Primary dapsone resistance in Bamako and Chingleput: final report; THELEP

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

Summary Approximately 37% of the 131 patients with lepromatous leprosy admitted into the THELEP controlled clinical trials in Bamako and Chingleput, whose *Mycobacterium leprae* obtained from pretreatment biopsy specimens could be tested in mice, have been found to harbour dapsone-resistant organisms, and are thought to represent instances of primary resistance to dapsone. The majority of these patients harboured strains of a low degree of resistance, one-fifth of these patients harboured organisms of an intermediate resistance, and no patient was found to harbour *M. leprae* of a high degree of resistance. No relationships were discerned between patient age, number of *M. leprae*, or disease classification on one hand, and primary resistance to dapsone on the other.

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Introduction

Because of the apparently increasing prevalence of primary resistance to dapsone that has been observed in the course of formal surveys, this problem has received increased attention of late. Two surveys, of which a preliminary report has already been published,[1] were carried out as one facet of the controlled clinical trials of combined chemotherapy mounted by THELEP (the Scientific Working Group on the Chemotherapy of Leprosy of the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases) among previously untreated patients with lepromatous leprosy in Bamako, Mali, and Chingleput, South India.[2]

The preliminary report[1] presented data based on examination of the *Mycobacterium leprae* isolated from the pretreatment biopsy specimens of the first 112 patients recruited into the two trials—45 in Bamako and 67 in Chingleput. Thirty-five % of the Bamako patients and 37·5% of the Chingleput patients, the susceptibility of whose organisms could be determined, were found to harbour dapsone-resistant *M. leprae*. The last patients recruited into the two trials were admitted in the latter part of 1983, so that complete information on the susceptibility to dapsone of the *M. leprae* of 215 patients—99 in Bamako and 116 in Chingleput—is now available.

Materials and methods

The patients recruited into the two trials, and the methods employed in the trials, including those employed to test the susceptibility to dapsone of the pretreatment isolates of *M. leprae*, are those already described.[1,2] In brief, patients with LL, LI

* Deceased

or BL* leprosy were recruited who denied prior treatment, and in whose urine no dapsone could be detected. Before treatment was begun, biopsy-specimens were obtained from skin lesions and shipped by air on wet ice to London. In the Department of Medical Microbiology, St George's Hospital Medical School, the specimens were homogenized, and *M. leprae* were recovered, counted, diluted, so as to provide an inoculum of 10^4 organisms per foot-pad, and inoculated into the right-hind foot-pads 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 6 months after inoculation, several control mice (those administered the drug-free diet) were sacrificed, and *M. leprae* were harvested from the inoculated foot-pads. If *M. leprae* were found not to have multiplied after 6 months, harvests were again performed from control mice after 9 months. 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.

Viable *M. leprae* were judged to be present if organisms were found to have multiplied to a level $\geq 10^5$ per foot-pad. *M. leprae* were determined to be resistant to dapsone in a given concentration if they multiplied in at least one mouse administered dapsone in that concentration. The *M. leprae* were considered to be susceptible to dapsone if two criteria were met: 1, the organisms did not multiply in a single mouse administered dapsone in any concentration; and 2, comparison of the numbers of untreated mice showing multiplication or no multiplication with those of mice administered dapsone in the smallest concentration (0·0001 g per 100 g diet) by means of the Fisher one-tailed test[4] yielded a value for $P \leq 0·05$ (*i.e.* the probability that the two sets of results had been drawn from the same population of results was no larger than 1 in 20). Finally, when the organisms did not multiply in a single treated mouse, but multiplied in so few untreated mice that the comparison described in criterion 2 (*v.s.*) yielded a value for $P > 0·05$, the susceptibility of that patient-strain of *M. leprae* to dapsone could not be determined.

Data were analysed by means of a number of statistical techniques. Nonparametric techniques were employed, because these may be applied to data that are not distributed normally. The techniques employed were[4–6]: 1, the χ^2 and Fisher exact probability techniques for comparison of frequencies in contingency tables (*e.g.* the frequency of the histopathological classification LI among patients with susceptible and resistant strains); 2, the Mann-Whitney U test, for comparison of two independent groups according to a variable, the measurement scale of which is at least ordinal (*e.g.* the pretreatment bacteriological index (BI)[7]

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* The Ridley–Jopling classification of leprosy, a system based on clinical, histopathological and other criteria, employs the following terminology: BL, borderline-lepromatous; LI (or LL$_s$), sub-polar lepromatous leprosy; LL (or LL$_p$), polar lepromatous leprosy[3]

of patients harbouring dapsone-susceptible or -resistant strains of *M. leprae*); and 3, the one-sample-runs test, to examine the order in which events (*e.g.* the admission of patients harbouring dapsone-resistant organisms) occurred.

Results

Prevalence of dapsone resistance

It was possible to assess the susceptibility to dapsone of the *M. leprae* recovered from 131 of the 215 biopsy-specimens obtained before treatment was begun. The *M. leprae* recovered from 82 specimens judged to contain dapsone-susceptible organisms multiplied to a level $\geq 10^5$ in a large proportion of control mice, and in no mouse administered dapsone in any concentration. Thirty-seven of 64 (58%) Bamako patients and 45 of 67 (67%) Chingleput patients were shown to harbour dapsone-susceptible organisms.

M. *leprae* recovered from 49 specimens were judged to be dapsone-resistant. The organisms recovered from 39 specimens multiplied to a level $\geq 10^5$ *M. leprae* per foot-pad in at least one mouse administered dapsone in the smallest concentration, but failed to multiply in a single mouse administered dapsone in a larger concentration. These pretreatment specimens represent the patients whose strains of *M. leprae* demonstrated a low degree of resistance to dapsone. Organisms from 10 specimens demonstrated an intermediate degree of resistance to dapsone, multiplying to a level $\geq 10^5$ per foot-pad in mice administered dapsone in the smallest concentration, and in at least one mouse administered the drug in the concentration of 0·001 g per 100 g diet. No specimen yielded *M. leprae* capable of multiplying in mice administered dapsone in the concentration of 0·01 g per 100 g diet (the definition of strains demonstrating a high degree of resistance to dapsone).

The results of study of the *M. leprae* recovered from the pretreatment biopsy specimens of 215 patients admitted to the THELEP trials in Bamako and Chingleput are summarized in Table 1. Considering the 131 patients in both centres, of whose *M. leprae* the susceptibility to dapsone could be determined, the prevalence of primary dapsone resistance was found to be 27 of 64 (42·2%) Bamako patients, and 22 of 67 (32·8%) Chingleput patients; these estimates of prevalence did not differ significantly between the two treatment centres ($P=0·27$), nor from the results reported earlier.[1] It is also apparent that the majority of the 49 patients harbouring dapsone-resistant *M. leprae* (21 of 27 (78%) in Bamako; 18 of 22 (82%) in Chingleput) harboured organisms exhibiting only a low degree of resistance to dapsone. As shown in Table 2, the proportion of patients harbouring dapsone-resistant *M. leprae* did not differ significantly among the regimens* ($P=0·13$ for Bamako, 0·34 for Chingleput).

* Patients were allocated among six treatment-groups, three in each centre; however, one of the

Table 1. Distribution of dapsone-resistant patients and degree of resistance between centres

Category of patient	Bamako (No. of patients)	Chingleput (No. of patients)
Total	99	116
Susceptible	37	45
Resistant	27	22
0·0001 g %	21	18
0·001 g %	6	4
0·01 g %	0	0
Susceptibility undeterminable	35	49

The frequency of resistant patients did not differ significantly between centres.

Table 2. Distribution of dapsone-resistant patients among regimens

	Regimen*					
	Bamako (No. of patients)			Chingleput (No. of patients)		
Category of patient	A_2	C	E_2	A_1	C	D_1
Total	12	44	43	39	39	38
Susceptible	7	10	20	18	11	16
Resistant	3	14	10	8	9	5
Susceptibility undeterminable	2	20	13	13	19	17

The frequency of resistant patients did not differ significantly among regimens within centre.

* See text

five regimens (regimen C) was employed in both centres. The regimens were, in Bamako, A_2: rifampicin, protionamide and dapsone, each in a daily dose of 600, 500, and 100 mg, respectively, for two years; C: rifampicin, in a single initial dose of 1,500 mg, and dapsone, 100 mg daily for two years; E_2: rifampicin, 900 mg once weekly, and protionamide, 500 mg daily for the first three months, together with dapsone, 100 mg daily for two years; and in Chingleput, A_1: rifampicin, clofazimine and dapsone, each in a daily dose of 600, 100, and 100 mg, respectively, for two years; C: as for Bamako; D_1: rifampicin, in a single initial dose of 1,500 mg, clofazimine, in a daily dose of 100 mg for the first three months, and dapsone, 100 mg daily for two years[2].

Table 3. Median ages of patients harbouring susceptible, dapsone-resistant, and undeterminable strains of *M. leprae*

Category of patient strain	Median age (years)	
	Bamako	Chingleput
Total	25	29
Susceptible	25	25
Resistant	26	30
Determinable	26	27
Undeterminable	25	30

Patient age did not differ significantly among categories.

As discussed in the earlier report,[1] it is not absolutely possible to exclude the possibility that some of the patients judged to harbour primary dapsone-resistant *M. leprae* may actually have concealed previous treatment. However, such patients might be expected to be older on the average than those patients who had not been previously treated; they should have responded to the treatment and only subsequently relapsed. As shown in Table 3, the ages of the patients harbouring dapsone-resistant *M. leprae* did not differ significantly from those of the patients harbouring dapsone-susceptible organisms at either centre. The

Table 4. Age of the patients and dapsone resistance as functions of admission order

Admission quartile	Median age		Proportion resistant	
	Bamako	Chingleput	Bamako	Chingleput
1	28	30	0·20	0·20
2	26	31	0·44	0·42
3	29	30	0·53	0·41
4	30	30	0·50	0·33

The proportion resistant (the proportion of patients harbouring dapsone-resistant *M. leprae*) is calculated as the fraction:

$$\frac{\text{number resistant}}{\text{number resistant} + \text{number susceptible}};$$

those patients harbouring strains, the susceptibility of which could not be determined, are ignored.

Table 5. Relationship of dapsone resistance to pretreatment patient characteristics

Pretreatment characteristic	Bamako		Chingleput	
	Resistant	Susceptible	Resistant	Susceptible
Median BI*	4·7	4·5	4·3	4·3
Median LIB	5·6	5·3	5·4	5·5
Median LAFBPG	8·5	8·4	8·7	8·3
Proportion with CLINCLAS LI	0·74	0·78	0·64	0·73
Proportion with HISTCLAS LI	0·81	0·84	0·95	0·93

No significant difference of any pretreatment characteristic was found between patients with dapsone-resistant and those with susceptible organisms in either centre.

* Abbreviations: BI, bacteriological index; LIB, logarithmic biopsy index; LAFBPG, $logarithm_{10}$ of the number of acid-fast bacilli per g tissue; CLINCLAS, clinical classification according to Ridley and Jopling; HISTCLAS, histopathological classification according to Ridley and Jopling.

possibility that some previously treated patients who had relapsed with secondary resistance to dapsone had concealed the earlier treatment was also examined by considering patient age and the frequency of dapsone resistance as functions of admission order, on the assumption that, in order to increase the rate at which patients were recruited, admission criteria may have been relaxed in the course of the trials. In fact, as shown in Table 4, no relationships could be demonstrated between admission order, on the one hand, and age or resistance to dapsone on the other. Moreover, as shown in Table 5, no significant differences could be discerned between those patients of either centre harbouring susceptible *M. leprae* and those harbouring strains resistant to dapsone, with respect to BI, logarithmic biopsy index (LIB),[8] $logarithm_{10}$ of the number of acid-fast bacilli per g tissue (LAFBPG),[9] and the proportions of patients classified clinically or histopathologically as LI.

Susceptibility not determinable

The susceptibility to dapsone could not be assessed of the strains of *M. leprae* obtained from the pretreatment biopsy-specimens of 35 of 99 (35%) Bamako patients and 49 of 116 (42%) Chingleput patients. None of these strains multiplied in dapsone-treated mice. The organisms recovered from 35 specimens failed to multiply to the level of $\geq 10^5$ in even a single untreated mouse. The *M. leprae* recovered from the remaining 49 specimens multiplied to a level $\geq 10^5$ in at

Table 6. Strains of undeterminable susceptibility as a function of admission order

Admission quartile	Proportion undeterminable	
	Bamako	Chingleput
1	0·40	0·31
2	0·36	0·59
3	0·24	0·41
4	0·42	0·30

A significant relationship was found between strains of undeterminable susceptibility and order of admission to Chingleput, whereas no significant relationship was found for Bamako.

least one untreated mouse, but multiplication was found to have occurred in so few control mice, that the failure of the organisms to multiply in dapsone-treated mice could not be taken as evidence of susceptibility to the drug. Thus, although the inocula prepared from these specimens contained viable *M. leprae*, the viable organisms must have represented so small a proportion of the total, that multiplication occurred in some mice but not in others.

Because the 84 patient strains of *M. leprae* of indeterminable susceptibility to dapsone represent two-fifths of all of the patients studied in the trials, an explanation for this phenomenon was sought. No significant difference could be demonstrated between the 84 patients harbouring these strains and those whose organisms were determined to be either susceptible or resistant to dapsone, according to centre ($P=0·30$) or regimen ($P=0·12$ for Bamako, $0·36$ for Chingleput). The failure of the organisms to multiply in large proportions of inoculated mice may be the result of some effective prior treatment; however, as shown in Table 3, no significant difference could be demonstrated between these and the remaining patients according to median age ($P=0·46$ for Bamako, $0·10$ for Chingleput). As shown by the data of Table 6, the proportion of Chingleput patients yielding *M. leprae* of undeterminable susceptibility was related to the order in which the patients were admitted to the trial ($P=0·05$); however, the proportion of such patients appears to have diminished in successive admission quartiles, beginning with the second quartile, a result inconsistent with the possibility that admission criteria had been relaxed in order to increase the rate at which patients were recruited. No relationship between undeterminable suscepti-

Table 7. Relationship of strains of undeterminable susceptibility to pretreatment patient characteristics

| Pretreatment characteristic | Susceptibility | | | |
| | Bamako | | Chingleput | |
	Determined	Undeterminable	Determined	Undeterminable
Median BI*	4·7	4·5	4·3	4·3
Median LIB	5·4	5·1	5·5	5·6
Median LAFBPG	8·4	8·2	8·4	8·4
Proportion with CLINCLAS LI	0·77	0·71	0·70	0·67
Proportion with HISTCLAS LI	0·83	0·74	0·94	0·98

The LIBs for Bamako patients harbouring *M. leprae*, the susceptibility of which could be determined, and those harbouring organisms of undeterminable susceptibility differed significantly. No significant difference was found for any other pretreatment characteristic in either centre.

* Abbreviations as for Table 5.

bility and admission order could be discerned for admissions to the trial in Bamako.

As shown by the data of Table 7, patients at both centres whose pretreatment biopsy-specimens yielded organisms of undeterminable susceptibility did not differ from the remaining patients, whose specimens yielded dapsone-susceptible or -resistant organisms, with respect to the BI, the LAFBPG, and the proportions of patients classified clinically or histopathologically as LI. On the other hand, Bamako patients harbouring *M. leprae* of determinable susceptibility, but not Chingleput patients, differed from those harbouring organisms of undeterminable susceptibility with respect to the LIB ($P = 0.05$).

Discussion

Analysis of the data on susceptibility to dapsone of 1.31 strains of *M. leprae* isolated from the pretreatment biopsy-specimens of the patients admitted to the THELEP controlled clinical trials of combined chemotherapy of lepromatous leprosy yielded estimates of the prevalence of primary dapsone-resistance of 42 per 100 patients at risk in Bamako and 33 per 100 patients in Chingleput. As was the case in the earlier analysis,[1] no evidence was found to support the possibility that the high estimates of prevalence resulted from the inadvertent admission into the trials of previously treated patients who had relapsed because of secondary dapsone-resistance.

Inspection of the available information with respect to the patients' pretreatment characteristics failed to reveal significant differences between those of patients harbouring dapsone-resistant and those harbouring dapsone-susceptible *M. leprae*, or between the pretreatment characteristics of patients whose pretreatment specimens yielded only small proportions of viable organisms and those whose specimens yielded large proportions of viable organisms. Thus, none of these characteristics, related to patient age, numbers of organisms or disease classification, could be employed to identify those patients who had been initially infected with strains of *M. leprae* resistant to dapsone.

The subject of primary resistance to dapsone has recently been carefully reviewed.[10] The prevalence of primary resistance to dapsone measured in Bamako and Chingleput is consistent with the results of surveys of primary resistance carried out among smaller numbers of patients in several leprosy-endemic areas, as is the finding that the majority of dapsone-resistant strains manifested a low degree of resistance.

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