MYCOBACTERIUM LEPRAE PERSISTERS AFTER TREATMENT WITH DAPSONE AND RIFAMPICIN

S. R. Pattyn[a, b], P. Dockx[b], M. T. Rollier[c], R. Rollier[c] and E. J. Saerens[b]

[a] Institute for Tropical Medicine, Antwerp, Belgium
[b] University of Antwerp, Belgium
[c] Service National de Lèpre, Casablanca, Maroc

Waters et al (1974) showed that it is possible to recover persistent M. leprae in small numbers from lepromatous patients treated for long periods with dapsone.

Rifampicin is a rapidly bactericidal drug for M. leprae (Rees et al, 1970; Shepard et al, 1972, 1974), but it is not known if some leprosy bacilli escape its bactericidal action, as is the case with dapsone.

We therefore decided to investigate this aspect of leprosy treatment on some patients who participated in the controlled clinical trial on initial three months continuous and intermittent rifampicin therapy, previously reported (Pattyn et al, 1975).

PATIENTS AND METHODOLOGY

The present study concerned 13, 8 and 10 patients each, belonging to the groups who had taken either rifampicin 450 mg daily (RMP 450), rifampicin 900 mg once weekly (RMP 900) and dapsone 100 mg daily (DDS), during the first three months of treatment and are now taking dapsone for various periods, since the end of the first trimester.

The patients were hospitalized in Casablanca for a few days and 3 biopsies taken from each of them: from a skin site, from the underlying muscle (deltoid or muscle of the forearm or quadriceps, and using a separate set of instruments), from a superficial branch of the radial nerve at the wrist.

Biopsies on ice were air-mailed to Antwerp. Suspensions were prepared and diluted, if necessary, so as to contain 10^3 - 5 x 10^3 per 0.03 ml and inoculated into mouse footpads, 9 mice per suspension.

After one year, 630 mice were examined, 180 harvests in pools of 3 footpads and 90 footpads individually in histologic sections. All examinations were done in blind and the correlation with the treatment was not revealed until all the results were available. Passage into new mice were done with most harvests; these results will be available in the future.

RESULTS

The biopsy fragments were not weighed but the skin and muscle fragments were estimated at 100 mg and the nerve fragments at 10-20 mg.

All skin biopsies were very rich in acid-fast bacteria (AFB). Table 1 shows the mean of the number of bacilli per ml of the suspensions. Because of two patients whose biopsies were extremely rich in AFB (more than 10^7), the mean of the number of leprosy bacilli in the skin of DDS patients was higher than in the RMP patients.

Muscle fragments belonged to one of three categories: those where a smear of the suspension was negative for AFB; those where only a few AFB were found and those allowing counts to be made. Some suspensions from muscle fragments were negative, although serial histologic sections in some cases revealed sometimes a few AFB. These results were presented elsewhere (Pattyn et al, 1975).

None of the nerve biopsies were negative, a majority were rich in AFB (Table 1). The RMP 450 group had a significantly higher number of bacilli than the other groups.

Table 2 shows the results of the harvests and histologic examinations of the inoculated footpads. Multiplying bacilli were demonstrated in material from three patients: two treated with dapsone (one muscle and one nerve biopsy) and one patient (nerve biopsy) treated during the first 3 months with rifampicin once weekly. In the first two cases only one and two mice, respectively, were found positive in histologic sections; the corresponding harvests of four pools of footpads were negative. In the case of the rifampicin-treated patient, one pool of three mouse footpads out of two examined showed multiplying leprosy bacilli, while the histology of the remaining footpads remained negative. From two other patients (one treated with dapsone and a second with rifampicin daily) doubtful results were obtained: the smears of the harvests showed 12 and 15 AFB, respectively. These and many other harvests (revealing 1-3 AFB per smear and considered negative) were passaged into new mice.

DISCUSSION

Our results confirm those of Waters et al (1974) who found persistent M. leprae after long-term DDS therapy. However, where these authors discovered living M. leprae in 7 out of 12 patients (53%) treated with dapsone, we found them in only 2 out of 10 patients. The three takes we

Table 1. M. lepraе in skin, muscle and nerve biopsies from patients treated for three months with DDS or rifampicin followed by regular DDS.

No. patients in treatment schemes	Skin	Muscle			Nerve		
		neg.	few	numerous	neg.	few	numer
10 DDS Mean: no. bacilli	1.2×10^6	2	3	5		2	8 3.3 x
13 RMP 450 Mean: no. bacilli	6.2×10^6	7	3	3		6	6(1.2 x
8 RMP 900 Mean: no. bacilli	1.9×10^6	1	7			2	6 4.4 x

(x) In one patient the radial nerve was not biopsied.

Table 2. Results of mouse inoculations of biopsies taken after three months continuous or intermittent RMP or DDS and since on DDS treatment.

	Treatment schedule	DDS Skin	DDS muscle	DDS nerve	RMP 450 Skin	RMP 450 muscle	RMP 450 nerve	RMP 900 Skin	RMP 900 muscle
Overall results	no. patients examined	10	10	10	13	13	12	8	8
	no. positive patients/total	-	1	1 2/10	-	- 0/12	-	-	-
Analysis positive cases	positive harvests (pools of 5 mice)/ no. pools harvested	-	0/2	0/2	-	-	-	-	-
	mice pos. in histol. sections/no. examined	-	1/2	2/3	-	-	-	-	-
	no. mice pos./no. examined		1/8	2/9					
Analysis doubtful cases	doubtful pos. cases[b]			1	1				
	pools doubtfully pos./ pools examined			1/2	1/2				
	mice examined in histol.	-			-				

[a] 1 is a minimal figure since mice were examined in pool.

[b] In the doubtful cases 12 and 13 AFB respectively were discovered in thick

observed were in footpads inoculated with biopsies from muscle and from nerve; one of the two doubtful cases was also from a nerve biopsy. In the study of Waters et al, the main source of takes in mice was skin and nerve (30% and 28% takes respectively).

We, therefore, think that for an investigation of this kind one could limit the efforts to nerve and muscle tissue.

In our study also only a few takes were noted in each group of mice, showing that the inocula contained only very limited numbers of living leprosy bacilli. The 3 patients in whom persistent bacilli were found were in their fifth or sixth month of treatment.

The main finding of the present investigation, however, is that in one patient out of ten treated with rifampicin once a week, living M. leprae were found, in a nerve. This patient had entered the study early in 1974 and thus had completed his three months of rifampicin treatment only recently when the present biopsy was taken.

One doubtful positive case was found among the patients treated with rifampicin daily.

Our results for the daily rifampicin treatment were inconclusive: we will have to wait for the results of passage made with the doubtful positive material.

The practical conclusion to be drawn from this investigation is that, although rifampicin is an extremely rapidly killing drug for M. leprae, it is unable to kill absolutely all of those present in multibacillary forms of the disease, at least within 3 months.

Even if treatment of lepromatous leprosy has been started with rifampicin there is no more hope to have cured the patient definitely than after dapsone treatment. This is the consequence of the persisting "dormant" state in which a small proportion of all parasitic mycobacteria seem to occur in animal tissue, a small proportion that constitutes an important absolute number in lepromatous and borderline-lepromatous leprosy. The patients with these forms of the disease, being unable to cope with small numbers of living leprosy bacilli, relapse after rifampicin therapy as well as after dapsone therapy.

The advantage of starting therapy of multibacillary forms of the disease with rifampicin is a more rapid clinical improvement (Pattyn et al, 1975) and hence rapid arrest of transmission (Rees et al, 1970; Shepard et al, 1972). If rifampicin is administered as an introductory treatment, it will have to be followed for years, preferably for life, by an antileprosy treatment that should be as sure and as cheap as possible. The form that fulfills these objectives best for the time being is acedapsone (Pattyn, 1972).

The above considerations do not apply to the tuberculoid spectrum of the disease, however, where the patient can eliminate some leprosy bacilli on his own, and where a short course RMP treatment is certainly worthy of consideration (Pattyn & Saerens, 1975).

Acknowledgments. This work was supported by the Damiaanfonds, Brussels, Belgium.

REFERENCES

1. Pattyn, S. R. Comments on the chemotherapy of leprosy as influenced by the present knowledge on Mycobacterium leprae. Lepr. Rev. 43 (1972) 126-136.

2. Pattyn, S. R., Rollier, M.-T., Rollier, R., Saerens, E. J. and Dockx, P. A controlled trial on initial three months continuous and intermittent rifampicin therapy in lepromatous leprosy. Final analysis. Lepr. Rev. 46 (Suppl.) (1975) 129-139.

3. Pattyn, S. R. and Saerens, E. J. Minimal inhibitory dosage of rifampicin in intermittent treatment of M. leprae in mice. Zentrbl. Bakt. Hyg. I Abt. Orig. A 231 (1975) 503-507.

4. Pattyn, S. R., Dockx, P., Jacob, W., Rollier, R. and Rollier, M.-T. Mycobacterium leprae in skeletal muscle. (In press)

5. Rees, R. J. W., Pearson, J. M. H. and Waters, M. F. R. Experimental and clinical studies on rifampicin in treatment of leprosy. Br. Med. J. I (1970) 89.

6. Shepard, C. C., Levy, L. and Fasal, P. Rapid bactericidal effect of rifampicin on Mycobacterium leprae. Am. J. Trop. Med. Hyg. 21 (1972) 446-449.

7. Shepard, C. C., Levy, L. and Fasal, P. Further experience with the rapid bactericidal effect of rifampicin on M. leprae 1974. Am. J. Trop. Med. Hyg. 23 (1974) 1120-1124.

8. Waters, M. F. R., Rees, R. J. W., McDougall, A. C. and Weddell, A. G. M. Ten years of dapsone in lepromatous leprosy: clinical, bacteriological and histological assessment and the finding of viable leprosy bacilli. Lepr. Rev. 45 (1974) 288-298.