INTERNATIONAL JOURNAL OF LEPROSY

Volume 67, Number 4
Printed in the U.S.A.
(ISSN 0148-916X)

Histopathological Activity in Paucibacillary Leprosy Patients after ROM Therapy[1]

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Almost two decades after the introduction of multidrug therapy (MDT) the treatment of leprosy and an intensive drive on the part of the World Health Organization (WHO) and the national leprosy programs to eliminate leprosy as a public health problem, there have been several reports of patients being detected at an early stage, presenting with just a single skin lesion ([4, 11]). In 1995 WHO passed a recommendation that patients with five skin lesions or less and/or only one nerve trunk involvement should be considered as paucibacillary (PB) leprosy patients ([15]). These patients were to be treated with fixed-duration MDT composed of dapsone 100 mg daily and rifampin 600 mg once a month for 6 months. During this time various clinical trials to check the efficacy of other antimicrobial drugs against leprosy had been undertaken ([1, 5]). These trials have confirmed that minocycline is highly effective both clinically and microbiologically in mouse foot pad models inoculated with *Mycobacterium leprae* ([2, 3, 6]). It is known that a single dose of rifampin (600 mg) exerts a strong bactericidal effect on *M. leprae* ([7, 10]) and together with ofloxacin (400 mg) and minocycline (100 mg) would form an effective drug combination (ROM) to treat any early PB leprosy lesion in which the bacterial population is expected to be well below 1 million ([13]). To our knowledge no histological studies have been conducted to find out the effectiveness of ROM to clear inflammatory granulomatous response in early skin lesions in leprosy patients.

MATERIALS AND METHODS

Serial skin biopsies from 13 PB leprosy patients who had been clinically diagnosed and classified according to the Ridley-Jopling classification as borderline tuberculoid (BT) patients attending the outpatient department of The Leprosy Mission Hospital, Naini, Utter Pradesh, India, were sent to us for histopathological examination. Each patient had a comprehensive clinical examination and the morphology and the site of skin lesions were recorded. Skin-smear examination for acid-fast bacilli (AFB) was done from routine and selective sites.

A single dose of rifampin 600 mg, ofloxacin 400 mg, and minocycline 100 mg (ROM) was given. Before giving these drugs an elliptical piece of skin was biopsied from an active patch using local anesthesia. The skin biopsies were fixed in 10% formalin and processed for paraffin sections. Serial sections of 5-μm thickness were cut and stained with hematoxylin and eosin (H&E) for routine study and a modified Fite-Faraco stain for AFB ([8]). Patients were grouped histopathologically in accordance with the Ridley and Jopling classification ([12]). At 6 and 12 months after ROM therapy, skin biopsies were taken from the same lesions.

All skin biopsies were assessed for type of granuloma, granuloma fraction, nerve inflammation and the presence or absence of AFB. The histological findings were graded as "active" when there were dermal infiltration by epithelioid granulomas and the granuloma fraction was more than 10% in the dermal tissue. It was graded as "resolving" when epithelioid granuloma was absent and the lymphohistiocytic infiltrate was less

[1] Received for publication on 27 April 1999. Accepted for publication in revised form on 7 September 1999.

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THE TABLE. *Histopathological activity in the skin of PB patients after ROM therapy.*

Patient	Age	Sex	No. skin lesions	HPC[a]	Initial biopsy		Biopsy at 6 mos.		Biopsy at 1 yr.	
					GF[b]	BIG[c]	GF	BIG	GF	BIG
1.	11	M	3	BT	90%	1+	70%	1+	20%	0
2.	30	M	2	BT	50%	0	50%	0	30%	0
3.	20	F	2	Ind	10%	0	5%	0	5%	0
4.	38	M	3	BT	30%	1+	10%	0	10%	0
5.	16	M	2	BT	20%	1+	10%	0	5%	0
6.	35	F	2	BT	50%	2+	10%	1+	5%	0
7.	34	M	3	BT	50%	1+	30%	1+	10%	0
8.	18	M	2	BT	30%	1+	10%	0	5%	0
9.	40	M	2	BT	20%	1+	10%	0	5%	0
10.	29	M	1	Ind	15%	0	5%	0	5%	0
11.	25	F	2	BT	50%	0	10%	0	5%	0
12.	38	F	3	BT	30%	0	10%	0	5%	0
13.	4	F	1+1Nr[d]	BT	80%	1+	5%	0	10%	0

[a] HPC = Histopathological classification.
[b] GF = Granuloma fraction.
[c] BIG = Bacillary Index of the Granuloma.
[d] Nr = Peripheral cutaneous nerve.

than 10% and "inactive" when the lymphohistiocytic infiltrate was less than 5%.

RESULTS

Of the 13 patients 8 were males and 5 were females. The age of the patients ranged from 4 to 38 years with a mean of 26 years. Four patients presented with three patches, seven patients presented with two patches, one patient had a single lesion and another patient presented with one skin patch and an enlarged left radial cutaneous nerve. Skin smears were negative in all of them.

Initial histopathological examination showed that 11 belonged to the BT type of the disease (The Table, Fig. 1). Seven of these 11 patients showed AFB only in the dermal nerves with a Bacillary Index of the Granuloma (BIG) of 1+. One patient showed AFB in nerves, granulomas and dermal smooth muscles with a BIG of 2+. Two were classified as indeterminate and neither of them showed AFB. The second histopathological examination, done 6 months after ROM therapy, showed that of the 11 histologically diagnosed BT cases, three continued to show granulomatous inflammation. Only lymphocytic collections occupying about 10% of the dermis were seen in seven (Fig. 2) and one was inactive (Fig. 3). AFB persisted within the nerves of these patients. Both indeterminate cases were found to be inactive.

Histological examination undertaken 12 months after ROM therapy showed that epithelioid granulomas were present only in 2 cases, collections of lymphocytes persisted in 3 cases and 8 patients were inac-

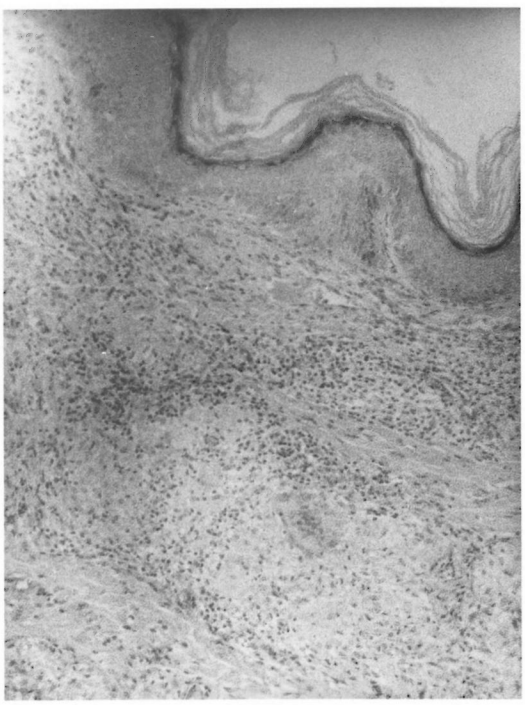

FIG. 1. Initial biopsy showing active tuberculoid granulomas composed of epithelioid cells, lymphocytes and a multinucleated giant cell in the upper dermis (H&E ×100).

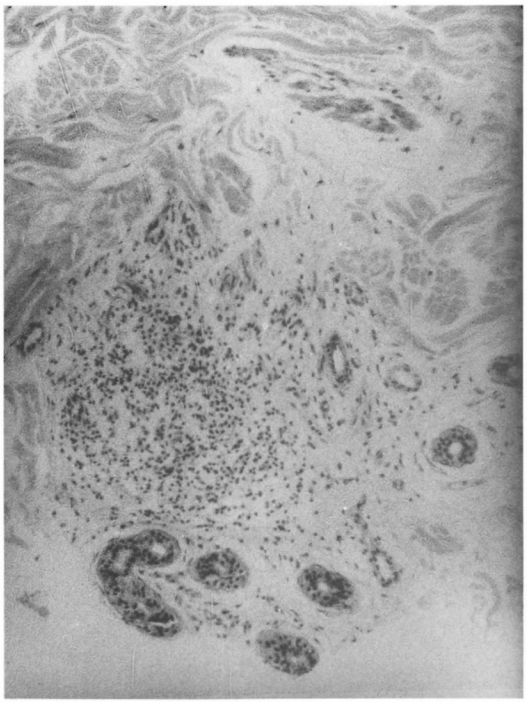

FIG. 2. Photomicrograph of a skin lesion 6 months after ROM therapy showing collections of lymphocytes around a sweat gland complex (H&E ×100).

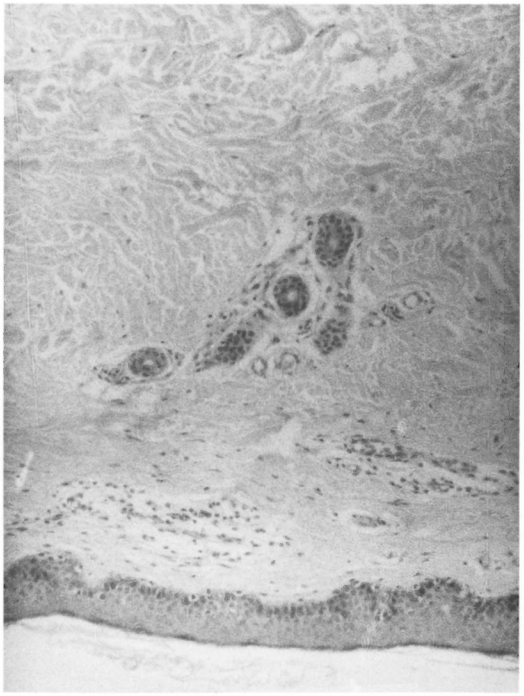

FIG. 3 Photomicrograph of a completely inactive lesion of a BT patient with only a few perivascular collections of histiocytes and lymphocytes 12 months after ROM therapy (H&E ×100).

tive. AFB were absent in all of them. It is interesting to note that of the two patients with unresolved granulomas one had three skin lesions and the other had two. Of the three biopsies with persisting granulomas one patient had three skin lesions and the others had two. All three biopsies with persisting lymphocytic collections were from patients with three lesions. The granuloma fractions in the skin of these leprosy patients before and 6 and 12 months after ROM therapy is contrasted in Figure 4.

DISCUSSION

Various clinical trials have been conducted with minocycline and other newer antibacterial drugs in order to reduce the duration of the current MDT regimens for leprosy patients. This would in turn result in increased cost effectiveness and compliance of patients.

In this study, out of the 13 cases treated with ROM, 11 of them could be confirmed as having BT histology while the other 2 showed features that were consistent with the Indeterminate type of leprosy. Initially AFB were present inside nerves in the tissues of seven patients, and in one patient bacilli were present in nerves, granulomas and smooth muscle. After 6 months, only three patients showed granulomatous lesions; the others showed only resolving or inactive lesions. AFB persisted in the nerves of three cases. At the end of 12 months, granulomas persisted in 2 out of 13 (15%) patients. No bacilli, however, were detected in any of them.

It has been reported that, clinically, the single dose multidrug regimen consisting of rifampin 600 mg, ofloxacin 400 mg and minocycline 100 mg (ROM) for single-lesion PB leprosy patients is as effective as the standard 6-month WHO-recommended PB-MDT regimen ([13]). In a previous study, 25% of the patients showed granulomas at the end of 12 months after having had 6 months of WHO-recommended therapy for PB patients ([9]). In our present study, it is clearly shown that after a single dose of ROM, granuloma clears in 85% of the pa-

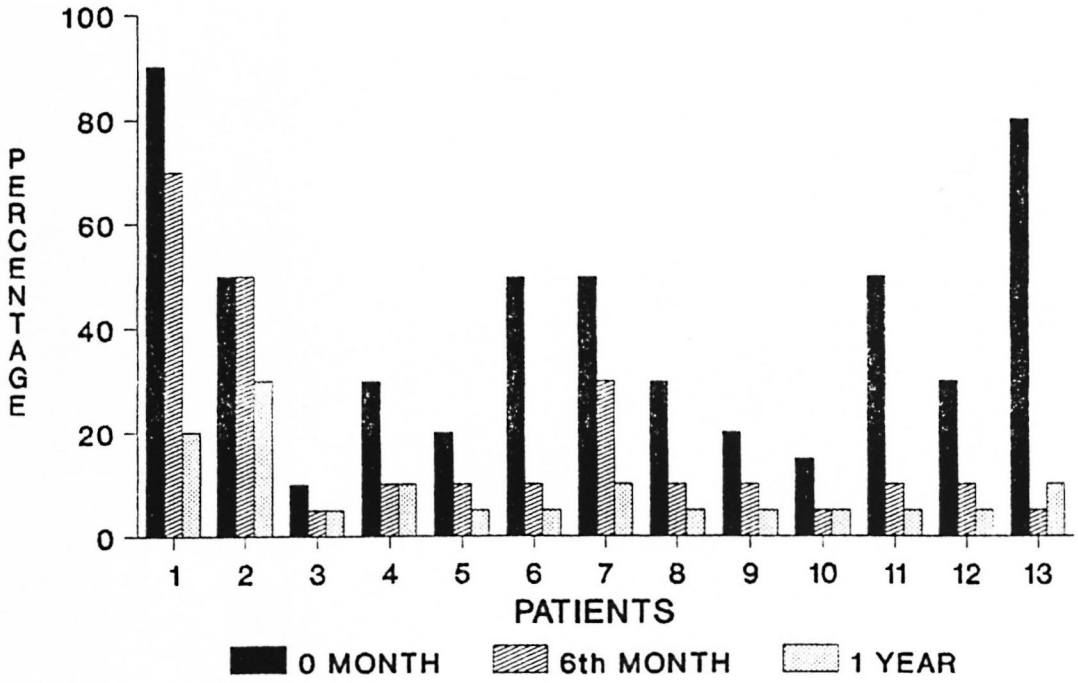

FIG. 4. Granuloma fractions in the skin after ROM.

tients in 12 months and detectable AFB are absent in all of them.

Since compliance, cost and delivery of MDT for a relatively long duration still pose problems and a sizable proportion of newly diagnosed leprosy patients present solely with early, single, PB skin lesions, the WHO has advocated using single-dose ROM for treating single-lesion PB leprosy, which is now considered to be a separate clinical entity ([14]). Granuloma persisted after 12 months in two patients (15%) who had three and two skin lesions and intraneural AFB persisted in three patients after 6 months. Are these AFB live? Will they multiply at a later date if there is any change in the immunological status of the patients? The number involved in this study is too small (only 13) and the follow-up period is too brief (only 12 months) to give firm answers to these questions. We suggest that skin biopsies be performed for proper classification when there is a clinical trial of drug regimens, and that a follow up of at least 5 years with repeat biopsies of patients with persisting active lesions be done so that a firm answer becomes available as to the effectiveness of these drug regimens.

SUMMARY

Histopathological activity was assessed in the skin tissue of 13 skin-smear negative, borderline tuberculoid leprosy patients after administration of a single dose of ROM (rifampin 600 mg, ofloxacin 400 mg and minocycline 100 mg) therapy. Biopsies taken just before therapy showed *Mycobacterium leprae* to be present in eight cases. After 6 months, only three showed granulomatous lesions and others showed only resolving or inactive lesions. Acid-fast bacilli (AFB) persisted in the nerves of three cases. At the end of 12 months, granulomas persisted in 2 out of 13 (15%) patients. No bacilli, however, were detected in any of them at the end of 12 months. This study demonstrated that 12 months after a single dose of ROM granuloma cleared in 85% of the patients and AFB were absent in all of them.

RESUMEN

Se analizó la actividad histopatológica en la piel de 13 pacientes con lepra tuberculoide subpolar que fueron negativos para bacilos alcohol-ácido resistentes (BAAR) después de administrar una sola dosis de ROM (rifampina 600 mg, ofloxacina 400 mg y

minociclina 100 mg). Las biopsias tomadas antes de la terapia mostraron *Mycobacterium leprae* en ocho casos. Después de 6 meses, sólo 3 biopsias mostraron lesiones granulomatosas mientras que las otras mostraron lesiones inactivas o en resolución. Los bacilos (BAAR) persistieron en los nervios de 3 casos. Las biopsias tomadas al final de 12 meses mostraron granulomas en 2 de 13 pacientes (15%), pero no se encontraron BAAR en ninguna de ellas. Este estudio demuestra que 12 meses después de aplicar una sola dosis de ROM, 85% de los pacientes resolvieron los granulomas y todos ellos eliminaron los bacilos.

RÉSUMÉ

L'activité lésionelle de 13 patients hanséniens borderlines tuberculoïdes ayant un examen du suc dermique négatif, après administration d'une dose unique de ROM (rifampine 600 mg, Ofloxacine 400 mg et minocycline 100 mg), fut évaluée par des examens histopatologiques de peau. Les biopsies prélevées juste avant le traitement ont démontré la présence de *Mycobacterium leprae* dans huit cas. Après une période de 6 mois, des lésions granulomateuses ne furent observées que dans 3 cas; les autres patients n'ont montré que des lésions inactives ou en involution. Des bacilles alcoolo-acido-résistants (AAR) persistaient dans les nerfs de 3 patients. A la fin du douzième mois suivant le traitement, des granulomes persistaient chez 2 des 13 (15%) patients. Aucun bacille AAR ne fut cependant détecté à 12 mois. Cette étude a montré que 12 mois après une dose unique de ROM, les lésions granulomateuses avaient disparu chez 85% des patients et qu'il n'y avait plus d'évidence de bacille AAR chez aucun d'entre eux.

Acknowledgment. This study was made possible through the biopsies sent from the TLM hospital at Naini. We are grateful to the staff and patients of that hospital for their contributions to this study. We also thank Mr. R. Soundararajan for his technical help and Mrs. Reeny S. Charles for secretarial assistance.

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