

INFLUENCE OF THE RAPID ACETYLATOR PHENOTYPE ON THE EMERGENCE OF DDS RESISTANT *Mycobacterium leprae*

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ABSTRACT

Twenty one lepromatous cases with suspected diaminodiphenyl sulfone (DDS) resistance had their isoniazid acetylator phenotype determined. The resistance of *Mycobacterium leprae* to this sulfone was investigated by means of foot pad tests in BALB/c mice. Results indicate that the emergence of complete resistance to DDS in *M. leprae* is more probable in rapid than in slow acetylators. It appears that rapid acetylators require higher doses of DDS than slow acetylators since the bacillary concentration in the lesions of the patients with DDS sensitive *M. leprae* was more than 17 times higher among rapid than among slow acetylators.

INTRODUCTION

Diaminodiphenyl sulfone (DDS) is monoacetylated in man by the same enzyme system that acetylates isoniazid (INH) and sulphametazine (SMZ) (Gelber *et al.*, 1971). As the genetic polymorphism for acetylation of INH and SMZ also applies to DDS, individuals classified as INH or SMZ rapid or slow acetylators will exhibit a correspondent DDS phenotype. However, unlike acetyloniazid or acetylsulphametazine, the monoacetylated DDS compound (MADDS) is concurrently deacetylated, yielding a MADDS/DDS plasma ratio which is constant for each individual, slow acetylators exhibiting significantly lower MADDS/DDS ratios than rapid acetylators (Gelber *et al.*, 1971).

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Since the level of bacteriologically active INH decreases less rapidly among slow than among rapid acetylators (Bönicke and Reif, 1953; Hughes *et al.*, 1954), the efficacy of INH in the therapy of tuberculosis is influenced by the acetylator phenotype (Devadatta *et al.*, 1960; Tuberculosis Chemotherapy Center, Madras, 1970, 1973; Eidus *et al.*, 1974). Concerning DDS, Ellard *et al.* (1972) found no evidence that the acetylator phenotype is of prognostic value in the treatment of leprosy. In contrast, Peters *et al.* (1972) suggested that the rapid acetylator phenotype would favor the emergence of DDS resistant *Mycobacterium leprae* in leprosy patients, but this suggestion was not confirmed by Irudaya *et al.* (1988).

This controversy, as well as the worldwide steady increase of *M. leprae* resistance to DDS (Baohong, 1985), stimulated us to reopen the question of the possible influence of the rapid acetylator phenotype on the emergence of DDS resistant leprosy bacilli.

MATERIAL AND METHODS

The acetylator phenotype of 21 lepromatous patients (Madrid classification) was determined by investigating their capacity to acetylate INH by means of a simple urine test (Eidus *et al.*, 1973; Hodgkin *et al.*, 1974). All of them were attended for treatment at the *Instituto Lauro de Souza Lima* (Bauru, SP, Brazil) and exhibited a logarithmic index of bacilli (Ridley and Hilsen, 1967) of at least +3, in spite of being under treatment for many years. The mean period of DDS therapy of these patients was 25 years with a standard deviation of 10.96 years, the values ranging from 5 to 45 years (coefficient of variation of 44%). Although these patients showed some clinical improvement at the beginning of treatment, they did not show any clinical response to DDS at the time of this study, being suspected, on clinical grounds, to harbor DDS resistant *M. leprae*.

For the investigation of DDS resistance skin smears were obtained from the active lesions. From each patient the lesion that exhibited the highest number of acid fast bacilli with morphological integrity was chosen for biopsy with a 5 or 6 mm punch. After weighing the biopsies and recording the number of acid fast bacilli per mg, they were processed for foot pad tests in BALB/c mice, as recommended by Shepard (1960) and Pettit and Rees (1964).

RESULTS AND DISCUSSION

Among the 21 patients 52% were rapid acetylators, the remaining being slow acetylators. Since these proportions do not differ significantly from those found among Caucasoids and Negroids in Brazil (Beiguelman *et al.*, 1977) there is no indication of an

association between this genetic polymorphism and lepromatous leprosy. Also no significant difference could be assigned to the mean number of years of DDS therapy of rapid acetylators ($\bar{x} = 24$; $s = 11.14$) as compared to slow acetylators ($\bar{x} = 26$; $s = 11.25$), since $t = 0.409$; 19 D.F.; $0.60 < P < 0.70$.

When the patients are classified according to both the acetylator phenotype and the presence or not of DDS resistant leprosy bacilli in the foot pad test (Table I), one is inclined to conclude that these two traits are independent, since the proportions of patients with *M. leprae* resistant or sensitive to DDS are practically the same among rapid and slow acetylators ($\chi^2 = 0.043$; 1 D.F.; $0.80 < P < 0.90$). However, when the resistance to DDS is subclassified according to its intensity, rapid and slow acetylators cannot be considered as identical with respect to DDS resistance, since, as seen in Table I, all rapid acetylators with resistant bacilli showed complete resistance, while the slow acetylators with DDS resistant bacilli included two patients with complete, one with intermediate and two with partial DDS resistance. Therefore, in spite of the small sample size, these data suggest that the emergence of complete resistance to DDS in leprosy bacilli would be more probable among rapid than among slow acetylators.

Table I - Distribution of patients according to both the acetylator phenotype and *M. leprae* resistance to DDS.

DDS resistance	Acetylator phenotype	
	Rapid	Slow
Complete	6	2
Intermediate	-	1
Partial	-	2
Absent	5	5

The difference between rapid and slow acetylators was even more striking among the patients with DDS sensitive bacilli since, according to Table II, the average number of acid fast bacilli per mg found in the biopsies of rapid acetylators was more than 17 times higher than that found in the biopsies of slow acetylators. By applying the Mann-Whitney non-parametric U-test (Siegel, 1956) for comparing the distribution of the bacillary concentrations in the biopsies of rapid and slow acetylators a significant difference between these two groups was found ($U = 1$; $P = 0.016$). Besides supporting the hypothesis that a direct relationship exists between the rapid acetylator phenotype

and the emergence of DDS resistant *M. leprae* among lepromatous patients (Peters et al., 1972), our data suggest that rapid acetylators require higher doses of DDS than slow acetylators.

Table II - Average number ($\times 10^6$) of acid fast bacilli per mg found in the biopsies of the patients classified according to both the acetylator phenotype and bacterial resistance to DDS.

DDS resistance	Acetylator phenotype	
	Rapid	Slow
Yes	591.37 <i>n</i> - 6	613.12 <i>n</i> - 5
No	786.34 <i>n</i> - 5	45.28 <i>n</i> - 5

The different levels of *M. leprae* resistance to DDS (partial, intermediate, and complete) seem to be determined by the amount of dihydropteroate synthetase produced by the bacilli, which links to DDS, thus preventing folate production. As the gradual increase of resistance to DDS seems to be achieved by step-wise development of mutants (Hastings, 1977), one may suppose that leprosy bacilli would find more opportunities for mutations in rapid than in slow acetylators, if the period of active DDS depends upon the rate of DDS acetylation in man.

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RESUMO

Determinou-se o fenótipo acetilador da insoniazida de 21 hansenianos suspeitos de terem desenvolvido resistência à DDS. A resistência do *Mycobacterium leprae* desses pacientes foi investigada por intermédio de testes no coxim plantar de camundongos da estirpe BALB/c. Nossos resultados indicam que o

surgimento de resistência à DDS no *M. leprae* dos acetiladores rápidos é mais provável do que nos bacilos dos acetiladores lentos. Parece plausível sugerir que os acetiladores rápidos recebam maiores doses de DDS do que os lentos, visto que a concentração bacilar nas lesões de pacientes com *M. leprae* sensível à DDS nos acetiladores rápidos foi mais de 17 vezes maior do que nos lentos.

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