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Oral Zinc as an Adjunct to Dapsone in Lepromatous Leprosy[1]

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In the therapy of lepromatous leprosy (LL) the main emphasis has been on antimicrobial agents. Although these drugs (clofazimine, dapsone, rifampin) are able to kill *Mycobacterium leprae,* they do not hasten the elimination of killed bacteria in the presence of suppressed cell-mediated immune (CMI) functions. These killed bacilli continue to release antigen for a prolonged period of time, resulting in recurrent episodes of erythema nodosum leprosum (ENL). Thus, without enhancing immunity, treatment with these antimicrobial agents alone has to be life long in order to avoid the risk of relapses due to resistant and persistent organisms ([26, 35]).

In order to promote CMI responses, diphtheria toxoid ([4]), transfer factor ([13]), allogenic lymphocytes ([21]), BCG ([25]), BCG along with killed *M. leprae* ([5]), and levamisole ([33]) have been tried but none showed consistent beneficial effects. Recently, Deo, *et al.* ([7]) have shown encouraging results with a vaccine made from ICRC bacilli.

The role of zinc as a immunostimulant is now fairly well established ([6, 8]). Low serum zinc levels have been shown in many conditions associated with depressed CMI ([1, 11, 12, 22]). There is suppression of CMI in lepromatous leprosy also, and recent studies have shown low serum zinc levels towards the LL end of the leprosy spectrum ([32]). It is not known, however, how much these low serum zinc levels in lepromatous leprosy influence CMI. In the present study, for the first time, we have tried oral zinc in combination with dapsone to enhance CMI in lepromatous leprosy.

MATERIALS AND METHODS

Twenty-five new multibacillary cases were included in the present study and classified according to Ridley ([28]) as: LLp, 13; LLs, 9; and BL, 3. These patients were divided into two groups: a) Group I: 15 cases, 9 males (3 LLp, 4 LLs, 2 BL) and 6 females (4 LLp, 2 LLs), all given dapsone 100 mg/day and zinc sulfate 220 mg/day orally after meals; b) Group II: 10 cases, 6 males (all LLp) and 4 females (3 LLs, 1 BL), all treated with dapsone 100 mg/day.

All of these patients were in the 20–50 age group and were of similar socio-economic and nutritional status. We present here the data for up to 18 months of therapy; these patients are still continuing on the same drug regimens.

Patients were subjected to clinical charting, slit-skin smears, and skin biopsies; initially, at three-month intervals for one year and subsequently at six-month intervals. These investigations were also performed whenever there was a reaction. The lepromin test and a serum zinc estimation were carried out before and after 18 months of therapy. Serum zinc levels were also estimated at the time of ENL.

Charting was done according to the method recommended by the Indian National Leprosy Organization ([34]). Edema, infiltration, erythema, and reactions were also recorded.

Slit-skin smears were prepared from 4 sites (2 ear lobules, 1 eyebrow, and 1 most prominent lesion) by the same technician throughout the study. These were stained by the Ziehl-Neelsen method ([29]) (cold method: 30 min of strong carbol fuchsin at room temperature). The bacterial index (BI) was recorded on the Ridley scale, and an

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TABLE 1. *Changes in the mean bacterial index (BI) with duration of treatment.*

Leprosy clinic no.	Type of leprosy	BI				
		Initial	6 mo.	12 mo.	18 mo.	Rate of fall/6 mo.
			Group I			
623	LLp	5.0	3.0	2.0	1.75	1.07
635	LLp	6.0	4.5	2.0	1.5	1.60
644	LLp	5.0	4.0	3.0	2.0	1.00
445	LLp	5.0	3.5	3.5	3.0	0.60
489	LLp	6.0	5.0	4.5	3.5	0.80
691	LLp[a]	5.0	5.0	5.0	4.0	0.30
503	LLp	5.0	4.0	3.0	2.5	0.85
409	LLs[b]	4.0	3.0	2.5	2.0	0.65
416	LLs[a]	5.0	5.5	3.5	4.0	0.50
461	LLs[b]	4.0	3.0	1.5	0.5	1.20
428	LLs[b]	3.0	0.5	Neg.	Neg.	0.95
760	LLs[b]	5.0	3.5	2.0	1.5	1.20
768	LLs	4.0	3.5	3.0	2.0	0.65
656	BL[b]	3.0	1.5	Neg.	Neg.	1.05
442	BL[b]	3.0	Neg.	Neg.	Neg.	0.90
			Group II			
488	LLp[a]	6.0	5.5	5.0	4.5	0.50
704	LLp	5.0	4.5	4.0	3.5	0.50
748	LLp	5.0	4.5	4.0	4.0	0.35
348	LLp	5.0	4.5	4.0	3.5	0.50
249	LLp[a]	5.0	4.0	3.5	3.0	0.65
543	LLp[a]	5.0	4.5	4.0	4.0	0.35
441	LLs	5.0	4.0	3.5	3.0	0.65
417	LLs	4.0	3.5	3.0	2.5	0.50
471	LLs[a]	3.0	2.5	2.5	2.0	0.30
567	BL[b]	3.0	3.5	2.0	1.5	0.60

[a] ENL reaction.
[b] Upgrading.

average BI from the four sites was calculated. Serial skin biopsies were taken from the same lesion, 2 cm away from the previous biopsy sites. Paraffin sections were subjected to hematoxylin and eosin (H&E) staining for routine histology and a modified Fite-Faraco method for acid-fast bacilli (AFB) staining. The bacterial index of granuloma (BIG) was recorded in granuloma ([27]). Both BI and BIG were assessed independently by a pathologist and a bacteriologist who did not know the drug regimens involved. The data were then subjected to statistical analysis by applying Student's *t* test.

The lepromin test was done using Dharmendra lepromin; 0.1 ml of lepromin was injected intradermally into the forearm 5 cm below the elbow joint. Results were read between 4–6 weeks after the intradermal injection ([14]). A serum zinc estimation was done by means of flame atomic absorption spectrophotometry ([18]).

Upgrading reactions were diagnosed on the basis of the rapid fall in the BI and BIG; edema of the dermis; appearance or increase in the number of lymphocytes, epithelioid cells, and giant cells; and a change in the lepromin reaction.

OBSERVATIONS

Clinical. Faster clinical improvement, suggested by a rapid decrease in erythema, edema, and infiltration, was seen in Group I as compared to Group II. Eyebrows which were absent in 8 out of 15 patients in Group I showed good growth which started after six months of zinc therapy. Eyebrows which were absent in 6 out of 10 patients in Group II did not show regrowth.

Bacteriological. Reductions in the BI and the BIG of individual patients are shown in Tables 1 and 2, respectively. The average BI and BIG of LLp and LLs patients at 6, 12, and 18 months are shown in Figures 1

TABLE 2. *Changes in the bacterial index in granuloma (BIG) with duration of treatment.*

Leprosy clinic no.	Type of leprosy	BIG				
		Initial	6 mo.	12 mo.	18 mo.	Rate of fall/6 mo.
			Group I			
623	LLp	5.0	4.0	3.0	2.0	1.00
635	LLp	6.0	4.0	3.0	2.0	1.30
644	LLp	6.0	5.0	4.0	3.5	0.85
445	LLp	5.5	4.0	3.0	2.0	1.15
691	LLp[a]	6.0	5.0	6.0	4.5	0.35
489	LLp	6.0	4.5	4.0	3.5	0.80
503	LLp	5.5	4.5	4.0	3.0	0.80
409	LLs[b]	4.0	3.0	3.0	1.5	0.75
416	LLs[a]	5.0	5.5	4.0	4.0	0.15
461	LLs[b]	4.0	2.0	2.0	1.0	0.90
428	LLs[b]	3.0	1.0	Neg.	Neg.	1.00
760	LLs[b]	5.0	3.0	2.0	1.0	1.30
768	LLs	4.5	3.0	2.5	2.0	0.80
656	BL[b]	3.0	1.0	Neg.	Neg.	1.00
442	BL[b]	3.0	Neg.	Neg.	Neg.	0.90
			Group II			
488	LLp[a]	5.0	5.0	5.0	4.5	0.15
704	LLp	5.5	5.0	4.5	4.0	0.50
748	LLp	5.5	5.0	5.0	4.5	0.30
348	LLp	5.0	4.5	4.5	4.0	0.30
249	LLp[a]	5.0	4.5	3.5	3.0	0.65
543	LLp[a]	5.5	5.0	4.5	4.0	0.50
441	LLs	4.0	3.5	3.0	3.5	0.20
417	LLs	5.0	5.0	4.0	3.5	0.55
471	LLs[a]	4.0	4.0	3.5	3.0	0.35
567	BL[b]	3.0	2.5	2.0	1.0	0.65

[a] ENL reaction.
[b] Upgrading.

and 2, respectively. The rate of fall per six months is significantly higher in Group I as compared to Group II, which is highly statistically significant ($p < 0.01$). Patients with borderline lepromatous leprosy were excluded from the statistical analyses.

Histopathological. The decrease in the size of the granuloma was found to be similar in both groups. At the end of the study all biopsies showed linear, perivascular, and periadenexial infiltrates composed of mononuclear cells, mainly macrophages and lymphocytes. Infiltration with lymphocytes gradually increased in both groups, but it was found to be greater and to occur earlier (also in clusters) in Group I as compared to Group II (Table 3). The epithelioid and giant cells were not present except in the biopsies of those patients who upgraded. A significant observation was the presence of neovascularization and of endothelial cell proliferation in all Group I patients, while it was not seen in the patients of Group II. Neovascularization started after three months of therapy and gradually increased thereafter (Figs. 3 and 4).

Reactions. ENL was seen in two Group I cases (13.3%) after about one year of therapy, and could be controlled by steroids given for a short period (1½–2 months) and in small doses (prednisolone 15–30 mg/day). One patient, who was also a diabetic, was treated with clofazimine. In Group II, four (40%) patients developed ENL after 9–12 months of therapy, and needed higher doses of steroids (prednisolone 30–60 mg/day) for variable periods of time (4–6 months). Three patients did not tolerate dapsone and were treated with clofazimine. Since clofazimine does not enhance the bacterial clearance more than dapsone ([15, 31]), it was not expected to affect the results. Hence these patients were not dropped from the study.

Upgrading. Four LLs and two BL patients

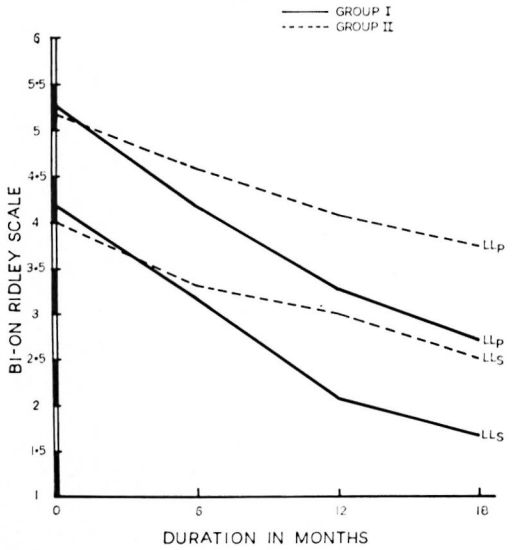

FIG. 1. Average BI with duration of treatment in LLp and LLs patients.

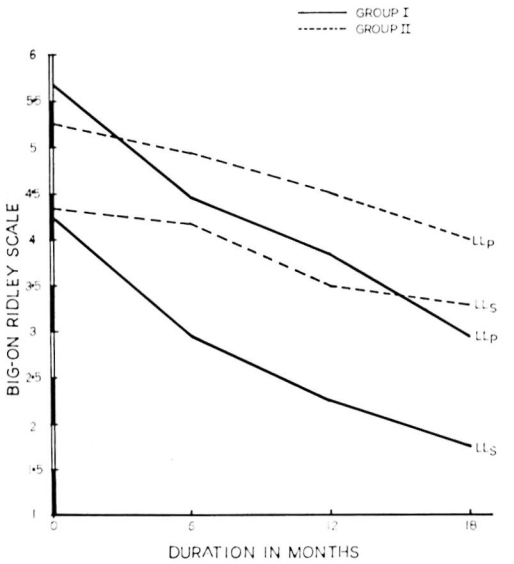

FIG. 2. Average BIG with duration of treatment in LLp and LLs patients.

in Group I upgraded both histologically and bacteriologically after 5–10 months of zinc therapy. Only one patient (BL) in Group II upgraded (Table 4).

Lepromin test. Five patients in Group I and one patient in Group II were found to be lepromin positive at the end of 18 months of therapy. These were the cases in whom upgrading was also observed histopathologically and bacteriologically (Table 4). The rest of the patients remained lepromin negative even after 18 months of therapy.

Serum zinc. The mean (±S.D.) serum zinc level in all of the patients at the commencement of therapy was 77.2 (±7.6) μg/100 ml. Serum zinc levels were noted to be slightly lower, 72.3 (±5.8) μg/100 ml, during the ENL episodes in the six patients who suffered from ENL. After 18 months of therapy, the mean serum zinc levels increased to 113.5 (±5.2) μg/100 ml in Group I, while they remained low, 80 (±5.0) μg/100 ml, in Group II. The mean serum zinc levels of the healthy controls of the same socio-economic and nutritional status from our center was 110.2 (±8.2) μg/100 ml (unpublished data).

Except for mild nausea in some patients, no side effects were observed during therapy. Urine examinations and total and dif-

TABLE 3. *Pattern of influx of lymphocytes in the lesion.*[a]

Patients	Appearance of lymphocytes			
	Initial	6 mo.	12 mo.	18 mo.
Group I				
LLp	−/±	+[b]	++/+++[c]	+++/++++[c]
LLs	±/+[b]	+/++[b]	++/+++[c]	++++[c]
Group II				
LLp	−/±	±/+[b]	+[b]	+/++[b]
LLs	−/±	+[b]	+/++[c]	++/+++[c]

[a] Percent of lymphocyte population in the lesion: − = absent; ± = occasional; + = 1–10%; ++ = 11–30%; +++ = 31–50%; ++++ = above 50%.
[b] Scattered.
[c] Clusters.

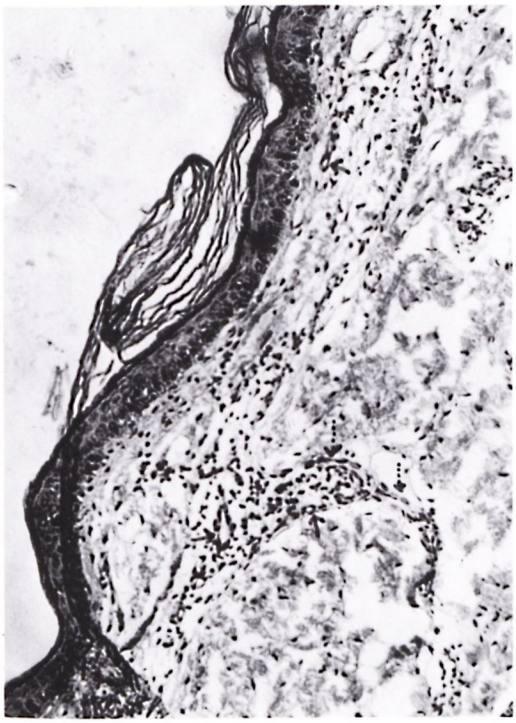

FIG. 3. Neovascularization (↑), endothelial cell proliferation (⇡), and lymphocytic infiltration after nine months in Case No. 635 (×150).

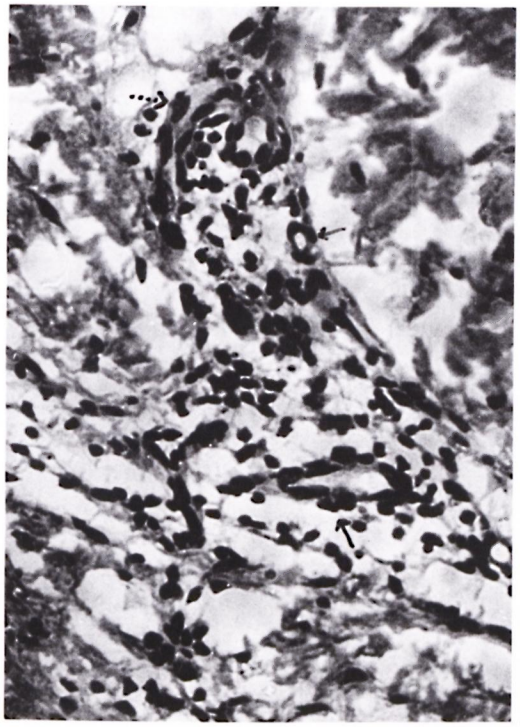

FIG. 4. Neovascularization (↑), endothelial cell proliferation (⇡), and lymphocytic infiltration after nine months in Case No. 635 (×300).

ferential leukocyte counts were normal before and after therapy.

DISCUSSION

Zinc has been successfully used to correct immunological defects in many conditions with low serum zinc levels ([1, 12, 20]). In leprosy there are independent reports showing a gradual fall in serum zinc levels ([32]) and depression of CMI ([2, 10]) from the TT pole to the LL pole of the leprosy spectrum, suggesting the possibility of their inter-relationship. In the present study with oral zinc therapy, we have observed a rapid clinical improvement in Group I cases which is comparable to that seen with clofazimine therapy. One most striking observation is the regrowth of eyebrows in Group I cases

TABLE 4. *Upgrading and the development of lepromin positivity.*[a]

Leprosy clinic no.	Initial type of leprosy	Type of leprosy after upgrading	Duration of treatment before upgrading occurred (mo.)	Lepromin positivity after 18 mo.
		Group I		
461	LLs	BL	9	Negative
409	LLs	BT	10	++ (6 mm)
656	BL	TTs	5	++ (7 mm)
428	LLs	BT	6	+ (5 mm)
760	LLs	BT	6	++ (6 mm)
442	BL	BT	9	++ (10 mm)
		Group II		
696	BL	BT	8	++ (7 mm)

[a] After upgrading, resolution occurred in upgraded type.

only. The mechanism of action of zinc in hair growth is not clear, however Wolowa and Jablonska have shown beneficial effects of zinc in alopecia areata ([36]).

The falls in BI and BIG were significantly more in patients taking dapsone with zinc sulfate than in those taking dapsone alone. This rapid fall with zinc seems to be due to T cell activation ([6, 8, 9]) which, in turn, activates macrophages to promote bacterial clearance. An enhanced rate of bacterial clearance has also been reported with transfer factor ([21]) and following the administration of a mixture of *M. leprae* and BCG ([5]).

Histologically, the earlier and greater influx of lymphocytes is similar to the effect of ICRC vaccine ([7]) and BCG ([30]), which further indicates the immunostimulant role of zinc. Another significant observation is the neovascularization and endothelial cell proliferation in Group I cases which was conspicuously absent in Group II cases. The cause of this phenomenon is not clear but appears to be due to the effect of zinc on DNA/RNA synthesis and cell replication ([24]). Neovascularization could explain the faster wound healing seen with zinc therapy ([3, 23]).

The low incidence of ENL in Group I patients could be because of the marked reduction of bacterial load, combined with immunomodulation ([6, 8]) and anti-inflammatory ([28]) actions of zinc. We have also used zinc in cases of recurrent ENL along with other drugs and have found it to be effective ([17]). The incidence of upgrading and the development of lepromin positivity is much higher in patients receiving zinc than in the controls. However, upgrading has not been observed in any case of LLp with or without zinc therapy, suggesting that zinc has the potential to correct CMI in LLs and BL only. This is compatible with the concept that LLs patients have some immunocompetency to revert ([16]), while LLp patients are completely anergic to *M. leprae* with no tendency towards reversion.

SUMMARY

Oral zinc was tried in 15 cases of multibacillary leprosy as an immunostimulant in addition to conventional antileprosy drugs. Results were compared with those in ten similar cases treated with dapsone alone. Cases treated with zinc showed faster clinical improvement, regrowth of eyebrows, rapid fall in the bacterial index (BI) and in the bacterial index in the granuloma (BIG), early and greater influx in lymphocytes in the granuloma, and neovascularization. Upgrading occurred in 6 out of 15 patients taking zinc, but in only 1 out of 10 patients in the control group. Five out of six patients who showed upgrading in the treated group became lepromin positive. Only one patient (BL) showed lepromin conversion in the control group.

RESUMEN

Quince pacientes con lepra multibacilar recibieron zinc oral además de las drogas antileprosas convencionales. Los resultados se compararon con los obtenidos en 10 casos similares tratados sólo con dapsona. Los casos tratados con zinc mostraron una más rápida mejoría clínica, el recrecimiento de las cejas, una caída rápida en el índice bacteriana (BI) y en el índice bacteriano de los granulomas (BIG), un mayor y más temprano influjo de linfocitos en el granuloma y neovascularización. En 6 de 15 pacientes bajo tratamiento con zinc, y sólo en uno de 10 pacientes del grupo control, se observó una reacción sugerente de mejoría ("upgrading"). Cinco de los 6 pacientes tratados con zinc que presentaron tal reacción se tornaron positivos a la lepromina. Sólo un paciente (BL) mostró conversión a la lepromina en el grupo control.

RÉSUMÉ

Dans 15 cas de lèpre multibacillaire, on a essayé le zinc par voie orale comme immuno-stimulant, en complément des médicaments antilépreux habituels. Les résultats ont été comparés avec ceux obtenus chez dix cas similaires traités uniquement par la dapsone. Les cas dont le traitement comprenait du zinc ont montré une amélioration clinique plus rapide, la repousse des sourcils, une chute rapide de l'index bactérien (BI) et de l'index bactérien au niveau des granulomes (BIG), de même qu'une mobilisation précoce et plus importante des lymphocytes dans le granulome, et une néovascularisation. Cette amélioration est survenue chez 6 des 15 malades qui prenaient du zinc, mais seulement chez un des dix malades du groupe témoin. Cinq des six malades qui ont présenté une amélioration dans le groupe en traitement sont devenus lépromino-positifs. Un seul malade (BL) a présenté un virage de l'épreuve à la lépromine dans le groupe témoin.

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