Discontinuous Administration of Clofazimine (B663) in *Mycobacterium leprae* Infections (35653)

CHARLES C. SHEPARD, LAURA L. WALKER, ROSALIND M. VAN LANDINGHAM, AND MARTHA A. REDUS

Center for Disease Control, Health Services and Mental Health Administration, Public Health Service, United States Department of Health, Education, and Welfare, Atlanta, Georgia 30333

The rimino-compounds were synthesized by Barry and colleagues and studied for their antimycobacterial activity (1, 2); clofazimine (B663) was the most active compound against *M. tuberculosis* in mice. It is active against *M. leprae* in mice (3, 4) and in man (5), and is now generally accepted as the drug of choice in the treatment of human infections with sulfone-resistant *M. leprae*. Its usual dosage in human leprosy is 100–300 mg daily. However, the minimal effective dosage in mice was found to be very low, 0.0001% in the diet, or about 0.1 mg/kg/day (6). Clofazimine is highly insoluble in water but soluble in fat. It is transported in the blood by lipoproteins and is deposited in the tissues, especially in fat and macrophages; in the latter it eventually appears as crystals (7, 8).

The deposition of clofazimine in the tissue and its slow elimination from the body, along with its low minimal effective dosage against *M. leprae*, suggest that spaced administration might be as effective as daily dosage in human therapy and that it might also be useful in chemoprophylaxis. Several investigators (2, 9, 10) have studied the potentials of clofazimine in discontinuous therapy and prophylaxis of experimental murine tuberculosis. The possibilities in *M. leprae* infections would appear to be greater because of the lower minimal effective dosage.

Materials and Methods. The strain of *M. leprae* used was in mouse passage and had originated from an untreated patient. The methods have been described (11–13). In brief, mice were treated for only a limited period during the course of their infection. By means of counts of acid-fast bacteria in the infected foot pads, the growth curve of the *M. leprae* in the untreated controls was compared to that in treated mice, and the amount of growth delay in the treated mice was estimated from the graphed results. There were 30 mice in each group, and three control groups. Each count represented the pooled harvest from four mice, except in some instances when fewer than four remained in the group. For the control curve, the harvests in the three control groups were averaged arithmetically. A level near the end of the logarithmic phase of growth ($10^{5.6}$ bacilli/mouse) was taken for the estimate of growth delay. In instances where the count in the treated group did not rise significantly, a minimal estimate of the growth delay was made by assuming that growth had occurred at full logarithmic rate (12.5 days/generation) (14) from the last plotted point.

Results. Several schedules of administration of clofazimine were tried (Fig. 1, Table I). When administered continuously from Day 70 to Day 140 after infection, no subsequent growth of *M. leprae* was seen. When given only 2 days a week over the 7-week period, growth eventually occurred but only after a delay of more than 400 days. When given only 2 days every 4 weeks, growth was delayed 189 days1 A single 2-day period of therapy was not effective when given 70 days after infection, or 35 days before infection, but it had a distinct effect when given on the day of infection. If the mice consumed 3 g of diet per day, they would have ingested 0.6 mg of the drug in 2 days.

The injection of drug on the day of infection was very effective. One milligram delayed growth more than 200 days, and 10

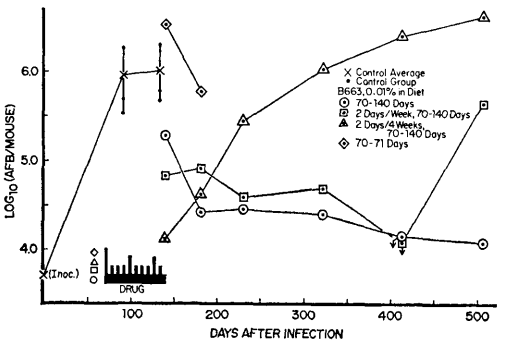

FIG. 1. Effect of clofazimine (B663) when administered discontinuously. The values marked by arrows indicate maximal estimates in cases in which no bacilli were found during the counting procedure.

mg delayed it more than 498 days.

Discussion. In earlier work with *M. tuberculosis* infections in mice, Barry and Conalty(2) found that prophylactic administration of 10 mg/kg/day of clofazimine for 14 days ending either 1 week or 4 weeks before infection was effective in prolonging the median survival time. Administration of 2 mg/kg/day for 14 days ending 1 week before infection prolonged the median survival time somewhat, but the same administration ending 4 weeks before infection did not. The total intake in the 14 days would have been 140 and 28 mg/kg, respectively. In experiment B, the dosage of 0.01% in the diet for 2 days administered approximately 20 mg/kg, and it was not effective when given 34 and 35 days before infection, but was effective when given on the day of infection. Thus, the prophylactic value of clofazimine against *M. leprae* would appear to be roughly the same as that against *M. tuberculosis* in mice.

TABLE I. Effect of Discontinuous Administration of Clofazimine on Multiplication of *M. leprae*.

Dosage	Time administered[a]	Growth delay[b]
0.01%	70–140	>498
0.01%	2 days/wk, 70–140	426
0.01%	2 days/4 wk, 70–140	189
0.01%	70–71	0
0.01%	0–1	90
0.01%	—35—34	0
1 mg[c]	0	219
10 mg[c]	0	>498

[a] Days after infection, *i.e.*, 70–140 indicates that drug was started on Day 70 after infection and stopped on Day 140.

[b] Days of delay relative to control curve.

[c] Injected intraperitoneally in Hanks' balanced salt solution.

Spaced administration of clofazimine in *M. tuberculosis* infections in mice was studied by Noufflard and Berteaux (9, 10). They found that administration of 10 mg/kg on the day before infection had slight but significant effect; 10 mg/kg on the day before infection plus Day 21 after infection had greater effect, and administration at increasing frequencies during these 21 days had progressively greater effects. Against *M. leprae,* a somewhat greater dose (2 days of 0.01% drug in the diet, or approximately 20 mg/kg), when given at 4-week intervals, had distinct effect and delayed growth 189 days.

Grumbach (15) compared orally and subcutaneously administered clofazimine against tuberculosis infections in mice and found the subcutaneous drug to be about one-tenth as effective as oral drug. In that work, the drug was given 6 days a week from the day of infection until Day 21, the time when half the controls were dead; the surviving mice were then killed and the extent of the tuberculosis lesions was compared. Against *M. leprae,* however, 1 mg intraperitoneal clofazimine on the day of infection had much more effect than approximately 0.6 mg consumed in the diet on the first 2 days after infection. In both experiments the drug was able to reach the site of infection only by systemic transport, but with *M. leprae* the longer time course of the infections would make it possible for more slowly transported drug to exert an effect. Clearly the effect of site of administration and vehicle deserves further study; injected clofazimine might have more favorable repository effect than oral drug.

With drugs, such as dapsone and rifampin, which are fairly evenly distributed in the body, it is possible, through measurements of drug concentrations in blood and tissue in mice and man, to estimate the minimal inhibitory concentration and thereby predict, with some confidence, the effect of a particular dosage against *M. leprae* in man. In this manner, the minimal effective dosage of dap-

sone in man was predicted in a useful way (16, 17). However, clofazimine is so unevenly distributed that such an approach is not feasible, so the mouse experiments can only provide general orientation to experiments in man. Some studies of several schedules of spaced administration in the therapy of human leprosy are now in progress. If effective, spaced administration would facilitate the provision of therapeutic coverage.

Summary. The prophylactic and spaced administration of clofazimine was studied. The drug had an effect when given orally for 2 days immediately after infection, but had no effect when given 5 weeks before infection. When given once every 4 weeks from Day 70 to Day 140 after infection, the drug had a distinct effect. It was also active when injected intraperitoneally on the day of infection.

This work was supported in part by an interagency agreement with the National Institute of Allergy and Infectious Diseases, National Institutes of Health, under the U.S.–Japan Cooperative Medical Science Program. Clofazimine (B663) was obtained through the courtesy of Dr. W. A. Vischer, J. R. Geigy S. A., Basel, Switzerland.

1. Barry, V. C., Belton, J. G., Conalty, M. L., Denneny, J. M., Edward, D. W., O'Sullivan, J. F., Twomey, D., and Winder, F., Nature (London) **179**, 1013 (1957).

2. Barry, V. C., and Conalty, M. L., Amer. Rev. Tuberc. Pulm. Dis. **78**, 62 (1958).

3. Shepard, C. C., and Chang, Y. T., Int. J. Lepr. **32**, 260 (1964).

4. Gaugas, J. M., Leprosy Rev. **38**, 225 (1967).

5. Browne, S., and Hogenzeil, L. M., Leprosy Rev. **33**, 182 (1962).

6. Shepard, C. C., Proc. Soc. Exp. Biol. Med. **122**, 893 (1966).

7. Barry, V. C., Buggle, K., Byrne, J., Conalty, M. L., and Winder, F., Irish J. Med. Sci. **416**, 345 (1960).

8. Barry, V. C., Sci. Proc. Roy. Dublin Soc. Ser. A. **3**, 153 (1969).

9. Noufflard, H., and Bertaux, S., Ann. Inst. Pasteur **95**, 449 (1958).

10. Noufflard, H., and Bertaux, S., Pathol. Biol. **9**, 1037 (1961).

11. Shepard, C. C., J. Exp. Med. **112**, 445 (1960).

12. Shepard, C. C., Int. J. Lepr. **35**, 429 (1967).

13. Shepard, C. C., Int. J. Lepr. **37**, 389 (1969).

14. Shepard, C. C., and McRae, D. H., J. Bacteriol. **89**, 365 (1965).

15. Grumbach, F., Ann. Inst. Pasteur **99**, 567 (1960).

16. Shepard, C. C., Tolentino, J. G., and McRae, D. H., Amer. J. Trop. Med. Hyg. **17**, 192 (1968).

17. Waters, J. F. R., Rees, R. J. W., and Ellard, G. A., Int. J. Lepr. **36**, 651 (1968).

Received Jan. 8, 1971. P.S.E.B.M., 1971, Vol. 137.