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The Activity of a Thiadiazole on *Mycobacterium leprae* (39475)

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A new compound, 2-amino-5-(1-methyl-5-nitro-2-imidazolyl)-1,3,4-thiadiazole (CL 64,855), was reported (1, 2) to exhibit a broad spectrum of antibacterial and antiprotozoal effects upon oral administration to chicks and rodents. The compound was not found active against *Mycobacterium tuberculosis* (H. Macdonald, personal communication). In a series of experiments, we have found that CL 64,855 is active against *M. leprae*. The drug produced either bacterial killing or prolonged bacteriostasis, an attribute of very few of the large number of compounds already studied (3).

Methods. CL 64,855 was incorporated into mouse meal (Wayne Lab Blox, Allied Mills, Inc., Chicago, Illinois) by the addition of appropriate volumes of a solution of the compound in 95% ethanol in a liquid-solid twin-shell blender (Patterson-Kelly Co., East Stroudsburg, Pennsylvania). The drug-containing diets were placed in powder feeders in the mouse cages and made available to the mice *ad libitum*.

Locally-bred male BALB/c mice were inoculated in both hind foot pads with $10^{3.7}$ *M. leprae* of the strain used for virtually all of the drug studies carried out in this laboratory. Drug-containing diets were administered for periods of approximately 90 days, beginning 60 to 75 days after inoculation, when the organisms were in logarithmic multiplication. *M. leprae* were harvested and enumerated at intervals from both untreated control mice and treated animals by published methods (4, 5).

Results. The results of five experiments in which CL 64,855 was administered to *M. leprae*-infected mice are presented in Fig. 1. In the first experiment (m 9-3-69), a harvest of *M. leprae* from the foot pads of untreated control mice performed just before drug administration was terminated yielded an average of $10^{5.7}$ organisms per foot pad. A harvest performed on the same day from the foot pads of mice to which CL 64,855 had been administered in a dosage in the diet of 0.2 g% yielded $10^{4.3}$ acid fast bacilli (AFB) per foot pad. Two subsequent harvests from treated mice, performed at intervals after termination of the treatment, yielded numbers of AFB no larger than that inoculated. Finally, a harvest performed 398 days after inoculation and 228 days after drug administration had been stopped yielded $10^{6.1}$ AFB per foot pad. The organisms appear to have multiplied at the same rate as in untreated mice during the period between 285 and 398 days after inoculation.

In the second experiment (m 7-31-71), dosages of CL 64,855 ranging from 0.00002 to 0.02 g% appear to have been without effect on the multiplication of *M. leprae*. The results of the third experiment (m 1-11-72) suggest that the drug had no effect when administered in a concentration of 0.05 g%, whereas it may have exerted a modest effect on multiplication of *M. leprae* when it was administered in a concentration of 0.1 g%. Because of an insufficient supply of the drug and because there was no reason to anticipate so steep a dose-response curve as that implied by these results, the concentration of CL 64,855 previously found effective—0.2 g%—was not retested concurrently with the testing of the lower concentrations.

In order to be certain that the effect on multiplication of *M. leprae* observed in the first experiment could be confidently attributed to the action of the drug, 0.2 g% CL 64,855 was administered in a fourth experiment (m 4-4-74). Profound inhibition of growth of the organisms was again observed. Finally, in the fifth experiment (m 9-11-75), the titration of the drug was repeated, on this occasion concurrently with the administration of 0.2 g% CL 64,855. As shown in the right-hand panel of Fig. 1, the drug exerted an effect on multiplication of *M. leprae* when administered in dosages of 0.1 and 0.2 g%; in addition, the administration of 0.05 g% of the drug appears to have

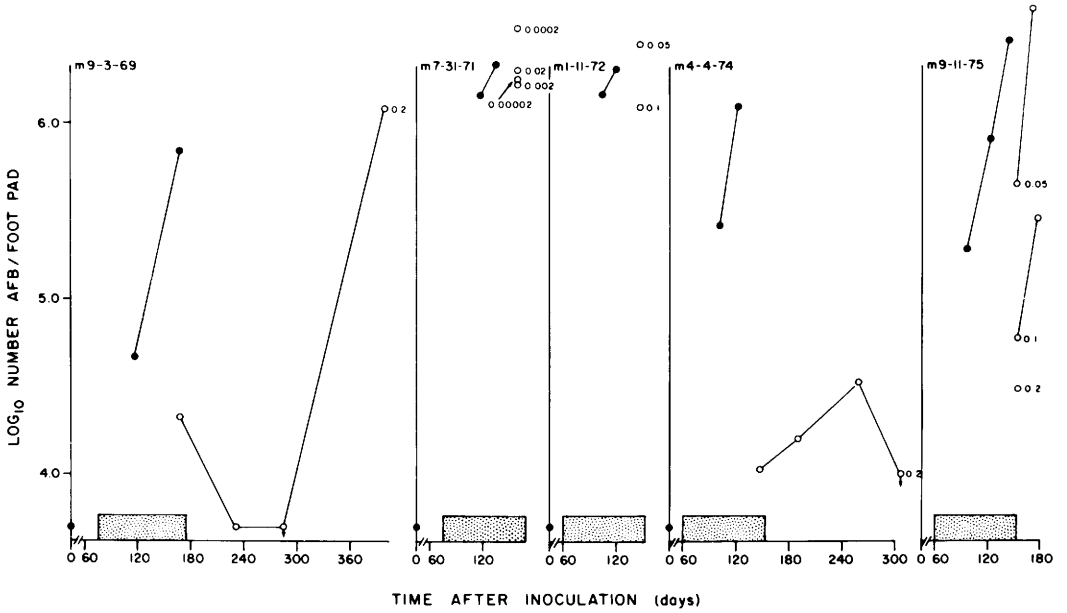

FIG. 1. Log_{10} number AFB per foot pad as a function of time after inoculation. Mice were inoculated with $10^{3.7}$ *M. leprae* in both hind foot pads (point on each ordinate). AFB were harvested at the intervals shown from the pooled tissues of four foot pads: closed circles—control mice; open circles—mice treated with CL 64,855 for the periods shown by the shaded bars along the abscissa. The concentrations in g% of drug administered to the mice are shown by the numbers appearing next to the points representing harvests from treated mice. Those points with downward-extending arrows represent harvests from which no AFB were recovered; the results were calculated as if one organism had been counted.

produced a modest effect. The administration of neither 0.05 nor 0.1 g% CL 64,855 was associated with a delay in resumption of multiplication of *M. leprae*. Unfortunately, failure of the automatic watering system in the animal house caused the loss of so many of the mice in this experiment that none of the animals treated with 0.2 g% of the drug remained for later harvest.

Discussion. The purpose of this study was to screen a new compound for activity against *M. leprae* in the mouse foot pad infection and to attempt to characterize the antimicrobial effect. The results demonstrate that CL 64,855, a thiadiazole derivative with a broad spectrum of antimicrobial activity but no activity against *M. tuberculosis*, exerts a profound effect on the multiplication of *M. leprae*.

The drug was without effect when administered in the mouse chow in concentrations smaller than 0.05 g%. In a dosage of 0.05 g%, the drug was without effect on one occasion and produced modest inhibition of multiplication of the organisms on a second. Administration of CL 64,855 in a concentration of 0.1 g% was modestly effective on one occasion, and demonstrated a definite effect in another experiment. When administered in a dosage of 0.2 g%, the drug exerted a profound effect on multiplication of *M. leprae* in three experiments.

After multiplication of *M. leprae* in the mouse foot pad has been interrupted by the administration of an effective drug and drug administration is subsequently terminated, the organisms resume multiplication in one of three ways (3). Immediate resumption is characteristic of the action of a drug that has exerted only a bacteriostatic effect, whereas the failure of multiplication may be taken as evidence of a bactericidal effect that has resulted in eradication of the *M. leprae* infection. More difficult to interpret is the situation in which the organisms resume multiplication only after a delay longer than can be attributed to the continued presence of the drug in an effective concentration. This situation is exemplified by the results of administration of CL 64,855 in the largest concentration studied. The delay before resumption of multiplication may be attrib-

uted to a prolonged bacteriostatic effect of the drug; the administration of dapsone frequently produces such a delay (6, 7). Alternatively, the delay may be attributed to a bactericidal effect of the drug that falls short of eradication of the infection. In this latter instance, the delay occurs because multiplication resumes from only the surviving fraction of the population of *M. leprae*, and, although the surviving organisms may resume multiplication immediately upon withdrawal of the drug, the first generations result in numbers of AFB that are too small to be detected. This interpretation appears to fit best the data shown in Figure 1. Particularly in experiment m 4-4-74, the suggestion that CL 64,855 has exerted a bactericidal effect is supported by the failure to find any organisms in the foot pad tissues of the mice sacrificed after 305 days, whereas small numbers of AFB had been enumerated in earlier harvests. It appears likely that the infection had indeed been eradicated in the foot pads of some of the mice.

In a survey of drugs studied for activity against *M. leprae* in the mouse foot pad infection, Shepard pointed out (3) the importance of those few agents capable of producing a delay of resumption of multiplication. This demonstration that CL 64,855 in sufficient dosage produces such a delay suggests that this drug or analogous compounds may potentially have value in the treatment of patients with leprosy. And the large dosages well tolerated by the mice suggest that the compound is relatively nontoxic. Unfortunately, the drug has been withdrawn from human trial because of its carcinogenicity on long-term administration to rats (H. Macdonald, personal communication). Perhaps some analog will prove to be both effective against *M. leprae* and free of carcinogenic effects.

Summary. A new broad-spectrum antimicrobial, 2-amino-5-(1-methyl-5-nitro-2-imidazolyl)-1,3,4-thiadiazole, reported inactive against *Mycobacterium tuberculosis*, inhibited multiplication of *M. leprae* in the mouse foot pad when administered orally to the mice. The dose–response curve was very steep: 0.2 g% of the drug exhibited considerable activity, whereas 0.05 g% was only modestly active in one experiment and inactive in another. This drug appears to be one of the few that is bactericidal for *M. leprae*.

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1. Berkelhammer, G., and Asato, G., Science **162,** 1146 (1968).
2. Redin, G. S., Kuck, N. A., and Forbes, M., Antimicrob. Ag. Chemother. **534** (1968).
3. Shepard, C. C., Int. J. Leprosy **39,** 340 (1971).
4. Shepard, C. C., J. Exp. Med. **112,** 445 (1960).
5. Shepard, C. C., and McRae, D. H., Int. J. Leprosy **36,** 78 (1968).
6. Levy, L., Proc. Soc. Exp. Biol. Med. **135,** 745 (1970).
7. Levy, L., Proc. Soc. Exp. Biol. Med. **139,** 263 (1972).

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