INTERNATIONAL JOURNAL OF LEPROSY Volume 51, Number 3
Printed in the U.S.A.

The Significance of Dapsone (DDS)-resistant *Mycobacterium leprae* in Untreated Patients[1]

Joel G. Almeida, Chinoy J. G. Chacko, and Melville Christian[2]

The finding that untreated leprosy patients can harbor *Mycobacterium leprae* which withstand the action of dapsone (DDS) ([7, 10, 14, 16–18]) has caused alarm ([5]). We report a study done in a stable rural population of South India, to find how many previously untreated patients harbored such resistant *M. leprae*.

The area studied, Gudiyatham Taluk, is the leprosy control zone of the Schieffelin Leprosy Research and Training Centre, Karigiri, and has a population of 480,000 (1981 census). DDS monotherapy, given as domiciliary treatment, has been extensively used in the area since 1963. Intensive case detection by regular surveys and health education, careful maintenance of individual patient records, and a continuing search for treated patients harboring resistant *M. leprae* are features of the control program. A total of 7157 patients were on the treatment register of the institution on 31 December 1980. This background made it convenient not only to study the occurrence of resistant *M. leprae* in untreated patients, but also to relate the findings to the picture of leprosy in the community as a whole.

PATIENTS AND METHODS

Between 1 May 1980 and 10 August 1981, all residents of Gudiyatham Taluk newly discovered to have borderline lepromatous (BL) or lepromatous (LL) leprosy had skin biopsies taken. Those who could reasonably be suspected of having previously taken DDS and those with a Bacterial Index (BI) <2+ were excluded from the mouse foot pad test. The mouse foot pad test, to detect *M. leprae* resistant to DDS, is usually not successful on specimens with a BI <2+. Altogether, 18 subjects qualified for the test: 5 were clinically diagnosed to have LL leprosy; 13, to have BL leprosy.

Ethical considerations did not allow DDS to be withheld from patients once they were diagnosed to have leprosy. Biopsies could sometimes only be taken after DDS therapy had been started. In no case did the delay exceed a month.

Biopsies were processed for the mouse test by methods described previously ([1]). Growth of *M. leprae* in mice treated with DDS was taken to indicate the presence of *M. leprae* resistant to DDS at the concentration used. If no growth occurred in even the untreated control mice, the concerned test was regarded as a failure, allowing no decision about the occurrence of resistant *M. leprae*.

A list was made of all treated patients in the area who were already shown to harbor DDS-resistant *M. leprae* by the mouse test. The places of residence and of work during the preceding ten years were noted for each of these patients, as well as for each of the 18 subjects. Treated patients shown to harbor resistant *M. leprae* were considered as "contacts" who had possibly passed resistant *M. leprae* to a subject, if they had shared a workplace, or even a village, with the subject, during the preceding ten years. In addition, a separate list was made for each of the 18 subjects, of all leprosy patients who had at some time in the preceding ten years, actually lived in the same house as the subject.

RESULTS

The Table indicates that 12 mouse tests yielded a result; 5 tests detected *M. leprae*

[1] Received for publication on 8 March 1983; accepted for publication in revised form on 25 April 1983.

[2] J. G. Almeida, M.B.B.S., Research Medical Officer, Schieffelin Leprosy Research and Training Centre (SLR&TC), Karigiri 632106, N.A. Dt., Tamil Nadu, India. C. J. G. Chacko, M.D., Ph.D., Head, Division of Laboratories, SLR&TC, and Professor of Pathology, CMC Hospital, Vellore, India. M. Christian, M.B.B.S., D.T.M. & H., D.Epid., Head, Department of Epidemiology and Leprosy Control, Schieffelin Leprosy Research and Training Centre, Karigiri 632106, N.A. Dt., Tamil Nadu, India. Reprint requests to: Dr. C. J. G. Chacko, SLR&TC, Karigiri, Vellore N.A. Dt., Tamil Nadu 632106, South India.

resistant to DDS, while 7 tests did not. Among the 5 tests that detected resistant bacilli, 3 detected bacilli resistant to the highest concentration of DDS used (0.01% w/w DDS in mouse diet); while in 2 tests the bacilli were found resistant to only a lower concentration of DDS (0.001% w/w DDS in mouse diet). It is not possible to decide whether any resistant bacilli were present in the six patients who failed to yield growth of *M. leprae.*

Among the 12 subjects successfully tested, 4 had shared the house of at least 1 treated leprosy patient, and none of the 4 yielded resistant *M. leprae*; whereas of the 8 who had not shared the house of a leprosy patient, 5 (62.5%) yielded resistant *M. leprae.* The difference however, is not statistically significant ($p > 0.10$, Fisher's exact test).

A comparison was made among the 12 subjects successfully tested, between those who did have identifiable contact with a treated patient shown to harbor resistant *M. leprae,* and those who did not. Of 9 subjects with contact, only 3 yielded resistant *M. leprae*; whereas of 3 subjects without contact, 2 yielded resistant *M. leprae.* However, the difference is not statistically significant ($p > 0.10$, Fisher's exact test).

Among the 9 subjects who did have identifiable contact with a treated patient shown to harbor resistant *M. leprae,* the 3 who yielded resistant *M. leprae* had, on an average, only 3.0 such "contact" patients each; whereas the 6 who yielded no resistant *M. leprae* had, on an average, 6.0 such "contact" patients each. The difference, however, is not statistically significant (*t* test, $p > 0.1$).

THE TABLE. *Results of the mouse test for the detection of* M. leprae *resistant to DDS.*

No. untreated patients tested	Growth of *M. leprae* in mice fed (g% DDS in diet)				Detected *M. leprae*
	Nil	0.0001	0.001	0.01	
3	+	+	+	+	Resistant to 0.01
2	+	+	+	−	Resistant to 0.001
7	+	−	−	−	Sensitive to 0.0001
6	−	−	−	−	(Failure of test)

DISCUSSION

Leprosy patients treated with DDS may possibly transmit *M. leprae* which are resistant to DDS. If this happened commonly, it might be cause for alarm.

The data presented do not support the hypothesis that treated patients are likely to be the only source, or even the major source, of resistant *M. leprae* in untreated patients. Known contact with a treated patient in the ten years preceding the diagnosis of leprosy did not significantly increase the risk of DDS-resistant *M. leprae* occurring in an untreated, newly diagnosed patient. These data share the limitations inherent in any study of the spread of leprosy, and involve small numbers of patients; however, no other data of this nature appear to be available.

The findings are easily explained by analogy with tuberculosis. Tubercle bacilli resistant to isoniazid (INH) were isolated in 1948 ([9]), several years before INH was first used for the treatment of tuberculosis. This is consistent with the bulk of evidence accumulated in bacteriology, which indicates that bacterial populations include mutants that develop before exposure to a selective agent ([3, 4, 6, 11, 12]). In the case of drug resistance, this means that resistant bacteria exist in bacterial populations before any contact with the drug. The prevalent concept, implying that all "strains" of *M. leprae* which have not come into contact with DDS form homogeneous groups in which there is no real variation in sensitivity to DDS, seems to ignore the bulk of evidence available ([2–4, 6, 8, 9, 11–13, 15–18]).

There are at least two possible explanations for resistant bacilli in an untreated patient: 1) the bacterial population in the patient may contain naturally resistant mutants or 2) the resistant bacilli may have been acquired from a treated patient. The evidence in tuberculosis indicates that the latter "rarely happens" ([13, 15]). Further, the finding in untreated patients of tubercle bacilli resistant to INH (sometimes called "primary INH resistance") ceased to cause alarm when it was pointed out that the "primary resistance" showed no increase over several years ([2, 8, 13]).

The limited evidence available in leprosy does not support the view that treated patients are the major source of resistant *M.*

leprae in untreated patients. It would seem important to know whether the proportion of untreated patients who harbor DDS-resistant *M. leprae* has been increasing. *M. leprae* from untreated patients were reported to grow in mice treated with DDS as early as 1965 ([16, 17, 18]). In recent years there have been more reports of DDS-resistant *M. leprae* in untreated patients ([7, 10, 14]). It is not clear whether these reflect an actual increase in the occurrence of resistant *M. leprae,* or merely an improved surveillance.

The monitoring of drug resistance among untreated patients, especially before a drug is pressed into general use in an area, is of great aid to subsequent evaluation of drug resistance in that area. It is hopefully not too late to do this for rifampin and clofazimine, which are being used increasingly in leprosy.

SUMMARY

In a stable rural population of South India, 18 consecutive untreated persons newly discovered to have leprosy with a Bacterial Index (BI) ≥2+ were tested for *Mycobacterium leprae* resistant to dapsone (DDS) by the mouse foot pad test. Of 12 successful tests, five detected resistant *M. leprae.* Known contact with a treated patient in the ten years preceding the diagnosis of leprosy was not found to increase the risk of DDS-resistant *M. leprae* occurring in an untreated, newly diagnosed patient.

This data is consistent with the bulk of evidence in the field of bacteriology, which makes it seem unlikely that treated patients are the only source, or even the major source, of resistant *M. leprae* in untreated patients. Bacterial mutants resistant to a drug have been shown to precede initial use of the drug. Tests for drug-resistant bacteria in untreated patients before a drug is widely used in a community are likely to be important for subsequent evaluation of resistance to the drug in that community.

RESUMEN

Usando el método del cojinete plantar en el ratón, se investigó la presencia de *M. leprae* resistentes a la dapsona (DDS) en 18 personas con lepra no tratada de reciente diagnóstico y con un Indice Bacterial de 2+ o mayor. El estudio se realizó en una población rural estable del sur de la India. En 5 de 12 pruebas exitosas se encontraron *M. leprae* resistentes al DDS. Las personas en contacto por 10 años o más con un paciente tratado no aumentaron el riesgo de que los casos recientes de lepra, no tratados, desarrollaran *M. leprae* resistentes a la dapsona.

Este dato indica que es poco probable que los pacientes tratados sean la única o la principal causa de la aparición de *M. leprae* resistentes al DDS en los pacientes de reciente diagnóstico aún no tratados. Se ha demostrado que las mutantes resistentes a la droga aparecen antes de que ésta haya sido utilizada. Las pruebas para establecer la resistencia a una droga de las bacterias de pacientes no tratados, antes de administrar esa droga en una comunidad, podrían ser importantes para la evaluación subsecuente de la resistencia a la droga en esa comunidad.

RÉSUMÉ

On a procédé à une étude de la résistance de *Mycobacterium leprae* à la dapsone (DDS), au moyen de l'épreuve sur coussinet plantaire de la souris, chez 18 malades consécutifs non traités, et récemment découverts porteurs d'une lèpre avec Index Bacterien (BI) ≥2+. Cette étude a été menée dans une population rurale stable de l'Inde méridionale. Parmi 12 tests pratiqués avec succès, cinq ont permis de mettre en évidence des *M. leprae* résistants. Il est apparu qu'un contact connu avec un malade traité au cours des dix années ayant précédé le diagnostic de la lèpre, n'entraînait pas une augmentation du risque de résistance à la DDS de *M. leprae,* chez des malades non traités récemment diagnostiqués.

Ces données sont en accord avec tout ce que nous savons dans le domaine de la bactériologie, dont il ressort qu'il est peu probable que les malades traités soient la seule source, ou même la source principale, de *M. leprae* résistant chez les malades non traités. On a montré que des mutants bactériens résistant à un médicament pouvaient apparaître avant l'utilisation initiale du médicament. Avant qu'un médicament soit largement utilisé dans une communauté, il paraît important de procéder à des épreuves portant sur la résistance médicamenteuse des bactéries chez des malades non traités, et ceci afin de permettre une évaluation ultérieure de la résistance au médicament dans cette communauté.

Acknowledgments. This study received support from the Chemotherapy of Leprosy (THELEP) component of the UNDP/WORLD BANK/WHO Special Programme for Research and Training in Tropical Diseases. The views expressed do not necessarily reflect official policy of the World Health Organization.

We thank Mr. S. Joseph and Mr. G. Sarangapani for expert technical assistance; Mr. J. Samuel and all the staff of the branch of leprosy control; and Mr. Raja Rao and Mr. N. Christopher for secretarial assistance.

REFERENCES

1. Balraj, V., Jesudasan, K., Chacko, C. J. G., Christian, M., Taylor, P. M., Fritschi, E. P. and Job, C. K. Prevalence of secondary dapsone resistance in Gudiyatham Taluk, the leprosy control area of the Schieffelin Leprosy Research and Training Centre, Karigiri. 1. Preliminary report. Int. J. Lepr. **48** (1980) 397–401.
2. Canetti, G., Fox, W., Khomenko, A., Mahler, H. T., Menon, N. K., Mitchison, D. A., Rist, N. and Smelev, N. A. Advances in techniques of testing mycobacterial drug sensitivity and the use of sensitivity tests in tuberculosis control programmes. Bull. WHO **41** (1969) 21–43.
3. Cavalli, L. L. Genetic analysis of drug-resistance. Bull. WHO **6** (1952) 185–206.
4. Cavalli-Sforza, L. L. and Lederberg, J. Isolation of preadaptive mutants in bacteria by sib selection. Genetics **41** (1956) 367–381.
5. Chemotherapy of leprosy for control programmes. WHO Tech. Rep. Ser. 675, 1982.
6. Demerec, M. Origin of bacterial resistance to antibiotics. J. Bacteriol. **56** (1948) 63–74.
7. Girdhar, B. K., Sreevastsa and Desikan, K. V. Primary sulphone resistance, a preliminary report. Lepr. India **50** (1978) 352–355.
8. Hobby, G. L., Johnson, P. M., Lenert, T. F., Crawford-Gagliardi, L., Greetham, L. and Ivaska, T. A continuing study of primary drug resistance in tuberculosis in a veteran population within the United States. Am. Rev. Respir. Dis. **89** (1964) 337–349.
9. Hobby, G. L. and Lenert, T. F. Resistance to isonicotinic acid hydrazide. Am. Rev. Tuberc. **65** (1952) 771–774.
10. Jacobson, R. R. The present status of sulfone therapy of leprosy. Proc. 1st Int. Workshop on Chemotherapy of Leprosy in Asia, Manila, Philippines. Tokyo: Sasakawa Memorial Health Foundation, 1977, pp. 25–36.
11. Lederberg, J. and Lederberg, E. M. Prelica plating and indirect selection of bacterial mutants. J. Bacteriol. **63** (1952) 399–406.
12. Luria, S. E. and Delbruck, M. Mutations of bacteria from virus sensitivity to virus resistance. Genetics **28** (1943) 491–511.
13. Miller, A. B., Tall, R., Fox, W., Lefford, M. J. and Mitchison, D. A. Primary drug resistance in pulmonary tuberculosis in Great Britain: Second national survey, 1963. Tubercle **47** (1966) 92–107.
14. 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) 183–200.
15. Prevalence of drug resistance in previously untreated patients. United States Public Health Service Cooperative Investigation. Am. Rev. Respir. Dis. **89** (1964) 327–336.
16. Rees, R. J. W. A preliminary review of the experimental evaluation of drugs for the treatment of leprosy. Trans. R. Soc. Trop. Med. Hyg. **61** (1967) 581–595.
17. Rees, R. J. W. Recent bacteriologic, immunologic and pathologic studies on experimental human leprosy in the mouse foot pad. Int. J. Lepr. **33** (1965) 646–657.
18. Shepard, C. C., McRae, D. and Habas, J. A. Sensitivity of *M. leprae* to low levels of 4,4′-diaminodiphenylsulfone. Proc. Soc. Exp. Biol. Med. **122** (1966) 893–896.