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Growth of Sulfone-Resistant *M. leprae* in the Foot Pads of Mice Fed Dapsone (39976)

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Since 1970, 123 viable isolates of *M. leprae* from skin biopsies of leprosy patients have been tested at Carville for sulfone resistance in the mouse foot pad. This large number of isolates provided an opportunity to analyze in some detail the growth patterns of *M. leprae* in mice fed dapsone. The present report concerns this analysis and its implications relative to the optimal clinical dosages of dapsone.

Materials and methods. Locally bred BALB/c mice were inoculated with 5×10^3 *M. leprae* in the hind foot pad and continuously fed dapsone until the time of sacrifice and enumeration of acid-fast bacilli in the inoculated foot pad (1). Routinely, concentrations of 4,4′-diaminodiphenyl sulfone (DDS, dapsone) (Sigma Chemical Company, St. Louis, Missouri) in powdered diets (Purina Laboratory Chow, Ralston Purina Company, St. Louis, Missouri) are 0.01, 0.001, and 0.0001% (w/w). For the purposes of analysis, these 123 strains of *M. leprae* were arbitrarily divided into four groups on the basis of whether or not growth occurred in the animals fed the three concentrations of dapsone: (i) If all three concentrations of dapsone prevented multiplication of *M. leprae*, the strain was designated "sensitive."

(ii) If growth occurred only in animals fed 0.0001% dapsone and not at 0.01 or 0.001% dapsone, the strain was designated as having "partial resistance."

(iii) If growth occurred in mice fed 0.0001 and 0.001% dapsone but not at 0.01% dapsone, the strain was designated as having "intermediate resistance."

(iv) If multiplication occurred at all three concentrations of dapsone, the strain was designated as having "full resistance."

Results. An analysis of the results of harvests from the 75 sulfone-resistant strains is of considerable interest (Table I). *M. leprae* with partial resistance multiplied in mice fed 0.0001% (w/w) dapsone, but harvests were significantly lower than in controls. Organisms with intermediate resistance were partially inhibited by 0.001% dapsone in the diet, but not by the lower concentration (0.0001%). Even in strains of *M. leprae* which show full resistance to dapsone, there is partial inhibition by 0.01% dietary dapsone, but not by lower concentrations.

This pattern of growth indicates that with each degree of sulfone resistance, there is a threshold above which dapsone still can inhibit multiplication of the resistant strain in the mouse foot pad. The increases in these thresholds of minimum inhibitory concentrations of dapsone in different sulfone-resistant strains of *M. leprae* cover a very wide range. In short, resistance to sulfones in *M. leprae* is not on an all-or-none qualitative basis, but is a graded or quantitative phenomenon.

Discussion. The sulfones (like the sulfonamides in other organisms) almost certainly act as competitive inhibitors of the paraaminobenzoate (PABA)-utilizing enzyme involved in the de novo folate synthesis pathway of *M. leprae* (2), competing with the natural substrate for this enzyme, PABA. By definition, in competitive enzyme inhibition, the degree of inhibition is proportional to the concentration of the inhibitor (sulfone) relative to the concentration of the natural substrate (PABA). The mechanism of sulfone resistance in *M. leprae* may well be on the same basis as the mechanism of sulfonamide resistance in some organisms, namely, the resistant organism may be resistant because it synthesizes larger amounts of PABA, thus shifting the ratio of inhibitor (sulfone) to substrate (PABA) back toward favoring the synthesis of folate.

One can speculate that *M. leprae* probably develops resistance to sulfones by mutation and that the first-step mutants, as in the case of many organisms developing re-

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TABLE I. NUMBERS OF ACID-FAST BACILLI HARVESTED PER FOOT PAD ($\bar{X}$ ± SEM) × 10^5.

	N	Days from inoculation to harvest ($\bar{X}$ ± SEM)	Controls	Dapsone (w/w) in diets		
				0.01%	0.001%	0.0001%
Full resistance	20	252.6 ± 24.5	6.30 ± 0.77	2.30 ± 0.49*	4.41 ± 0.91	4.51 ± 0.53
Intermediate resistance	22	244.6 ± 9.8	6.54 ± 1.25	No growth[a]	2.46 ± 0.50**	5.36 ± 0.84
Partial resistance	33	227.7 ± 6.7	4.60 ± 0.64	No growth[a]	No growth[a]	1.32 ± 0.17*
Sensitive	48	230.6 ± 6.4	4.30 ± 0.62	No growth[a]	No growth[a]	No growth[a]

[a] $<6.45 \times 10^3$ acid-fast bacilli per foot pad.
* $P < 0.001$, Student's t test, compared to controls.
** $P < 0.01$, Student's t test, compared to controls.

sistance to penicillin, are of low and uniform resistance. Second-step mutants, arising from first-step mutants, might be expected to have uniformly slightly higher resistance, and so on, until finally the majority of the bacilli is not inhibited by concentrations of sulfones attainable by conventional clinical dosages. If, as is the case with most organisms, the mutants arise independently of exposure to the drug, then a given dose of sulfones, by inhibiting susceptible bacilli, is selecting those mutants of *M. leprae* which are resistant to that given concentration of sulfones. If the development of sulfone resistance in *M. leprae* involves a penicillin type of development, then clearly this process can be minimized by a concentration of sulfones which will inhibit both the original population of bacilli and the first-step mutants with a uniform low level of resistance. Even if the process is already underway, the further selection for higher degrees of sulfone resistance can still be minimized theoretically by maintaining continuous levels of sulfones high enough to inhibit both the original population of *M. leprae* and all the first-, second-, third-, etc., step mutants having minimal to even intermediate levels of resistance.

Thus from the points of view of (a) efficacy in patients already harboring mutants of *M. leprae* with partial or intermediate levels of resistance to sulfones, (b) achieving maximal competition with PABA in both native and mutant strains of *M. leprae*, and (c) preventing the step-wise development of mutants with higher levels of sulfone resistance, it seems clear that maximal subtoxic dosages of dapsone are indicated in all leprosy patients with multibacillary disease (lepromatous or borderline type disease with numerous bacilli) treated with the drug.

Summary. One hundred twenty-three viable isolates of *M. leprae* from skin biopsies of leprosy patients have been tested for sulfone resistance in the mouse foot pad since 1970. In 33 strains, growth occurred in animals fed 0.0001% (w/w) dapsone, but not at higher concentrations; in 22, growth occurred at 0.001 and 0.0001% (w/w) dapsone, but not at the higher concentration; and in 20 isolates, growth occurred at all three concentrations, 0.01, 0.001, and 0.0001% (w/w) dapsone. In each group, in animals fed the highest concentration of dapsone at which growth occurred, the number of bacilli harvested was significantly less than that in controls. Thus 75 strains of *M. leprae* had some degree of sulfone resistance, and with each degree of sulfone resistance, there was a threshold above which dapsone could still inhibit multiplication of the resistant strain in the mouse foot pad. This finding, in light of the probable mechanism of action of sulfones and mechanism of bacterial resistance to sulfones, strongly implies that maximal subtoxic dosages of dapsone are indicated in all leprosy patients with multibacillary disease treated with this drug.

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