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Studies on Sulfone Resistance in Leprosy

3. A Case of "Partial" Resistance[1]

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Recent papers from Malaysia ([2, 4]) have reported the cases of four patients with leprosy resistant to sulfone therapy. This resistance was detected during a study of nine patients who had active lepromatous leprosy despite at least 13 years of treatment with sulfone. All these patients were admitted to the Leprosy Research Unit at Sungei Buloh for a six-months rigorously controlled trial period on sulfone (4,4'-diaminodiphenyl sulfone, DDS) in a dosage of 300 mgm. twice weekly by injection. At the completion of this period the response of the patients was assessed clinically, bacteriologically and histologically. Four patients failed to respond satisfactorily, and sensitivity tests in the mouse foot pad (using bacilli obtained from skin biopsy specimens taken at the start of the trial) showed that the strains of *Mycobacterium leprae* from these four patients were insensitive to DDS. The remaining five patients improved during the trial period, and as their bacilli were shown to be sensitive to DDS we concluded that they were not infected with sulfone-resistant organisms, and therefore their treatment with DDS was continued. The subsequent progress of one (case 1 in Refs. 2,4) was unsatisfactory, with fluctuations in the bacteriologic and clinical findings. We report here, in greater detail, the clinical history of the patient and the subsequent studies that showed the presence of "partial" sulfone resistance.

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CASE HISTORY AND CLINICAL FINDINGS

Patient No. 5075, Indian male. This patient was admitted to Sungei Buloh Settlement in 1937 at the age of 14. He was treated until 1948 with hydnocarpus oil and from 1948 until 1961 with injectable DDS, 400 mgm. twice weekly. Although the records surviving from this period are scanty, they show that skin smears in 1953 and again in 1961 were strongly positive. On two occasions in 1959 he was admitted to hospital with a febrile illness characterized by backache, diarrhea and vomiting, which on the second occasion appeared to respond to oral tetracycline but not to injections of stibophen (antimony pyrocatechin sodium sulfonate, Fuadin). Investigations then included urinalysis, "x-rays of the kidneys," and a barium meal, all of which appear to have been negative. The attacks were not then recognized as leprosy "reactions."

Until 1961 he responded reasonably satisfactorily, but early in 1961 multiple small nodules appeared on most of his body, and a biopsy showed active lepromatous leprosy. Because of this his treatment was changed in May 1961 to thiambutosine (DPT, Ciba 1906) in a dosage of 1 gm. twice daily by mouth, and this was continued for 16 months[3] until August 1962. In June 1961 he was admitted to hospital with ulceration of the arms and legs, which was diagnosed as "lepra reaction." He was treated with steroids for about three weeks. In January 1962 an additional biopsy showed a "typical foamy leproma," with large numbers of acid-fast bacilli with a morphologic index (MI: percentage of solid-staining bacilli) of 0.

[3] In Ref. 2 this period was erroneously stated to be 10 months, through an error in the manuscript.

In September 1962 his treatment was changed back to DDS by injection, 400 mgm. twice weekly. The reason for this is uncertain, as no further biopsies were made and no smear reports are available from the period of thiambutosine therapy. But in March 1963, when he was referred to the Leprosy Research Unit as a possible case of sulfone resistance, he appeared to have extensive untreated nodular lepromatous leprosy. Skin smears from six sites showed an average bacterial index (BI) of 4.8 and MI of 37, and a biopsy specimen (part of which was used to obtain bacilli for sensitivity tests in the mouse foot pad) showed "definitely active leproma." He was treated with DDS, 300 mgm. twice weekly by injection, and at the end of six months showed some clinical improvement; his biopsy was reported as indicating quiescent disease and the MI had fallen to 12.

Treatment with DDS was continued for six months more, but the MI rose to 19 at nine months, and in March 1964, after a full year's detailed observation, it was still 15. Clinically there was now little evidence of improvement, for, although the nodules were smaller, diffuse infiltration of the skin had increased. The unsatisfactory response to 12 months' treatment strongly suggested that the patient was resistant to DDS in spite of the failure to detect resistance in the mouse foot pad test with bacilli obtained at the beginning of the trial. In May 1964 further foot pad tests were set up from a repeat biopsy, DDS was discontinued, and the patient was started on sulformethoxine (4-sulfanilamide-5, 6-dimethoxypyrimidine, Fanasil) a long-acting sulfonamide. Initially he was given 500 mgm. sulformethoxine daily, and after two weeks 500 mgm. twice weekly.

Ten days after starting sulformethoxine he developed what appeared to be an upper respiratory tract infection, with fever, a cough, and sore throat, but two days later definite ENL appeared. The fever failed to respond to antibiotics, but settled within 24 hours on ACTH, 25 units twice daily. Treatment with sulformethoxine was continued and ACTH was given as necessary to control the ENL. After about one month glycosuria appeared, but it was controlled fairly well by oral hypoglycemic drugs, except when his ENL was severe and he required large doses of ACTH.

From May to December 1964 the ENL fluctuated, being moderate most of the time, with intermittent severe bouts of fever and polyarthritis mainly affecting the hands and feet. During this period the MI, which had fallen to 4 in the first three months, failed to maintain this progress and fluctuated wildly (Table 1). Because of the severity of the reaction sulformethoxine was discontinued in November. His condition deteriorated and in December the patient became seriously ill with a very severe bout of ENL, which lasted for about two weeks but then subsided rapidly. By early January 1965 ACTH was discontinued and the glucosuria ceased about two weeks later.

The patient had received no antileprosy therapy since November 1964 and the MI rose rapidly from 7 in October 1964 to 10 in December 1964 and 28 in February 1965. It was considered essential that he be given an effective antileprosy drug, and in February 1965 treatment was recommenced using the riminophenazine derivative B.663, 100 mgm. thrice daily for six days a week. His response to this drug has been satisfactory and confirms our previous experience in patients with DDS resistance ([3]).

DDS SENSITIVITY TESTS

These tests were undertaken at the National Institute for Medical Research, London. Suspensions of bacilli for infecting the mouse foot pad were obtained from biopsy specimens reaching London from Malaysia by air on wet ice. The specimens were processed and inoculated into mice within 48 hours from the time they were obtained from the patient. Groups of six to 12 mice were either used as untreated controls or were treated with various concentrations of DDS or sulformethoxine administered in the diet. Details of the technic used have been described in previous papers ([2, 4, 6, 7]).

The initial DDS sensitivity tests were undertaken in March 1963 at the time of the patient's entry into the trial period on

TABLE 1. *Treatment and progress of the patient during the study period.*

Date	Treatment	Morphologic index (MI)[a]		ENL[b]	Biopsies
		Average	Range		
1963					
Jan.	Inj. DDS 400 mgm.				
Feb.	twice weekly	37	12–16		Definitely active
Mar.	"				leproma
Apr.	Inj. DDS 300 mgm.				
May	twice weekly				
June	"				
July	"	10	5–18		
Aug.	"	9	1–25		
Sept.	"				
Oct.	"	12	9–18		Quiescent (one lesion
Nov.	"				more than the other)
Dec.	"				
1964					
Jan.	"	19	1–34		
Feb.	"				
Mar.	"				
Apr.	"	15	1–25		Definitely active
May	Sulformethoxine 500 mgm. daily			+++	
June	Sulformethoxine			++	
July	500 mgm. twice	4	0–7	+++	
Aug.	weekly	19	7–24	+++	
Sept.	"	2	1–4	++	
Oct.	"	7	1–30	+++	
Nov.	"			++++	
Dec.	nil	10	1–20	++++	
1965					
Jan.	"			+	
Feb.	B. 663, 100 mgm.	28	5–37		Definitely active
Mar.	t.d.s				
Apr.	"	9	3–20		
May	"	5	1–11		No evidence of
June	"	1	0–2		active spread
July	"				
Aug.	"	1	0–2		Quiescent

[a] The bacteriologic index (BI) showed no significant change throughout this period.
[b] Grading after Waters[11].

DDS; the mice received 0.1 per cent DDS in their diet. At this level of DDS the bacilli failed to grow, indicating that they were DDS-sensitive. Because subsequent clinical and bacteriologic studies showed no improvement, two more biopsies were made, in May 1964 and February 1965, for DDS and sulformethoxine sensitivity tests. The tests in May 1964 were carried out in mice receiving 0.1 and 0.025 per cent DDS in their diets, and those in February 1965 in mice receiving 0.025 and 0.006 per cent DDS and 0.04 per cent sulformethoxine in their diet. The results showed in each test that the bacilli were resistant to DDS at 0.025 per cent or less in the diet, and also resistant to sulformethoxine at 0.04 per cent in the diet. The results are shown in full in Table 2.

DISCUSSION

Clinical findings. This patient was included with eight others in a study of DDS resistance in leprosy ([2, 4]) because he presented clinically and bacteriologically active disease despite prolonged treatment with DDS. Nevertheless there is good evidence that his disease was not always DDS-resistant. First, his clinical history was that of response followed by relapse, and second, he probably suffered from ENL on several occasions while under treatment with DDS. ("Reaction" was definitely diagnosed only in 1961, but the symptoms of his illnesses in 1959 were strikingly similar to those that accompanied definite ENL when he was under close observation in the Leprosy Research Unit.) Both these facts suggest that DDS did some good at first, and that his relapse was due to the emergence of drug resistance.

There was slight clinical improvement by the end of the original six months test period, but in retrospect the failure of the MI to fall below 5 was the first definite evidence of the emergence of DDS resistance. Of all the criteria used to assess response to chemotherapy the MI is the most sensitive, and a fall to between 0 and 5 in the first six months can be expected in

TABLE 2. *DDS and sulformethoxine sensitivity as shown by mouse foot pad infection with* M. leprae[a] *obtained from the patient on three occasions during treatment.*

Date of test	Proportion of foot pads showing multiplication of *M. leprae*[b]					Result of sensitivity test
	Untreated mice	Treated mice (% drug in diet)[c]				
		DDS			Sulformethoxine	
		0.1	0.025	0.006	0.04	
March 1963	7/9	0/6	—	—	—	Sensitive to DDS (0.1%)
May 1964	10/10	2/8	6/6	—	—	Sensitive to DDS (0.1%) Sensitive to DDS (0.025%)
February 1965	12/12	—	6/10	7/12	10/12	Resistant to DDS (0.025 and 0.006%) and to sulformethoxine (0.04)%

[a] Foot pad inoculated with 10^4 *M. leprae*.
[b] Yield of 2×10^5 *M. leprae* or more per foot pad in animals killed between 6 and 12 months after inoculation.
[c] Per cent of drug in diet; treatment started day of inoculation and continued throughout.

patients responding satisfactorily to treatment with DDS ([12]). However, it was felt that this small difference did not warrant an immediate change of treatment, and he was given DDS for another six months. Moreover this decision appeared to be justified because the first sensitivity tests showed bacillary inhibition by 0.1 per cent DDS in the diet.

But after a year on DDS, during which there was no clinical improvement and the MI had reached 15, it became certain that at least some of the patient's bacilli were DDS-resistant. This was supported by further biopsies, which once again showed active lepromatous leprosy. Further drug sensitivity tests were therefore set up and a six months period of treatment with sulformethoxine was started. The initial fall in MI and the development of severe ENL ([5]) indicate that this drug was active at the high dose used at first.

However, after the first three months on sulformethoxine, the MI began to behave in a most unusual way. Despite the presence of severe ENL the average MI at six sites rose steadily, but at individual sites it varied widely. Successive MI's from the left ear lobe, for instance, were 0, 21, 1, 30, 7, and 35, and when the MI was low at one site it would be high at another. This was the first time we had seen such lability in the MI, and the combination of severe ENL, high and very variable MI's at different sites, and a rising average MI, is unique in our experience. Our interpretation is that in the initial period of treatment with sulformethoxine, when a high dose was used, there was heavy bacterial killing, which precipitated ENL, and that later, at a lower dose, there remained foci of DDS-resistant bacilli capable of multiplying in the presence of sulformethoxine. These foci might be microscopic, and it would be a matter of chance if one was or was not hit in any particular skin smear. The presence of sulformethoxine resistance was confirmed by the final sensitivity tests. We now realize that changing the treatment from DDS to sulformethoxine was illogical, as these drugs have a similar mode of action, and cross resistance could have been anticipated.

The initial response to sulformethoxine can be accounted for by the higher concentration of drug that was obtained: sulformethoxine at 200 mgm. daily gives 5-10 times higher blood levels than DDS at 300 mgm. twice weekly ([1]). The reduction of the dose of sulformethoxine from 500 mgm. daily to 500 mgm. twice weekly allowed remaining viable bacilli to multiply and the MI rose again, slowly at first, but rapidly as soon as treatment was stopped altogether. At this stage it was clearly essential to change treatment to an entirely different drug, and the one chosen was the riminophenazine derivative B.663, to which other patients with more clearly defined DDS resistance had already been found to respond ([3]). This patient too has done well on it.

Experimental findings. In 1963, when this patient and others were first studied for drug resistance, the animal tests were carried out at only one level of DDS in the diet, viz., 0.1 per cent. This level of DDS, which is the maximum tolerated by the mouse, was chosen because strains of *M. leprae* from previously untreated patients were sensitive to it. Moreover, the bacilli from the patients in the first study who failed to show clear clinical and bacteriologic improvement when treated with 600 mgm. DDS/week were resistant to 0.1 per cent DDS fed in the diet of mice ([2]). However, later a possible fallacy in the use of only this one level of DDS in the diet for detecting resistance was revealed when the experimental studies on the present patient showed apparent drug sensitivity whereas his clinical condition was deteriorating. To attempt to resolve this anomaly DDS sensitivity tests were carried out on bacilli from two further biopsies, using lower concentrations of DDS (0.025 and 0.006 as well as 0.1 per cent). The results showed that the patient's organisms were still sensitive to 0.1 per cent but resistant to both the lower concentrations, which from later studies are known to inhibit strains of *M. leprae* from previously untreated patients ([8, 9, 10]).

These findings confirm that this patient showed true DDS resistance. But if they are to be correlated with each other they must be expressed in terms of serum DDS

levels achieved in the patient during treatment and in the mouse during the drug tests. The figures for man are well known, and fortunately the relevant data in mice have recently become available ([8, 9, 10]); they are summarized in Table 3. It will be seen that the bacilli multiplied in mice in the presence of about 3-4 μgm./ml. of DDS, and in this patient in the presence of 1-5 μgm./ml., a remarkably close correlation. Multiplication in mice was inhibited by 10-15 μm./ml., but such levels could not be achieved in man, except as occasional peaks, without serious toxic effects. In other words, this patient's organisms multiplied in the presence of the drug at a concentration above that achieved by the dose administered, which was close to maximal. This can be considered a practical definition of drug resistance.

TABLE 3. *Concentrations of DDS in the sera of mice fed different levels of drug in the diet and in man on standard treatment.*[a]

Dose	Concentration of DDS in serum (μgm./ml.)
Mouse (% in diet)	
0.1	10–15
0.025	2.6–4.0
0.006	0.4–0.7
0.0001[b]	0.01–0.03
Man	
100 mgm./day	1–5

[a] Data extracted from Refs. 9 and 10.
[b] Dose of DDS inhibiting the growth of *M. leprae* from untreated patients.

General studies on the emergence of drug resistance show that different strains of bacilli do not show a uniform degree of resistance. Our findings confirm this in the field of DDS-resistant leprosy. The bacilli from our first three patients showed a very high degree of DDS resistance ([2]); they multiplied in mice fed with doses of DDS that were the maximum tolerated and that gave serum DDS levels far higher than could be obtained constantly in man. But in this patient the degree of resistance was less, and we have chosen the term "partial" resistance to describe his clinical and bacteriologic picture.

We treated this patient with B.663, and it is now clear that patients with this degree of "partial" resistance will always require treatment with a nonsulfone-like drug, for if DDS were used it would have to be given in doses that are known to be toxic for man. It is not clear from this case (because bacilli from the third biopsy were not tested against 0.1 per cent DDS in the diet) whether his bacilli were becoming increasingly DDS-resistant during the period of observation, but this might happen.

This patient is the first in whom "partial" DDS resistance has been demonstrated, but it is likely that more cases exist, possibly with lesser degrees of "partial" resistance. In order to recognize them it is essential that, in the future, drug sensitivity tests using the mouse foot pad infection include a range of DDS concentrations in the diet that more than covers the serum DDS concentrations attainable in man. Our present data indicate that at least three concentrations (0.025, 0.01 and 0.001 per cent DDS in the diet) should be used. Bacilli multiplying at one or both of the high concentrations must be treated as resistant, whereas if multiplication occurs only at the lowest concentration the bacilli can be considered DDS-sensitive, and the patient should respond to full doses of DDS. Nevertheless, these "sensitive" bacilli would be considerably less sensitive than strains from previously untreated patients, which are inhibited in the mouse foot pad by DDS concentrations in the diet of 0.0001 and sometimes even 0.00001 per cent ([9, 10]). Therefore patients from whom such bacilli are grown should be considered as potentially resistant, for even a slight increase in their resistance would make them nonresponsive to DDS. It is clear that if such patients are treated with DDS, special precautions will be needed to ensure that they receive regular treatment and prolonged follow-up, and it would probably be wise to treat all patients with DDS-resistant bacilli, whatever the degree of resistance, with a nonsulfone-like drug.

SUMMARY

Proof that a patient is suffering from sulfone-resistant leprosy depends on demonstrating that his bacilli can multiply in the mouse foot pad even when the mice are fed sulfone in the diet. Hitherto the maximal dose of DDS tolerated by the mouse has been used in such tests. This paper concerns a patient whose bacilli multiplied in mice fed lower doses of DDS, but were inhibited when the maximal dose was used. His clinical features are distinctive and probably characteristic of this type of "partial" resistance. It is likely that more cases of this type will be found. Recommendations are made concerning the investigation of possible DDS-resistant leprosy patients and their treatment.

RESUMEN

La pruebe que un enfermo sufre de lepra resistente a la sulfona descansa en demostrar que su bacilo puede multiplicarse en el colchón plantar del ratón, aún cuando los ratones son alimentados con sulfonas en su dieta. Por esto la dosis máxima de DDS tolerada por el ratón ha sido empleada en dichas pruebas. Este trabajo se relaciona con un paciente cuyo bacilo se multiplicó en ratones alimentados con dosis bajas de DDS, pero que fueron inhibidos cuando se usaron dosis máximas. Sus rasgos clínicos son distintos y probablemente característicos de este tipo de resistencia "parcial." Es posible que se encuentren más casos de este tipo. Se hacen recomendaciones relativas a la investigación de posibles enfermos de lepra resistentes a DDS y a su tratamiento.

RÉSUMÉ

La preuve qu'un malade souffre d'une lèpre résistante aux sulfones est basée sur la démonstration que les bacilles de ce malade peuvent se multiplier dans la sole plantaire de la souris, même lorsque les animaux reçoivent des sulfones dans leur alimentation. En conséquence, la dose maximale de DDS tolérée par la souris a été utilisée pour de telles épreuves. Cet article se rapporte à un malade dont les bacilles se multipliaient chez des souris nourries avec des doses de sulfones inférieures, mais qui étaient inhibées lorsque la dose maximale était utilisée. Les particularités cliniques de ce malade sont distinctes et probablement caractéristiques de ce type de résistance "partielle." Il est vraisemblable que davantage de cas de ce type seront décelés. Des recommandations sont faites en ce qui concerne l'investigation des malades atteints d'une lépre qui pourrait être résistante à la DDS, et de leur traitement.

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