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Primary Dapsone-resistant Leprosy in San Francisco[1]

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Although clinicians suspected sulfone resistance in the 1950s, dapsone-resistant leprosy was first proved in Malaysia in 1964 ([10]). This report described three Malaysian patients who were being treated with full-dosage dapsone and, despite that, relapsed with new active lesions. Organisms from these lesions were found to be fully dapsone resistant since they grew in mice fed 0.1% w/w dapsone in their diet. Since that time, secondary dapsone resistance has been reported from every locale where it has been sought. The risk of lepromatous patients developing sulfone resistance has been estimated to be 2.5% in Malaysia ([7]), 7% in Costa Rica ([9]), and 19% in Ethiopia ([5]).

In 1977 Pearson ([6]) described in Ethiopia the first cases of primary dapsone-resistant leprosy, i.e., resistance in individuals who had not previously received sulfone therapy. In this first report 5 of 8 patients studied were found resistant. Three of these isolates were resistant to only 0.0001% dapsone, and two were also resistant to 0.001% dapsone. These strains were not tested to 0.01% dapsone. Since that time these studies have been expanded—in the Addis Ababa area, 5 out of 9 patients tested were resistant and, in other parts of Ethiopia, 11 out of 15 were resistant (overall prevalence = 67%) ([3]). At the National Hansen's Disease Center, Carville, Louisiana, U.S.A., Jacobson and Hastings ([3]) reported that 9 of 33 (37%) previously untreated lepromatous leprosy patients' bacilli grew in mice fed 0.0001% dapsone. Six of these strains were inhibited by 0.001% dapsone, two of the strains grew at 0.001% dapsone but not at 0.01% dapsone, and one grew at 0.01% dapsone. However, Guinto ([2]) found that only 2 of 55 (3.6%) previously untreated patients' bacilli were partially dapsone resistant. One strain was resistant to only 0.0001% dietary dapsone, and the other to 0.001% dapsone as well. In a recent report from Bamako and Chingleput ([12]) 35 of 96 (36%) previously untreated lepromatous patients harbored *Mycobacterium leprae* that were to some extent dapsone resistant. Only one of these strains, however, was fully resistant to dapsone, i.e., to 0.01% in mouse chow, which is equivalent to the usual human dose of 100 mg/day.

We had been looking for primary dapsone resistance in all new multibacillary patients (BL, LLp, LLs) from 1978 until the close of the United States Public Health Service Hospital in San Francisco in late 1981. This report details those studies.

MATERIALS AND METHODS

The dapsone sensitivity of *M. leprae* from a total of 54 newly diagnosed lepromatous leprosy patients without a history of previous therapy was studied. Of these 54 patients, 27 were from Mexico, 10 from the Philippines, 6 from Vietnam, 3 from Laos, 3 from Samoa, and 1 each from China, Cambodia, Burma, Indonesia, and Fiji. Nine patients studied were female, and 45 were male. The patients' ages ranged from 17 to 82 years, with a mean of 41 years. For this study skin punch biopsies of 4 mm diameter or greater were taken, minced, and treated by the standard procedures of Shepard ([4]) for enumeration of *M. leprae*. Four groups of mice (15 control and 10 in each of three treatment groups) were inoculated with 5×10^3 *M. leprae* in both hind feet, and from the time of inoculation continually fed a diet containing no dapsone (control), 0.0001% dapsone, 0.001% dapsone, and 0.01% dapsone. Diets were prepared by dissolving dapsone (4,4'-diaminodiphenyl sulfone) in 95% ethanol and evenly distributing it in mouse chow by use of a Patterson-Kelley twin-shell diet mixing machine. After six months to one year, when control counts of *M. leprae* from two animals (four feet) reached 10^6/foot pad, treated mice were harvested and *M. leprae* enumerated. Initially, bacterial counts were made from

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TABLE 1. *Growth of* M. leprae *in mice from* 5×10^3 M. leprae *inocula.*

Subject	*M. leprae* per foot pad[a]			
	No diet	0.0001% DDS	0.001% DDS	0.01% DDS
TA	9.88×10^5	2.75×10^5	$<4.53 \times 10^3$	$<8.26 \times 10^3$
EW	1.99×10^6	$<6.09 \times 10^3$	$<6.09 \times 10^3$	$<5.45 \times 10^3$
JV	6.01×10^5	$<4.80 \times 10^3$	$<8.02 \times 10^3$	$<8.23 \times 10^3$
JV	1.05×10^6	$<4.94 \times 10^3$	$<7.80 \times 10^3$	$<8.23 \times 10^3$
RP	8.89×10^5	7.79×10^3	$<7.80 \times 10^3$	$<8.47 \times 10^3$
JT	5.41×10^5	1.70×10^4	$<4.53 \times 10^3$	4.53×10^3
TE	2.97×10^5	$<4.53 \times 10^3$	$<4.79 \times 10^3$	$<4.66 \times 10^3$
MM	1.26×10^6	$<4.91 \times 10^3$	$<5.05 \times 10^3$	$<5.03 \times 10^3$
MF	7.62×10^5	$<5.19 \times 10^3$	$<5.03 \times 10^3$	$<4.77 \times 10^3$
BD	2.11×10^6	$<4.79 \times 10^3$	$<4.79 \times 10^3$	$<4.53 \times 10^3$
AM	2.59×10^6	4.66×10^3	$<8.23 \times 10^3$	$<7.80 \times 10^3$
MC	1.58×10^6	$<9.52 \times 10^3$	$<8.23 \times 10^3$	$<8.23 \times 10^3$
MH	1.95×10^6	$<7.41 \times 10^3$	$<7.84 \times 10^3$	$<7.62 \times 10^3$
MA	2.14×10^6	$<4.79 \times 10^3$	$<4.77 \times 10^3$	5.03×10^3
BC	6.51×10^5	$<7.84 \times 10^3$	$<5.10 \times 10^3$	4.53×10^3
GS	1.44×10^6	$<4.94 \times 10^3$	$<4.53 \times 10^3$	$<5.48 \times 10^3$
TS	1.75×10^6	4.53×10^3	$<4.77 \times 10^3$	$<4.79 \times 10^3$
JN	9.74×10^5	5.2×10^3	$<5.0 \times 10^3$	$<4.8 \times 10^3$
PO	4.71×10^5	$<4.94 \times 10^3$	$<7.62 \times 10^3$	$<7.62 \times 10^3$
PS	1.51×10^6	9.3×10^3	$<7.02 \times 10^3$	$<7.41 \times 10^3$
RB	2.58×10^6	$<4.77 \times 10^3$	$<8.24 \times 10^3$	$<4.94 \times 10^3$
BM	4.26×10^5	1.48×10^4	$<7.62 \times 10^3$	7.41×10^3
NV	3.29×10^4	$<7.39 \times 10^3$	$<5.93 \times 10^3$	$<1.16 \times 10^4$
TT	1.27×10^6	$<9.81 \times 10^3$	$<1.15 \times 10^4$	$<5.18 \times 10^3$
KS	1.50×10^5	5.33×10^3	$<5.48 \times 10^3$	$<6.09 \times 10^3$

[a] Counts generally from 6–12 months after inoculation.

groups of mice receiving all three dietary concentrations, but after 25 studies were complete and no discrepancies found (counts were never greater in mice treated with higher dapsone concentration), if counts were $<1.5 \times 10^4$/foot pad in mice treated with 0.0001% dapsone, *M. leprae* from groups of mice treated with higher dietary dapsone concentrations were not counted, and the patients were considered fully dapsone sensitive.

The reliability of the dietary dapsone concentrations was assessed by bleeding groups of mice receiving five different batches of each of the three dietary concentrations of dapsone and analyzing the resultant plasma concentrations. These were graciously done by Dr. John H. Peters of Stanford Research Institute, Palo Alto, California, U.S.A., by a modification of his high-pressure liquid chematographic technique ([8]).

RESULTS

Of the first 25 strains studied, in which harvests were performed on controls and on each of the three groups receiving a different dietary dapsone concentration, one strain was resistant to 0.0001% while sensitive to 0.001% (Table 1). All of the 29 others (Table 2) grew on the control diet to at least 5×10^5 but were inhibited by 0.0001% dapsone—counts $<2 \times 10^4$ acid-fast bacilli (AFB)/foot pad. Thus, the prevalence of primary dapsone resistance from 1977 to 1981 among patients seen in San Francisco was 2%.

Mouse plasma concentrations resulting from the prepared diets are presented in Table 3. These generally were in accord with the levels found in previous work on plasma dapsone concentrations in mice receiving the same dietary dapsone (DDS) concentrations ([2]). (A mouse dietary DDS concentration of 0.0001% results in mouse plasma levels that average 10 ng/ml, while correspondingly 0.001% dietary DDS yields 100 ng/ml and 0.01% yields 1000 ng/ml [[2]].) Only the 42 ng/ml and 20 ng/ml levels which resulted from 0.001% deviated by more than a factor of 2 from the expected level, but since none of the strains was resistant to 0.001% or higher dapsone concentration,

TABLE 2. *Growth of* M. leprae *in mice from 5 × 10^3* M. leprae *inocula.*

Subject	*M. leprae* per foot pad[a]	
	No diet	0.0001% DDS
RV	3.82×10^6	$<8.23 \times 10^3$
RC	7.32×10^5	$<1.44 \times 10^4$
LF	9.71×10^5	$<7.80 \times 10^3$
RR	1.68×10^6	$<5.03 \times 10^3$
JG	2.66×10^6	$<8.71 \times 10^3$
OG	1.26×10^6	$<4.41 \times 10^3$
LB	9.52×10^5	$<4.80 \times 10^3$
TH	1.50×10^6	$<4.53 \times 10^3$
TP	1.85×10^6	$<4.59 \times 10^3$
NP	1.12×10^6	$<4.80 \times 10^3$
MG	9.18×10^5	$<7.59 \times 10^3$
LT	4.16×10^5	$<4.29 \times 10^3$
TN	1.10×10^6	$<7.84 \times 10^3$
JC	3.67×10^5	$<4.66 \times 10^3$
DA	6.29×10^5	$<4.29 \times 10^3$
BV	4.34×10^5	$<4.41 \times 10^3$
RP	3.32×10^6	$<4.40 \times 10^3$
SC	1.04×10^6	$<8.84 \times 10^3$
SS	2.15×10^6	$<4.53 \times 10^3$
TT	9.40×10^5	$<4.41 \times 10^3$
ID	4.40×10^5	$<7.40 \times 10^3$
TL	8.73×10^5	$<4.53 \times 10^3$
MM	9.58×10^5	$<8.00 \times 10^3$
TL	5.13×10^4	$<8.53 \times 10^3$
LT	3.40×10^5	$<4.53 \times 10^3$
DD	2.63×10^6	$<7.79 \times 10^3$
CP	1.85×10^6	$<7.2 \times 10^3$
TA	2.58×10^6	$<4.79 \times 10^3$
FH	2.05×10^6	$<7.41 \times 10^3$

[a] Counts generally from 6–12 months after inoculation.

this did not seem to have prejudiced the results.

DISCUSSION

Because the incubation period of leprosy averages perhaps ten years, current prevalence figures for primary resistance reflect the incidence of transmission of secondary resistance ten years ago, and current individuals incubating the disease might reasonably be expected to harbor a greater prevalence of resistant bacilli. The low prevalence of primary dapsone resistance found in this study (2%) is similar to that published by Guinto from the Philippines (3.6%) and significantly different from those published from other centers. Since many of our patients have the same ethnic or national origins as those at Carville, our results were most unexpected. Different prevalences of primary dapsone resistance may be a true reflection of a necessarily increasing incidence resulting from standard dapsone monotherapy of the past four decades with different regional rates of development. However, although wild strains in the past have always been inhibited by 0.0001% dapsone, most are not inhibited by 0.00001% ([1, 4]), and some are not inhibited by 0.00003% ([4]).

TABLE 3. *Levels of dapsone (DDS) in mouse plasma (ng/ml).*[a]

Group	% DDS in diet		
	10^{-4}	10^{-3}	10^{-2}
1	19	73	1621
2	12	66	818
3	13	176	1050
4	5.3	42	882
5	6.5	20	1099

[a] Mean value of duplicate samples.

In this last cited study ([4]), unexplained reduced dapsone concentrations were found in animal feeders, and mouse plasma dapsone concentrations in animals fed on a diet presumably containing the same dapsone concentration were found to vary by 100%. In tropical locales, where mouse diets are not refrigerated, deterioration of dapsone in mouse chow might be a particular problem. There are thus a number of sources of variable bioavailability of dapsone to the mouse foot pad that might critically affect the interpretation of whether a particular strain was fully sensitive or partially resistant. Only the studies from San Francisco and the Philippines substantiated the accuracy of the mouse dietary dapsone concentrations.

Furthermore, noncompliant patients with lepromatous leprosy may deny vehemently any previous therapy, and the individuals thought to be suffering from primary dapsone resistance may indeed have secondary resistance. It must be underscored that the clinical significance of partial dapsone resistance, except possibly as a harbinger of complete dapsone resistance, is not clear. Mouse diets of 0.0001% result in plasma levels similar to that produced by 1 mg/day in man. On the other hand, the usual adult dose of 100 mg/day produces plasma levels equivalent to 0.01% dietary dapsone in mice. So far only two cases of primary dapsone resistance (one from Louisiana and one from Bamako) have been found in the mouse foot pad to be resistant to this level of dapsone.

Furthermore, all nine of the primary dapsone-resistant patients at Carville responded clinically and bacteriologically to full-dosage dapsone therapy.

This is not to say that dapsone monotherapy is to be recommended. Monotherapy with a bactericidal agent for tuberculosis, where specific cellular immunity is generally intact and the total number of bacilli and presumably drug-resistant population is much less, is unthinkable because of the resultant high incidence of resistant relapse in individuals so treated. It is remarkable that four decades of monotherapy with a bacteriostatic agent, dapsone, has thus far not resulted in universal sulfone resistance.

SUMMARY

The dapsone sensitivity of strains of *Mycobacterium leprae* from 54 multibacilliferous untreated leprosy patients presenting to the United States Public Health Service Hospital in San Francisco, California, U.S.A., from 1978 to 1981 was studied by mouse foot pad inoculation. *M. leprae* from 53 patients were found fully sensitive to dapsone. *M. leprae* from one patient were resistant to only the lowest dietary level of dapsone, 0.0001%, since growth of the bacilli was inhibited by higher and clinically easily achievable levels. Mouse plasma dapsone levels confirmed the reliability of the drug-containing diets.

RESUMEN

Usando la "prueba del cojinete plantar del ratón" se estudió la sensibilidad a la dapsona de las cepas de *Mycobacterium leprae* provenientes de 54 pacientes multibacilíferos con lepra no tratada atendidos en el Hospital del Servicio de Salud Pública de los E.U. en San Francisco, CA, U.S.A., de 1978 a 1981. Los *M. leprae* de 53 pacientes fueron completamente susceptibles a la dapsona. El *M. leprae* de un paciente fue resistente sólo al nivel dietario más bajo de la droga (0.0001%) ya que el crecimiento del bacilo fue inhibido por los niveles más altos facilmente alcanzados en la clínica. Los niveles de dapsona en el plasma de los ratones fueron proporcionales al contenido de droga en las dietas.

RÉSUMÉ

On a étudié par l'épreuve d'inoculation dans le coussinet plantaire de la souris, la sensibilité à la dapsone de souches de *Mycobacterium leprae* obtenues chez 54 malades atteints de lèpre multibacillaire et non traités, ayant fréquenté l'hôpital de l'U.S. Public Health Service, à San Francisco, Californie, entre 1978 et 1981. On a observé que les bacilles de 53 malades présentaient une sensibilité totale à la dapsone. Chez un malade, *M. leprae* était résistant aux doses les plus basses de dapsone incorporée à la nourriture, soit 0,0001%, car la croissance du bacille était inhibée par des taux plus élevés, semblables à ceux qui peuvent être facilement obtenus au cours du traitement clinique. Les taux de dapsone dans le plasma des souris confirmait la fiabilité des rations contenant le produit.

REFERENCES

1. Ellard, G. A. and Gammon, P. T. Studies on the determination of the minimal inhibitory concentration of 4,4'-diaminodiphenyl-sulfone (dapsone, DDS) against *Mycobacterium leprae.* Lepr. Rev. **42** (1971) 101–117.
2. Guinto, R. S., Cellona, R. V., Fajardo, T. T. and de la Cruz, E. C. Primary dapsone-resistant leprosy in Cebu, Philippines. Int. J. Lepr. **49** (1981) 427–430.
3. Jacobson, R. R. and Hastings, R. C. Primary sulfone resistant leprosy. Int. J. Lepr. **46** (1978) 116.
4. Levy, L. and Peters, J. H. Susceptibility of *Mycobacterium leprae* to dapsone as a determinant of patient response to acedapsone. Antimicrob. Agents Chemother. **9** (1976) 102–112.
5. Pearson, J. M. H., Haile, G. S., Barnetson, R. St.C. and Rees, R. J. W. Dapsone resistant leprosy in Ethiopia. Lepr. Rev. **50** (1979) 183–199.
6. Pearson, J. M. H., Haile, G. S. and Rees, R. J. W. Primary dapsone-resistant leprosy. Lepr. Rev. **48** (1977) 129–132.
7. Pearson, J. M. H., Rees, R. J. W. and Waters, M. F. R. Sulphone resistance in leprosy. A review of one hundred proven clinical cases. Lancet **2** (1975) 69–72.
8. Peters, J. H., Murray, J. F., Jr., Gordon, G. R. and Gelber, R. H. Dapsone in saliva and plasma of man. Pharmacology **22** (1981) 162–171.
9. Peters, J. H., Shepard, C. C., Gordon, G. R., Rojas, A. V. and Elizondo, D. S. The incidence of DDS resistance in lepromatous patients in Costa Rica: Their metabolic disposition of DDS. Int. J. Lepr. **44** (1976) 143–151.
10. Pettit, J. H. S. and Rees, R. J. W. Sulphone resistance in leprosy. An experimental and clinical study. Lancet **2** (1964) 673–674.
11. Shepard, C. C. The experimental disease that follows the injection of human leprosy bacilli into foot-pads of mice. J. Exp. Med. **112** (1966) 445–454.
12. Subcommittee on Clinical Trials of the Chemotherapy of Leprosy (THELEP) Scientific Working Group. Primary resistance to dapsone among untreated lepromatous patients in Bamako and Chingleput. Lepr. Rev. **54** (1983) 177–183.