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Primary Dapsone-Resistant Leprosy in Cebu, Philippines[1]

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During the period from 1 June 1975 to 30 June 1978, 42 patients with long-treated lepromatous leprosy whose disease had relapsed were suspected of secondary dapsone resistance. Lesions of 31 patients were biopsied, *Mycobacterium leprae* were recovered from the skin biopsy specimens and inoculated into mice, and the susceptibility to dapsone of the patient-strains of *M. leprae* was measured. All of the 31 patient-strains of *M. leprae* were resistant to dapsone; 13 multiplied in mice treated with 0.01 g% dapsone, 27 in mice administered 0.001 g% dapsone, and 31 in mice treated with 0.0001 g% dapsone, the minimal effective dosage of the drug.

These results established the presence of dapsone-resistant *M. leprae* in Cebu. However, because all of these patients lived in the "negative barrios"—settlements including about 700 former patients with lepromatous leprosy that surround the Eversley Childs Sanitorium (ECS), one could not extrapolate from these data to the prevalence of dapsone resistance in the area served by the ECS. A survey of the prevalence of dapsone resistance in the entire area was not feasible. Therefore, in an attempt to measure the proportion of the infectious patients in the area who served as sources of dapsone-resistant *M. leprae*, we have conducted a survey of the prevalence of primary dapsone resistance.

MATERIALS AND METHODS

The technique of Shepard ([8, 9]) was employed for recovery of *M. leprae* from biopsy specimens, their inoculation into the right hind foot pads of locally-bred CBA/J mice in a dose of 5000 acid-fast bacilli (AFB) per foot pad, harvest of organisms from the foot pads, and staining and counting of the AFB. Comparability of the results of mouse foot pad inoculation in the Leonard Wood Memorial-ECS laboratory with those of an established laboratory had been demonstrated earlier ([1]). The organisms from each specimen were used to inoculate 35 or 40 mice, which were then divided into four groups; one group of 10 mice served as untreated controls, whereas groups of from 8 to 10 mice were administered dapsone incorporated into the mouse diet in a concentration of 0.0001, 0.001 or 0.01 g per 100 g diet. Harvest of *M. leprae* from the pooled foot pad tissues of four control mice was performed when a monthly histopathologic section revealed significant multiplication of the AFB ([4, 8]). If the harvest from control mice revealed fewer than 5×10^5 AFB per foot pad, a second harvest was performed 60 days later; harvests of *M. leprae* from the pooled foot pads of treated mice were carried out as soon as multiplication of the organisms in control mice reached the level of 5×10^5 AFB per foot pad, or at the time of harvest from the last remianing control mice.

Dapsone-containing mouse diets were prepared by means of a liquid-solid twin-shell blender (Patterson-Kelly Co., East Stroudsburg, Pennsylvania); crystalline dapsone was diluted with lactose in the blender to achieve a concentration of 0.1 g dapsone per 100 g dapsone-lactose mixture; the mixture was then serially diluted in the blender with powdered mouse chow, in order to achieve the desired concentrations of dapsone in the mouse diets. Samples of dapsone-containing diets and of mouse plasma were frozen and shipped by air to the laboratory of Dr. John H. Peters, SRI International, Menlo Park, California, where dapsone assays were performed.

RESULTS

Fifty-eight consecutive patients with previously untreated leprosy classified BL, LI,

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TABLE 1. *Multiplication of* M. leprae *in dapsone-treated mice.*

No. patients	No. AFB per specimen	Time of harvest from control mice (days)	No. AFB recovered per foot pad ($\times 10^5$)			
			Concentration of dapsone in mouse diet (g%)			
			0	0.0001	0.001	0.01
53	3.88×10^7*	293*	58.8*	<0.1	<0.1	<0.1
1	1.17×10^8	256	75.2	7.5	<0.1	<0.1
1	1.24×10^7	261	19.8	4.8	1.2	<0.1

* Median values.

and LL were subjected to skin biopsy before initiation of chemotherapy, and the susceptibility to dapsone of the patient-strains of *M. leprae* was measured (see Table 1). The organisms recovered from the specimens obtained from three patients failed to multiply in untreated mice; those from a fourth specimen multiplied in mice administered 0.0001 g dapsone per 100 g diet; and those from a fifth specimen multiplied in mice administered 0.001 g dapsone per 100 g diet. *M. leprae* recovered from all of the remaining 53 specimens multiplied in untreated control mice, but in none of the mice administered dapsone. Thus, the prevalence of primary dapsone resistance may be calculated as 3.6 per 100 patients at risk; the 95% confidence limits around this estimate, calculated from the exact binomial probabilities ([2]), are 0 and 9.1 per 100.

Assays of the concentration of dapsone in the samples of mouse diet and plasma are presented in Tables 2 and 3. As shown in the upper panel of Table 2, the dapsone-containing diets as prepared were found to contain, on the average, 84% of the theoretical quantity of dapsone. As shown in the lower panel of Table 2, the diets recovered from the diet feeders after some days in the mouse cages were found to contain, on the average, only 54% of the theoretical concentration of dapsone. These data appear, on the whole, satisfactory. Because the dapsone-containing diets are prepared so that each diet contains 10 times the concentration of the next less-concentrated diet, and one-tenth the concentration of the next more-concentrated diet, diets that contain only 54% or 84% of the theoretical concentration of dapsone are not likely to produce confusing results. The one sample of diet found to contain only 3% of

TABLE 2. *Concentration of dapsone in mouse diets.*

	Theoretical concentration (g%)	Actual concentration[a] (g%)
A. Stock diets	0	0.000015
	0	0.00001
	0.0001	0.000064
	0.0001	0.000088
	0.001	0.00066
	0.001	0.00088
	0.01	0.0094
	0.01	0.0104
B. Recovered from cage	0.0001	0.000048
	0.0001	0.000052
	0.0001	0.000052
	0.001	0.00049
	0.001	0.00098
	0.01	0.0003
	0.01	0.0064
	0.01	0.0066

[a] Each value the mean of duplicate assays; variation in duplicate assays averaged 7.8%.

TABLE 3. *Concentrations of dapsone and monoacetyldapsone in mouse plasma samples*

Theoretical concentration of administered dapsone (g%)	Plasma concentration (ng/ml)[a]	
	Dapsone	Monoacetyl-dapsone
0	<1	<1
0	<1	<1
0.0001	16	<1
0.0001	21	<1
0.001	146	5
0.001	192	10
0.01	1580	53
0.01	2630	102

[a] Each value the mean of duplicate assays; variation in duplicate assays averaged 1.6%.

the theoretical concentration could indeed have led to confusion; *M. leprae* multiplying in mice administered this diet would be considered resistant to 0.01 g percent dapsone, when, in fact, what had actually been demonstrated was their resistance to <0.001 g percent dapsone.

The problem of disappearance of dapsone from the diet feeders during their residence in the mouse cages had been encountered earlier, and could not be explained ([5]). Because the concentration of dapsone in the plasma of mice sacrificed during the period of dapsone administration in the diet should reflect more truly the concentration of drug to which the *M. leprae* had been exposed, plasma samples were also submitted for analysis. As shown in Table 3, the concentrations of dapsone in the samples of mouse plasma show little variation among groups of mice administered dapsone in the same concentration in the diet. Moreover, there is good proportionality among the dapsone concentrations in the plasma of mice administered the different dietary dapsone concentrations, and the values are in the ranges expected ([6]). Finally, the concentrations of monoacetyldapsone are, on the average, 4% of the concentrations of dapsone in the same plasma samples, a value consistent with that in the literature ([3]).

DISCUSSION

A study of treated lepromatous patients living in settlements near the ECS yielded a prevalence rate of secondary dapsone resistance of approximately 6 per 100. Although this rate is not greatly different from those reported to have resulted from other prevalence surveys, it has very likely been biased; lepromatous patients whose disease has relapsed would appear more likely to remain in or return to such settlements than would those patients whose disease has responded favorably to treatment, and who remain free of relapse. A more true estimate of prevalence of secondary dapsone resistance would be obtained by examining all patients at risk of relapse with dapsone resistance in the area served by the ECS. However, this area includes many small islands and population centers at some distance from the ECS, access to many of which is very difficult. Thus, such a survey could be accomplished only with great effort. Assuming that the new patients presenting for treatment during a given time interval form a representative sample of all of the new patients in the area served by the ECS, a survey of the prevalence of primary dapsone-resistant leprosy among patients presenting for diagnosis and treatment at the ECS and the Cebu Skin Clinic appears to provide a much easier means of measuring the size of the pool of infectious dapsone-resistant patients in the area. In fact, it should be possible to maintain continuing surveillance for dapsone resistance among patients with newly diagnosed lepromatous leprosy wherever there is access to a mouse foot pad laboratory or an international airport.

Survey of primary dapsone resistance in Cebu yielded an estimated prevalence of 3.6 per 100, a figure much smaller than the estimate of about 50 per 100 reported from one treatment center in Ethiopia ([7]). However, any such comparison must be viewed with considerable caution.

Although the survey of primary resistance appears to offer the advantage of much greater ease, it suffers from at least one important disadvantage, in relation to a formal, area-wide survey of secondary resistance. The latter survey provides data that accurately represent the situation extant at the time of the survey; a survey of primary resistance, on the other hand, will provide data on the situation as it was at the time that the patients now presenting with newly diagnosed disease were first infected wtih *M. leprae*. Thus, continuing surveillance for primary resistance among newly presenting patients should provide a continuous record of the situation as it was 5 to 15 years earlier.

The description of the methods employed in this survey and of its results emphasize the importance of standardization of methods, if the survey data are to be compared with those of a survey in another area. In the description of methods, reference is made to an earlier comparison of the results of mouse foot pad inoculation with those of an established laboratory, and the results of dapsone assays of mouse diets and mouse plasma are presented. Because detection of drug resistance depends upon the

behavior of *M. leprae* in drug-treated mice, it is important, for the purpose of ensuring comparability of data, that the results of inoculation of mice with *M. leprae*, the procedures for preparing drugs-containing diets, and the metabolism of the drugs by the mice employed in the laboratory on which the survey is based be much the same as they would be, were the specimens shipped to some other laboratory.

SUMMARY

A survey of the prevalence of primary dapsone-resistant leprosy in Cebu, Philippines, has yielded an estimate of 3.6 per 100. Fifty-three of 55 patients proved to have *M. leprae* fully susceptible to dapsone. The organisms of two patients multiplied in mice administered the minimal effective dose of dapsone; and those of one of these patients also multiplied in mice administered dapsone in a 10-fold larger dose.

RESUMEN

Una investigación en Cebu, Filipinas, sobre la resistencia primaria a la dapsona en lepra, ha revelado que la prevalencia es del 3.6 por 100. Cincuenta y tres de 55 pacientes tuvieron *M. leprae* totalmente susceptible a la dapsona. Los organismos aislados de dos pacientes se multiplicaron en ratones a los que se administró la mínima dosis efectiva de dapsona; además, los microorganismos de uno de estos pacientes también se multiplicaron en ratones tratados con dapsona en una dosis 10 veces mayor que la mínima efectiva.

RÉSUMÉ

Une étude de la prévalence de malades présentant une résistance primaire à la dapsone à Cebu aux Philippines, a livré des chiffres s'élevant à 3,6 pour cent. Parmi 55 malades on a pu prouver que *M. leprae* était entièrement susceptible à la dapsone. Les microorganismes trouvés chez deux malades se sont multipliés chez des souris auxquelle on avait administré des doses minimales efficaces de dapsone. Chez un autre de ces malades, les bacilles se sont également multipliés chez des souris auxquelles on avait administré de la dapsone à des doses 10 fois supérieures.

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