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Activity of four clofazimine analogues against *Mycobacterium leprae*

L LEVY*
Leprosy Research Unit, Public Health Service Hospital, San Francisco, California 94118, USA

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Summary Four clofazimine analogues were found active against *Mycobacterium leprae* in the mouse footpad system, but none was as active as clofazamine itself. The results suggest the importance of the two *p*-chlorosubstituents that are a structural feature of clofazimine.

Introduction

Except for one compound [2-anilino-5-phenyl-8-chloro-3,5-dihydro-3-cyclohexyliminophenazine, B1912] studied by Shepard *et al*,[1] no analogue of clofazimine [2-(6-chloroanilino)-5-(6-chlorophenyl)-3, 5-dihydro-3-*iso*propyliminophenazine, B663] had been screened for activity against *Mycobacterium leprae*. Such a study became possible when the late Dr VC Barry, Medical Research Council of Ireland, Dublin, agreed to furnish four analogues; the number of analogues to be screened initially was limited by the need to resynthesize each in 10-g quantities. Therefore, the activity against *M. leprae* of clofazimine and the four analogues was investigated in the mouse footpad system by Shepard's kinetic method.[2,3] In addition, the half-time of disappearance of each analogue from the mouse carcass was determined, to permit more precise comparisons of the antimicrobial activity of the compounds.

Materials and methods

Locally-bred weanling BALB/c mice were inoculated in the hind footpad, each footpad receiving 5,000 *M. leprae* of the same strain. Compounds were administered, either incorporated into the mouse chow in several

*Requests for reprints should be addressed to L Levy, MD, Ph D, Department of Comparative Medicine, Hebrew University–Hadassah Medical School, POB 1172, Jeruselem, Israel.

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concentrations, or in weekly intraperitoneal doses, for periods of about 90 days, beginning 60 or 75 days after inoculation. Clofazimine was tested at a dietary concentration of 0.1 mg per 100 g chow, the minimal effective concentration for this strain of *M. leprae*,[4] and in an intraperitoneal dose of 25 μg (approximately 1 mg/kg body weight); the analogues were tested in dietary concentrations equimolar to 0.1, 1.0 and 10 mg clofazimine per 100 g mouse chow, and in intraperitoneal doses equimolar to that of clofazamine. Groups of untreated mice served as controls. Harvests of *M. leprae*, usually from a pool of four footpads, were performed at intervals by published methods,[5,6] and bacterial growth curves were constructed from the results of the harvests. Activity of the compounds is expressed in terms of the 'delay' of bacterial multiplication in treated mice compared to that in control mice;[2,3] a delay of at least 30 days may be considered significant. In separate experiments, mice were administered a single intraperitoneal dose of each of the analogues, dissolved in propylene glycol, equimolar to 4 mg clofazimine/kg body weight; pairs of mice were sacrificed at intervals during the following 21 days, the carcasses were homogenized, and the clofazimine analogues were extracted from the trichloracetic acid-precipitates of the homogenates, as previously described for clofazimine.[4] The concentrations of the analogues were determined spectrophotometrically by their native absorbance in 20% H_2SO_4, and the half-time of disappearance of each analogue from the mouse carcass was calculated.

Results

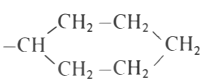
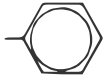
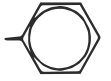
The results are summarized in Table 1. All four analogues of clofazimine demonstrated activity against *M. leprae*, but none was as active as clofazimine itself. Three analogues – B669, B749 and B775 – demonstrated only one-tenth the activity of clofazimine, whereas B1056 appeared only about 1/100 as active as clofazimine.

The half-time of disappearance of clofazimine from the mouse carcass had been determined previously to be 7 days.[4] Two of the analogues – B749 and B1056 – were found to disappear at about the same rate as did clofazimine, whereas the two remaining compounds disappeared more rapidly.

Discussion

None of the four analogues of clofazimine was as active against *M. leprae* as was clofazimine itself. In the cases of B669 and B775, the lesser degree of antimicrobial activity may simply reflect the more rapid disappearance of the two analogues. On the other hand, that B749 demonstrated roughly 10-fold the

Table 1. Studies of clofazimine analogues

Compound	R_1	R_2	Dosage	Delay* (days)	$T^{1/2}$† (days)
Clofazimine‡		$-CH(CH_3)_2$	25 μg§	160	7
			0.0001 g%‖	182	
				112	
B 669¶			23 μg	0	
			0.000094 g%	19	2
			0.00094 g%	182	
			0.0094 g%	150	
B 749¶		$-(CH_2)_2-N-(CH_2-CH_3)_2$	27 μg	6	
			0.00011 g%	11	7.5
			0.0011 g%	200	
			0.011 g%	455	
B 775¶		$-CH_2-CH-(CH_3)_2$	21 μg	0	
			0.000085 g%	13	0.4
			0.00085 g%	133	
			0.0085 g%	162	
B 1056¶**		$-(CH_2)_2-N-(CH_2-CH_3)_2$	25 μg	0	
			0.0001	23	8
			0.001	40	
			0.01	258	

*Delay in multiplication of *M. leprae* in treated mice compared to that in untreated mice, corrected for period of drug administration.
†Determined from analyses of mouse carcases after intraperitoneal administration.
‡Generously furnished by Dr W Vischer, CIBA–GEIGY, Basle, Switzerland.
§Dosages shown as quantities of compounds were administered up weekly between day 60 and day 142.
‖Dosages shown as concentrations of compounds were administered, incorporated in the mouse chow, between day 75 and day 165.
¶Generously synthesized and furnished by Dr Barry.
**Furnished as the hydrochloride salt.

activity of B1056 suggests the importance of the *p*-chloro-substituent on the two benzene rings; it may be that the *p*-chlorophenyl-analogues of B669 and B775 would have been more active than the latter compounds. It is interesting that, against murine infection with *M. tuberculosis*, all four analogues were much less active than was clofazimine; B775 was the most active of the four analogues, and B1056 the least active.[7] And B673 and B776, the *p*-chlorophenyl-analogues of B669 and B775, respectively, were each much more active in murine tuberculosis than the corresponding compound without the *p*-chloro-substituent.[7]

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