VACCINATION OF LEPROSY PATIENTS AND HEALTHY CONTACTS

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INTRODUCTION

In recent years there has been a growing interest in the development of a vaccine against leprosy. The main purpose of any vaccine is to initiate or boost the protective immune response in that part of the population which may develop the disease and possibly also to be of use in immunotherapy. Activation of the macrophages in lepromatous leprosy patients by immunotherapy to enable them to effectively bio-degrade *M. leprae* to prevent persister bacilli from acquiring drug resistance might be of great value.

Extensive skin test surveys have been conducted in Lalitpur district and adjacent districts of Nepal using Leprosin A, Tuberculin, the *vaccine*† and Scrofulacin[1]. A certain proportion of the healthy individuals and contacts of leprosy patients are persistently skin test negative after a single vaccination with *M. leprae* plus BCG.

The overall objectives of this study is to investigate the ability of *M. leprae* plus BCG to induce sensitivity to Leprosin A skin test in (i) multibacillary leprosy patients (BL/LL) (ii) Indeterminate patients and (iii) Healthy contacts. In the present communication results obtained with *M. leprae* plus BCG are reported with emphasis on Leprosin A skin test reactions, clinical and histopathological changes observed in the vaccinated sites.

PATIENTS AND METHODS

Patient Selection

Patients selected for vaccination had the details of the study explained to them and those who had given their consent were entered into the study. All the personal particulars of each patient were recorded and all underwent a thorough clinical examination. Patients were classified on the leprosy spectrum using the Ridley-Jopling scale[2]. Special emphasis was given to nerve examination. Sensory and voluntary muscle tests were

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†1. BCG—Glaxo, freeze dried 10^8 live organisms.

2. 10^7 killed *M. leprae*.

performed. The initial clinical classification of the patient was always confirmed histologically. Further information was sought as to BCG status, and history of tuberculosis. All the patients were receiving combined chemotherapy from Anandaban leprosy hospital.

The following Multidrug regimens were followed:

1. All the *multibacillary patients* were admitted to Anandaban Leprosy Hospital for supervised administration of drugs:

   Phase I : Rifampicin 600 mg o.d.
   Clofazamine 100 mg o.d.
   Dapsone 100 mg o.d.
   Duration : One month.

   After Phase I intensive therapy patients were discharged and advised to return to the skin clinic at Patan Hospital once a month for supervised intermittent therapy.

   Phase II : Rifampicin 600 mg 2 doses a month.
   Clofazamine 100 mg o.d.
   Dapsone 100 mg o.d.
   Duration : Till skin smears are negative.

2. *Indeterminate patients*
   Rifampicin 600 mg 2 doses a month.
   Dapsone 100 mg o.d.
   Duration : Six months.

Contact selection

Those residing in the same house as multibacillary patients were entered in the study. Clinical examination was performed and those apparently free of clinical lesions were included.

The following groups were included in the study.

(a) 40 Indeterminate patients.

(b) 94 Multibacillary patients (BL/LL) bacteriologically positive.

(c) 72 Multibacillary patients (BL/LL) bacteriologically negative after prolonged treatment with dapsone.

(d) 52 Healthy contacts.

Skin test reagents used in the study

Tuberculin and Vaccine : The reagents used were a range of new tuberculins prepared from live organisms grown on sautons agar. The organisms were broken with ultrasound. The sonicates were sterilised by serial filtration, the protein content measured and the final dilutions prepared in a borate buffer containing tween([3]). The concentrations used for skin testing were 20 μg protein/ml for Vaccine and 2 μg protein/ml for Tuberculin.

Preparation of Leprosin A: From batches of armadillo derived *M. leprae* prepared as per protocol 3/77([4]), the purified organisms were broken with ultrasound. The methods used for sterilisation, standardisation and dilution were the same as those for Tuberculin. The concentration for skin testing were 10 μg protein/ml.

Vaccines used in this study

1. BCG — Glaxo, freeze dried 10^6 live organisms.
2. 10^7 killed *M. leprae*.

Preliminary tests :

Skin tests : Prior to vaccination skin testing with Tuberculin 0.2 μg (0.1 ml) Leprosin A, 1.0 μg (0.1 ml) and Mitsuda Lepromin (A) 1.6×10^7 AFB (0.1 ml) were injected intradermally into the volar surfaces of the fore arms using a 1 ml. disposable syringe, fitted with size No. 26 intradermal needle. Skin tests were considered positive if the induration at 48 hours was 5 mm or more. In all subjects Leprosin A and Mitsuda lepromin were negative at 48 hours and 21 days respectively.

Blood : Samples of 5 ml. of blood were drawn from the majority of subjects in the study. Serum was obtained after separation and stored at —20°C until assayed by ELISA[5].

Skin Biopsies :

These were taken from the following:

(i) Leprosy lesions adjacent to each other; pre and post vaccination.

(ii) Positive Leprosin A skin test sites.

One percent Xylocaine was injected intradermally around, and subcutaneously below, but never directly into the site to be biopsied. The skin biopsy was divided into 2 portions, one was transfered into the formal-

Zenker([6]) fixative and the other portion was snap frozen in liquid nitrogen and stored in liquid nitrogen for demonstration of T-cell subsets, using the PAP method([7]).

Vaccination protocol :

This is designed to observe and record the conversion from negtive to positive of Leprosin A skin tests in as many patients and contacts as possible after vaccinating them with *M. leprae* plus BCG. Each time it was used the vaccine was freshly reconstituted and given in 0.1 ml intradermally over the deltoid muscles, initially over the right deltoid and subsequently alternate vaccinations were administered on either side. In some patients the vaccines were injected in the interscapular regions. Vaccination was administered at intervals of 8 to 12 weeks. All subjects were observed for a few hours after vaccination and then allowed to go home. They were advised to report to the clinic if any reaction occurs. In our experience, no toxic effects were noticed. The vaccine was safe, except for ulceration at vaccinated sites in some multibacillary patients whose initial tuberculin reactions were larger than 15 mm induration. However with application of local antiseptic cream they did not produce much additional discomfort to the subjects affected.

Re-skin testing with Leprosin A :

In multibacillary patients repeat skin testing was performed after every 4 vaccinations. In Indeterminate patients and healthy contacts first repeat skin testing was performed after 4 vaccinations and subsequently prior to every re-vaccination.

RESULTS

(a) **Skin test results :**

Table 1 shows the number of patients and contacts according to the position on the Ridley-Jopling scale and their bacteriological status.

Table 2, reveals the number of subjects converted to Leprosin A after vaccination with *M. leprae* + BCG. Among the indeterminate patients, 25 out of 40 (63%) were converted to positivity to Leprosin A. After vaccination, 34 out of 72 (47%) smear negative multibacillary patients were positive to Leprosin A. It is clear from the Table that the bacteriologically positive BL/LL patients were slower to convert than the smear

TABLE 1: Shows the number of patients and contacts according to type of leprosy and the bacteriological status

GROUP	TYPE	No.	%	BACTERIOLOGICAL STATUS	
				(+)	(—)
I *Patients*					
(a)	Indeterminate	40	(16)		—
(b)	Multibacillary patients (BL/LL)	72	(28)		—
(c)	Multibacillary patients (BL/LL)	94	(36)	+	
II *Contacts*		52	(20)		—
Total		258			

TABLE 2: Number of study subjects converted to Leprosin A

No.	GROUP	TYPE	NUMBER	CONVERTED TO LEPROSIN A	
				No.	%
I	*Patients*				
(a)		Indeterminate	40	25	63%
(b)		BL/LL (BI—)	72	34	47%
(c)		BL/LL (BI+)	94	22	23%
II	Contacts		52	32	62%
Total			258	113	

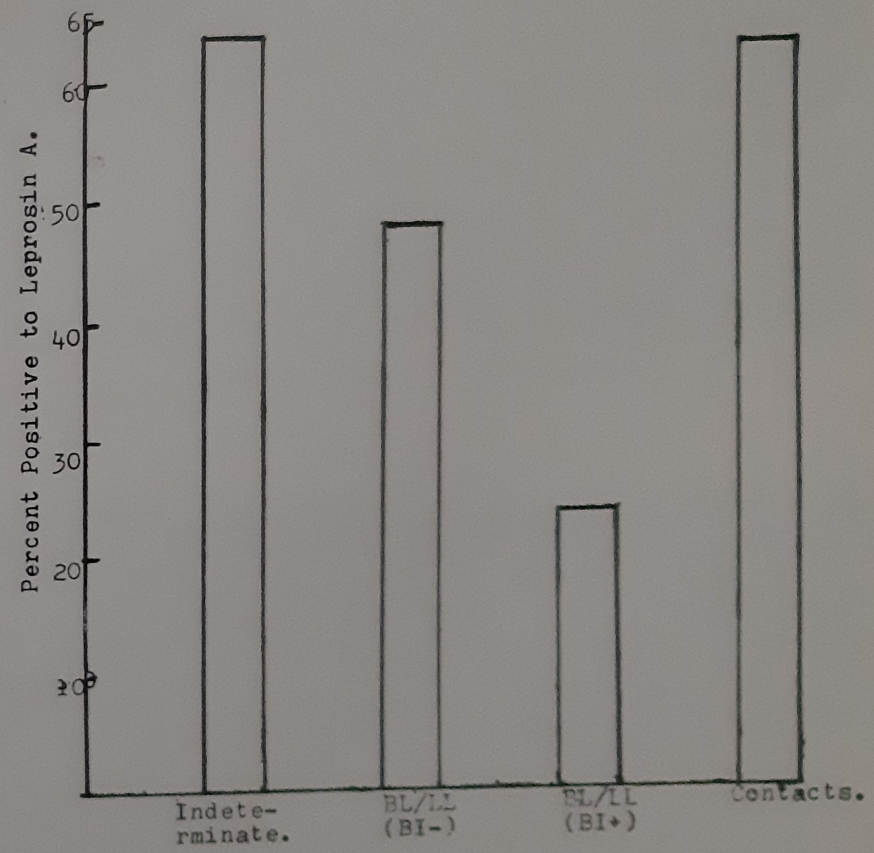

Fig. 1. Showing the frequency distribution of the skin reactions to Leprosin A after vaccination in patients and contacts.

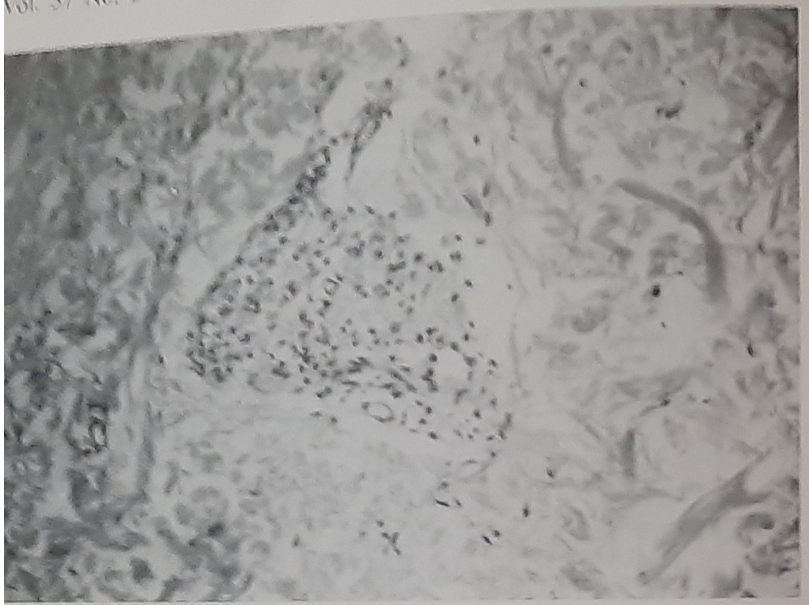

Fig. 2a. Biopsy taken from LL patient before immunotherapy. (× 240).

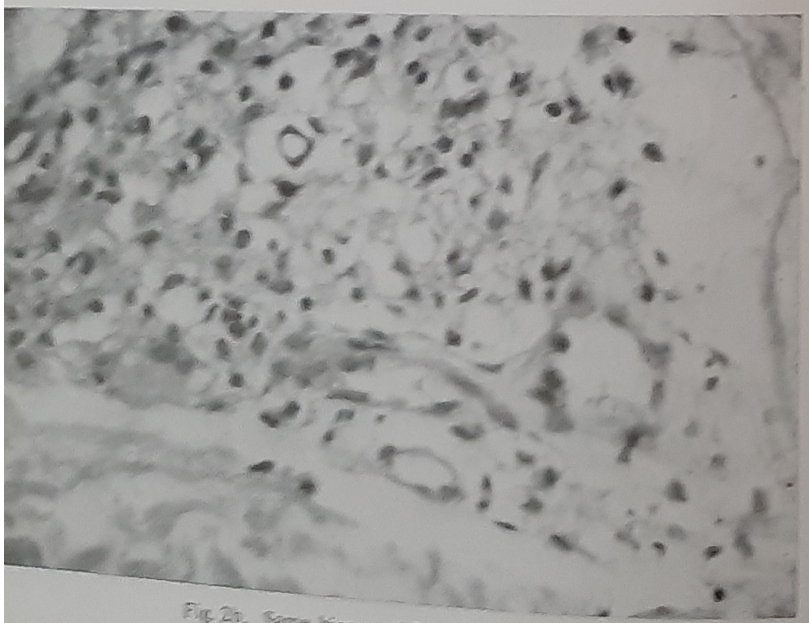

Fig. 2b. Same biopsy of Fig. 2a (× 650).

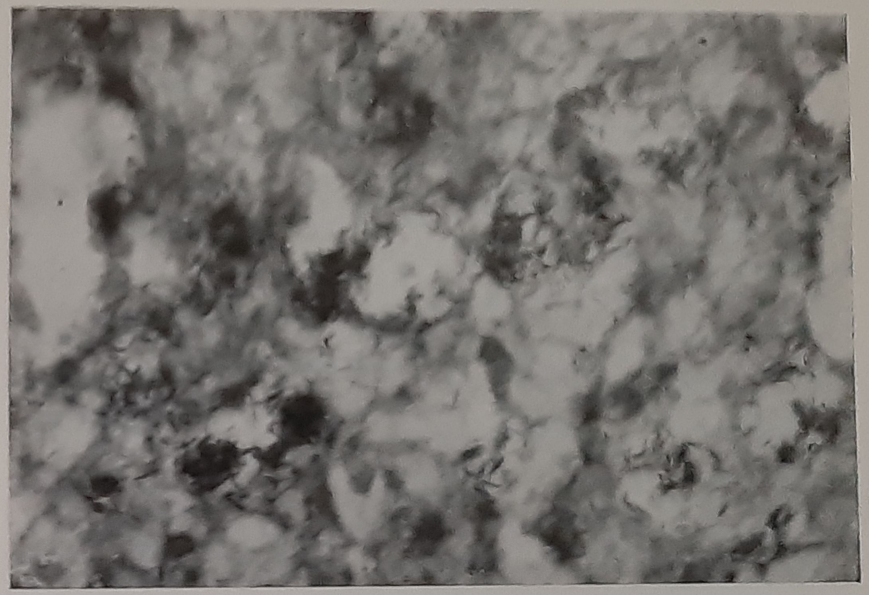

Fig. 2c. Same biopsy of Fig. 2a. (× 1000).

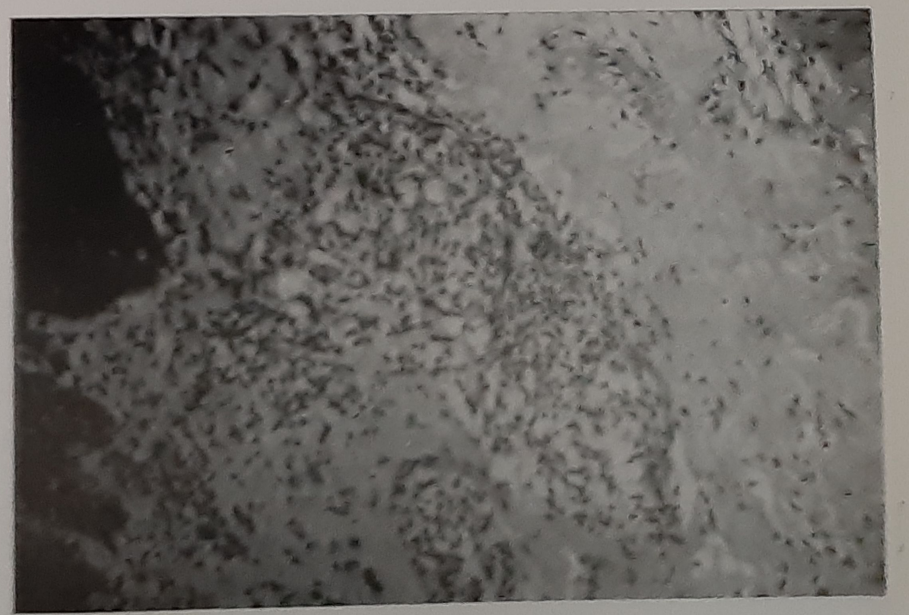

Fig. 3a. Biopsy taken from vaccinated site after eight injections of *M. leprae*+BCG. (×240).

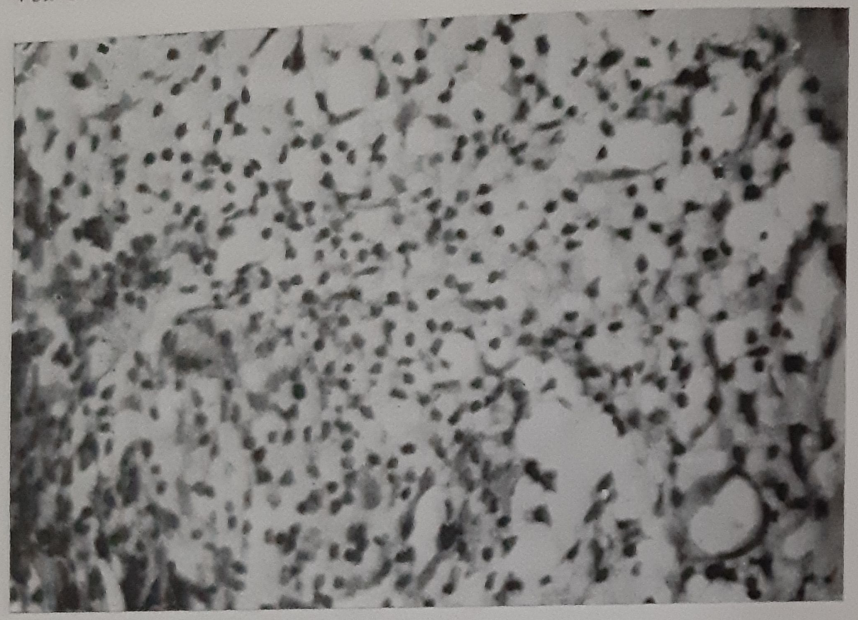

Fig. 3b. Same biopsy of Fig. 3a. (X 400).

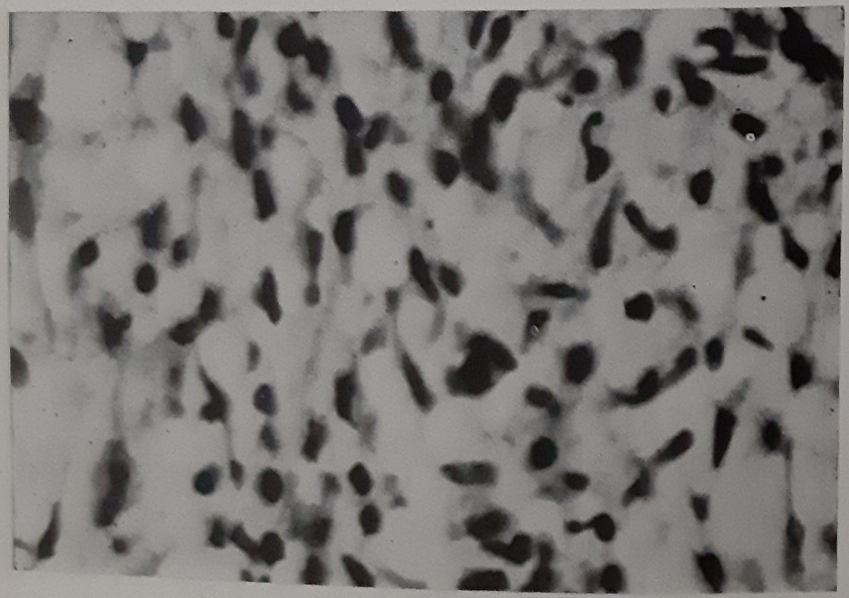

Fig. 3c. Same biopsy of Fig. 3a. (×1000).

negative BL/LL patients. The noteworthy observation is that among contact group of 52, 32 (62%) became positive to Leprosin A (Fig. 1); a group that may be incubating *M. leprae* and might benefit from vaccination to avert the development of clinical disease.

Table 3 shows the average number of vaccinations received by the patients and contacts. It is revealing that the maximum number were administered to multibacillary patients, in whom, the immunological defect appears greatest.

TABLE 3: **Shows the average number of vaccinations administered**

	GROUP	TYPE	AVERAGE NUMBER OF VACCINATIONS
I	*Patients*		
	(a)	Indeterminate	5
	(b)	BL/LL (BI—)	8
	(c)	BL/LL (BI+)	11
II	*Contacts*		4

(b) **Clinical changes :**

Amongst the Indeterminate patients, the hypopigmented lesions re- gained sensation. In four vaccinated patients tuberculoid macules was ob- served. In the multibacillary patients, BL/LL, the lesions showed reacti- vation and development of erythematous eruptions bordering the old le- sions and at the sites of vaccination. This is observed in those patients. whose smears for acid-fast bacilli were negative. In BL/LL smear posi- tive patients, the changes observed are of particular interest. Reactiva- tion of the lesions, formation of nodules were observed. Twelve smear positive patients developed new plaques. We have noticed 10 patients out of 72 developing type II reaction (14%). They were treated with Thalidomide. A significant observation amongst all patients is that an in- significant proportion of them showed nerve damage attributable to vacci- nation.

(c) **Histological changes :**

(i) **Post vaccination :** Marked changes were observed between pre and post vaccination biopsies from lepromatous leprosy patients (See

Fig 2a and 2b). After vaccination the vacuolated macrophages were devoid of acid-fast bacilli, and were surrounded by large numbers of lymphocytes. This change was seen throughout the dermis, however the numbers of mononuclear cells (i.e. lymphocytes and monocytes) in the infiltrate were variable. In some biopsies, formation of clusters of mononuclear cells were observed. These changes may enable the patients to be reclassified on the Ridley-Jopling scale as upgrading towards the borderline tuberculoid part of the spectrum.

(ii) **Positive Leprosin A sites :** The cellular infiltrate extended into the deep dermis and frequently involved the subcutis and spared the epidermis. This may be due to the antigen distribution within the skin[8]. The reactions were characterised by striking perivascular infiltration of mononuclear cells. The infiltration of mononuclear cells was peri appendageal as well. The intensity of mononuclear cellular infiltration was proportional to the size of the induration of the skin test.

DISCUSSION

A vaccine against leprosy has been successfully tested in animal models[9]. However, it's efficacy can only be confirmed in humans in whom natural suceptibility to leprosy occurs. Chemotherapy in lepromatous leprosy patients leads to the death of the bacteria but is unable to stimulate the bacteria-laden macrophages to lyse the bacilli.

The vaccination of patients has resulted in the disappearance of large vacuolated macrophages with bio-degradation of acid-fast bacilli and a massive influx of mononuclear cells at the vaccination sites.

Convit[10] reported that injection of 6×10^8 *M. leprae* with variable amounts of BCG at three sites per vaccination, resulted in positive responses to soluble skin test antigens among patients and contacts of leprosy. In the present study the amount of *M. leprae* and BCG administered is in a much smaller dose and was injected at one site per vaccination making it more practical and acceptable to the subjects. Nevertheless, the observations of this study are similar clinically and histologically to those made by Convit.

Among leprosy patients and their contacts, a proportion are unable to recognise and process the antigens of *M. leprae* in an effective manner. As well as the disease-specific defect continuous overload of environmental mycobacterial products may contribute to the suppressive effect. It should be possible to overcome this apparent defect by vaccination and the immuno-stimulating properties of *M. leprae* and BCG may perhaps partially restore the immunological state to normality.

The interesting observations of vaccination results in contacts and Indeterminate patients may enable the vaccination to be used as immunoprophylaxis in the control of the disease as well as in immunotherapy.

The precise mechanism operative is difficult to explain. However, the consequence of such vaccination may be the modulation of the suppressor mechanism from an excessive to an appropriate regulatory function. Preliminary studies[11] of cryostat sections of vaccination sites show an increase of anti-Leu-3a positive cells through which protective immune mechanisms might be induced.

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EFFECT OF CLOFAZIMINE AND DAPSONE ON RIFAMPICIN (LOSITRIL) PHARMACOKINETICS IN MULTIBACILLARY AND PAUCIBACILLARY LEPROSY CASES

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ABSTRACT : *A comparative pharmacokinetic study of Lositril (rifampicin) was carried out in six multibacillary and twelve paucibacillary leprosy cases. The type of leprosy had no significant effect on rifampicin pharmacokinetics.*

The effect of dapsone and clofazimine when given separately and in combination was studied on rifampicin pharmacokinetics in each group of six patients. Within group comparison revealed that clofazimine reduced rifampicin absorption significantly ($P<0.01$) and prolonged the time to reach the peak serum concentration ($P<0.01$). Since MCR and Ke were also reduced significantly in RC group, as compared with RDC group ($P<0.02$ and $P<0.05$ respectively), no significant alteration was seen in overall Auc and Cmax, although t0.05 was increased significantly ($P<0.02$) in RC Group.

Dapsone alone did not produce any significant alteration in rifampicin pharmacokinetics parameters, while dapsone with clofazimine reduced rifampicin 1h serum levels ($P<0.05$) and Auc ($P<0.05$) significantly.

Of the three groups, except RC group, both RDC and RD groups were homogenous Ka, avd, Cmax and Auc/t0.5 ratio of RC group were significantly different from those in RD group. While Ka and avd were significantly less ($P<0.05$ and <0.001 respectively) and Cmax and Auc/t0.5 ratio were significantly more ($P<0.01$) in RC group. Since clofazimine reduced rifampicin absorption, the difference in Ka and tp became more significant in the post-regimen phase ($P<0.01$).

ABBREVIATIONS USED IN THE ARTICLE

1. MCR — Metabolic Clearance Rate (ml/mt/kg = millilitre per minute per kilogram body weight).
2. Ke — Rate constant for elimination (h^{-1} = rate per hour).

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