarithromycin when given at the same dose, it is uncertain that the clinically tolerated dosage of minocycline will be more active against human leprosy than that of clarithromycin. Because the latter is better tolerated, and therefore, the tolerated dosage of clarithromycin is much higher than that of minocycline. An important observation is that the additive effects were demonstrated by the combination of clarithromycin plus minocycline and the combination of rifampin plus clarithromycin and minocycline. The effects of minocycline and dapsone or rifampin have also been reported to be additive (3), suggesting that it may be advantageous to combine clarithromycin and minocycline with both dapsone and rifampin in the treatment of human leprosy.

Although the pharmacokinetics of minocycline administered to mice by gavage have not been described, information is available from the administration of minocycline in the mouse diet (3). Based on these data, the peak concentration of minocycline is serum following an oral dose of 25 mg/kg in mice can be estimated to be well within the clinically achievable level, i.e., 2 μg/ml after a single oral dose of 150 mg of minocycline (7). Because the serum half-life of minocycline is approximately 16 h in humans (7), whereas in mice it is about 4 h (3), it appears likely that a daily dose of 100 mg of minocycline to humans may produce a therapeutic effect similar to that produced by a daily dose of 25 mg of minocycline per kg to mice. Peak concentrations of clarithromycin in mouse serum measured by microbiological assay were 1.5 and 4.3 μg/ml, and areas under the concentration-time curve were 130 and 372 mg·min/liter after single doses of 25 or 50 mg/kg, respectively, given by gavage. Corresponding values following single 400- and 600-mg doses of clarithromycin to humans are 1.13 and 2.03 μg/ml and 333 and 926 mg·min/liter, respectively (Abbott Laboratories). Thus, it appears likely that the therapeutic effect of 500 mg of clarithromycin daily in humans may be similar to that of 25 to 50 mg/kg daily in mice.

To increase the effectiveness and shorten the duration of current multidrug regimens (13), new bactericidal antileprosy drugs that act by mechanisms different from those of the currently available compounds should be developed (5). Because none of the established drugs, rifampin, dapsone, and clofazimine, acts at the level of the ribosome, both clarithromycin and minocycline may be considered potential components of new multidrug regimens. Clarithromycin has been well tolerated by patients in phase II and III trials under various clinical conditions (2), and the clinical use of minocycline has already been established. Therefore, based on the results described above, clinical trials should be conducted among patients with previously untreated lepromatous leprosy, aiming to measure the therapeutic effects of clarithromycin alone at a daily dose of 500 mg, minocycline alone at a daily dose of 100 mg, and the combination of clarithromycin at 500 mg daily and minocycline at 100 mg daily.

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REFERENCES


