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Characteristics of the multiplication of dapsone-resistant strains of *Mycobacterium leprae* in mice*

Subcommittee on Clinical Trials of the Chemotherapy of Leprosy (THELEP) Scientific Working Group of the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases

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Summary Twenty-seven per cent of the 49 strains of *Mycobacterium leprae* isolated in the course of the THELEP controlled clinical trials of combined chemotherapy of lepromatous leprosy in Bamako and Chingleput, and found to be resistant to dapsone multiplied in significantly fewer mice administered dapsone than in mice administered the dapsone-free diet.

Members of the THELEP Clinical Trials Subcommittee are:

Dr M Christian, Schieffelin Leprosy Research and Training Centre, Karigiri, South India,
Dr M J Colston, National Institute for Medical Research, London, UK,
Dr G A Ellard, National Institute for Medical Research, London, UK,
Dr C A P Ferracci, Institut Marchoux, Bamako, Mali,
Dr J H Grosset, Faculte de Medicine Pitie-Salpetriere, Paris, France,
Dr G Grossetete, Institut Marchoux, Bamako, Mali,
Dr C G S Iyer,† Central Leprosy Teaching and Research Insitute, Chingleput, South India,
Dr Ji Baohong, World Health Organization, Geneva, Switzerland,
Dr Kyaw Lwin, Ministry of Health, Rangoon, Burma,
Dr D L Leiker, Royal Tropical Institute, Amsterdam, the Netherlands,
Dr L Levy, Hebrew University-Hadassah Medical School, Jerusalem, Israel,
Dr S K Noordeen, World Health Organization, Geneva, Switzerland,
Dr S R Pattyn, Prince Leopold Institute for Tropical Medicine, Antwerp, Belgium,
Dr J M H Pearson, Dhoolpet Leprosy Research Centre, Hyderabad, India,
Dr R J W Rees, National Institute for Medical Research, London, UK,
Dr H Sansarricq, World Health Organization, Geneva, Switzerland,
Dr P S Seshadri, Central Leprosy Teaching and Research Institute, Chingleput, South India,
Dr J K Seydel, Borstel Research Institute, Borstel, Federal Republic of Germany,
Dr C C Shepard,† Centers for Disease Control, Atlanta, Georgia, USA,
Dr M F R Waters, Hospital for Tropical Diseases, London, UK.

Also participating in this study were Drs R D McDermott and G F R Hilson, Department of Medical Microbiology, St George's Hospital Medical School, London, UK. Mr J L Duppenthaler,

* This report was prepared by L Levy, M Anker and G A Ellard.

† Deceased.

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World Health Organization, Geneva, Switzerland, provided statistical consultation throughout the trials, and Mrs M Anker, World Health Organization, performed the statistical analyses.

Introduction

During the years 1978–1983, trials of combined chemotherapy were carried out among 215 patients with previously untreated multibacillary (LL, LI and BL) leprosy at the Institute Marchoux, Bamako, Mali, and the Central Leprosy Teaching and Research Institute, Chingleput, South India.[1]. Of the 131 patients, the susceptibility of whose strains of *Mycobacterium leprae* to dapsone could be determined, 49 (37·4%) were found to harbour strains resistant to the drug; all but 10 of these strains were resistant only to 0·0001 g dapsone per 100 g mouse diet, the smallest concentration of the drug tested, and no strain resistant to the largest concentration (0·01 g per 100 g mouse diet) was encountered.[2]

In the course of the study, it was observed that a proportion of the strains of dapsone-resistant *M. leprae* did not multiply in as many of the mice administered dapsone as in the mice administered drug-free diet. Because there was no ready explanation for this phenomenon, the relevant data have been analysed.

Materials and methods

The methods employed to test the susceptibility to dapsone of the pretreatment isolates of *M. leprae*, are those already described.[2] In brief, before beginning treatment by means of one of the trial regimens, a biopsy-specimen was obtained from a skin lesion of each patient and air-shipped on wet ice to London. In the Department of Medical Microbiology, St George's Hospital Medical School, the specimens were homogenized, and the *M. leprae* were recovered, counted, diluted to provide an inoculum of 10^4 organisms per footpad, and inoculated into the right-hind footpads of CD-1 mice. Groups of 8 mice were administered a drug-free diet, and groups of 5–7 mice were fed diets into which had been incorporated dapsone in concentrations of 0·0001, 0·001 or 0·01 g per 100 g diet. Approximately six months after inoculation, several control mice (those administered the drug-free diet) were killed, and *M. leprae* were harvested from the inoculated footpads. If *M. leprae* were found not to have multiplied after 6 months, additional harvests were performed from control mice 3 months later. Similarly, if *M. leprae* were found not to have multiplied after 9 months, harvests were performed from additional control mice after 12 months. When the organisms were noted to have multiplied in control mice, harvests were performed from all surviving treated mice. *M. leprae* were determined to be resistant to dapsone in a given concentration if they multiplied to $\geq 10^5$ per footpad in at least one mouse administered dapsone in that concentration. The data were analysed by means of the χ^2 and Fisher exact probability techniques for comparison of frequencies.[3]

Results

As demonstrated by the examples presented in Table 1, three patterns of multiplication were observed. The results of testing the pretreatment isolate from patient 1061 for resistance to dapsone reveal that multiplication had occurred in almost all of the mice administered the drug-free diet and the diet containing dapsone in the concentration of 0·0001 g per 100 g, and in none of the mice administered dapsone in the concentration of 0·001 g per 100 g diet. Thus, this strain, which is resistant to the smallest concentration of dapsone, and susceptible to the intermediate concentration, multiplied virtually as readily in mice administered the largest concentration of dapsone permitting multiplication as in those administered the drug-free diet.

Table 1. Results of dapsone-resistance measurement on pretreatment isolates of three patients

Concentration of dapsone (g%)	Results of mouse-by-mouse harvests ($\times 10^4$)	Proportion of mice showing multiplication	P*
Patient 1061†			
0	150, 98, 61, 39, 26, 14, 11, 8·4	7/8	0·28
0·0001	32, 25, 21, 13, 12, 8·8, 6·4, < 1·0	5/8	
0·001	5·4, 3·6, 3·6, 1·2, < 1·0, < 1·0, < 1·0	0/7	
Patient 1117			
0	37, 30, 15, 7·1, 4·9, 3·1	3/6	0·59
0·0001	120, 40, 16, 12, 3·6 2·7, 1·3	4/7	0·21
0·001	22, 16, 12, 7·1	3/4	
0·01	6·7, 4·0, < 1·0, < 1·0	0/4	
Patient 1001			
0	140, 130, 120, 81, 19, 10, 6·0, 4·4	6/8	0·05
0·0001	11, 5·2, 4·4, 4·0, 3·2, 2·4	1/6	
0·001	< 1·0, < 1·0, < 1·0, < 1·0, < 1·0, < 1·0	0/6	

* The probability, calculated by Fisher's exact test,[3] that the adjacent pairs of results have been drawn from the same population.

† The patient number consists of the number of the centre (1, Chingleput; 2, Bamako) and the three-digit number representing the order in which the patient was recruited.

A second pattern is exemplified by the results of testing the susceptibility to dapsone of the *M. leprae* isolated from the pretreatment biopsy-specimen of patient 1117. In this case, the organisms may be seen to have multiplied in only a fraction of the mice, but in much the same fraction of mice administered drug-free diet and those administered diets into which dapsone had been incorporated in concentrations of 0·0001 or 0·001 g per 100 g diet. This strain, which manifests an intermediate degree of resistance to dapsone, also multiplied as readily in the mice administered the largest concentration of dapsone permitting multiplication as in control mice, but, probably because the proportion of viable organisms in the inocula was small, multiplication occurred in an average of only 60% of the mice.

The third pattern is exemplified by the results obtained from the study of the pretreatment specimen of patient 1001. In this case, the *M. leprae* may be noted to have multiplied in 6 of 8 mice administered the drug-free diet, but in only 1 of 6 mice administered 0·0001 g dapsone per 100 g diet, a significantly smaller proportion ($P = 0{\cdot}05$).

The results of inoculating mice with *M. leprae* of the 49 dapsone-resistant strains are summarized in Table 2, in which are listed, for each strain, both the numbers of mice in which no evidence of multiplication was found, and the numbers of mice in which the organisms were found to have multiplied. With respect to those 39 strains exhibiting a low degree of resistance, 9 (23%) were found to have multiplied in significantly fewer dapsone-treated than control mice. Among the 10 strains that demonstrated an intermediate degree of resistance, 3 multiplied more readily in control mice than in mice administered dapsone in the smallest concentration; one of these strains and one additional strain multiplied more readily in control animals than in mice administered dapsone in the intermediate concentration. Thus, 13 of the 49 (27%) dapsone-resistant strains manifested the third pattern of multiplication, multiplying in significantly fewer treated that control mice.

In Table 3 are shown the distributions of specimens demonstrating different patterns of multiplication. The *M. leprae* of the majority [28 of 48 (58%)] of the strains demonstrating

Table 2. Strains of *M. leprae* manifesting resistance to dapsone

Patient No.	Number of mice yielding: Dapsone concentration (g/100 g diet) 0		0·0001		0·001	
	$<10^5$	$\geq 10^5$	$<10^5$	$\geq 10^5$	$<10^5$	$\geq 10^5$
Low-grade resistance						
1001*	2	6	5	1†	6	0
1008	0	6	5	2†	7	0
1025	1	7	5	2†	7	0
1028	3	2	2	3	7	0
1050	0	4	3	3	5	0
1053	5	3	7	1	4	0
1055	6	2	7	1	ND‡	
1060	4	4	4	4	5	0
1061	1	7	3	5	7	0
1062	2	6	2	4	7	0
1064	2	6	4	1	5	0
1067	6	2	6	1	5	0
1078	3	3	5	1	4	0
1093	2	5	6	1†	5	0
1097	3	3	4	3	5	0
1104	3	4	4	2	5	0
1108	0	4	3	2	5	0
1116	2	5	5	1	5	0
2008	0	3	8	1†	10	0
2018	6	3	6	1	7	0
2028	1	7	3	3	7	0
2030	0	6	5	2†	7	0
2032	6	3	7	1	6	0
2037	6	1	6	1	ND	
2043	0	3	2	3	5	0
2051	2	3	4	2	4	0
2054	1	5	5	1†	6	0
2057	1	4	5	1	4	0
2059	3	0	5	1	5	0
2060	3	3	5	1	4	0
2064	0	6	4	1†	5	0
2069	ND		5	1	10	0
2071	3	4	3	4	6	0
2075	3	4	6	1	5	0
2087	0	7	2	5	5	0
2089	1	6	6	1†	5	0
2090	3	4	5	1	5	0
2095	3	4	6	1	5	0
2098	2	8	2	6	10	0
Intermediate resistance						
1059	3	5	8	0†	4	1
1080	0	6	1	5	2	2
1037	0	5	1	4	2	5
1117	3	3	3	4	1	3
2025	1	6	6	2†	6	1†
2026	0	7	2	6	5	1†
2034	2	5	0	7	2	4
2072	3	3	6	1	4	1
2088	1	5	5	1†	4	1
2096	2	5	4	2	4	1

* The patient is shown in the first column. In the remaining columns are entered the numbers of mice yielding on harvest the number of organisms shown at the head of each column. In no case did *M. leprae* multiply in mice administered dapsone in the concentration of 0·01 g per 100 g diet.

† $P \leq 0{\cdot}05$ by Fisher's exact test,[3] when this distribution of mice demonstrating multiplication and those not showing multiplication is compared with that in the untreated control mice.

‡ Not done.

Table 3. Proportion of mice showing multiplication as a function of degree of resistance and concentration of dapsone

Degree of resistance	Dapsone concentration (g%)	Proportion of mice showing multiplication				
		<0·20	0·20–0·39	0·40–0·59	0·60–0·79	>0·80
		(Numbers of specimens)				
Low	0	2	5	11	5	15*
	0·0001	19	7	6	6	0*
Intermediate	0	0	0	2	3	5
	0·0001	3	2	1	1	3
	0·001	2	4	1	3	0

* For one strain, no harvests were reported from untreated mice.

resistance to the smallest or intermediate concentrations of dapsone were found to have multiplied in more than 60% of the mice administered dapsone-free diet, whereas, in the mice administered dapsone in the concentration of 0·0001 g per 100 g diet, this pattern of multiplication was found in the case of only 6 of 38 (16%) strains demonstrating resistance to dapsone at this level, and in the mice administered 0·001 g dapsone per 100 g diet, in the case of only 3 of 10 strains resistant at this level.

Discussion

That the *M. leprae* of 49 of the 131 strains multiplied in mice administered dapsone in some concentration provided an opportunity to analyse the patterns of multiplication of dapsone-resistant organisms in dapsone-treated mice. More than 25% of the strains multiplied in significantly fewer of the mice administered dapsone than in mice administered dapsone-free diet. Hastings has described[4] a similar study of 75 strains of *M. leprae* resistant to dapsone, in which harvests (presumably from pooled mouse footpad tissues) from mice administered the largest concentration of dapsone permitting some multiplication were reported consistently to yield fewer organisms than were obtained from simultaneous harvests from mice administered smaller concentrations of dapsone or control diet.

The explanation for this phenomenon is obscure. Some authors[4–9] have suggested the coexistence in the biopsy-specimen of more than a single strain of *M. leprae*, the strains exhibiting different degrees of resistance to dapsone. In the case of primary resistance, however, such an hypothesis does not appear tenable. Because infection with *M. leprae* probably involves only one or, at most, a very small number of viable organisms, it appears unlikely indeed that the patients were infected *ab initio* by *M. leprae* representing a mixture of drug-resistant and drug-susceptible strains. One might consider as an alternative the possibility of superinfection by a second strain differing in susceptibility to dapsone from the strain causing the first infection. Although superinfection has been proposed as the cause of some instances of relapse,[10] however, no evidence has been produced to show that superinfection occurs under any circumstances.

A second alternative explanation, that of phenotypic variation of susceptibility among the members of a genetically homogeneous population, has been proposed by Ji.[11] It should be possible to exclude infection by a mixture of susceptible and resistant strains of *M. leprae* by experiments in mice. Ji[11] suggested as one experimental approach, simply subinoculating organisms harvested from both control and dapsone-treated mice into new groups of mice, and repeating the test of

susceptibility on both isolates. If the pattern of multiplication in both control and untreated mice of the organisms isolated from treated mice was the same as that in the original test, i.e. greater multiplication in control than in dapsone-treated mice, one could exclude the possibility of infection by a mixture of strains.

It appears more likely that, in mice administered dapsone in or near the minimal effective dosage, *M. leprae* multiply more slowly than in control mice or mice administered dapsone in a less than maximally effective dosage. Such a phenomenon has been described by Seydel[12] in the case of a laboratory-derived, dapsone-resistant strain of '*M. lufu*'.

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