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Amino
Acids

The use of an amino acid as a precursor of a bioactive metabolite in combatting an infectious disease: the efficacy of a tryptophan-enriched diet in the treatment of leprosy

J. Mauron[1], R. Ganapati[2], P. R. Mahadevan[3], and K. Anantharaman[4]

[1] Vevey, Switzerland
[2] Bombay Leprosy Project, Bombay, India
[3] Foundation for Medical Research, Bombay, India
[4] Nestlé Research Centre, Lausanne, Switzerland

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Summary. Deoxyfructosylserotonin (DFS), a stable derivative of serotonin, was shown to have an anti-leprosy effect (Mester and Antia). Therefore, a tryptophan-enriched food (NAL) was devised to increase the concentration of free tryptophan as serotonin precursor in blood.

32 multibacillary lepromatous leprosy patients were divided in 3 groups receiving: 1) MDT (multi-drug therapy, control — 8 patients), 2) NAL (50 g/day — 13 patients) and 3) NAL + 1/2 MDT (11 patients). The clinical improvement (clinical score) was 24.2% for 1), 19.9% for 2) and 30.4% for 3). The loss of viability of the *M. leprae bacilli* in the mouse foot-pad test was 66% for 1), 75% for 2) and 70% for 3). The improvement by serodiagnosis (ELISA) was 19.4% for 1), 25.1% for 2) and 23.3% for 3).

These preliminary results (6 months) suggest that the NAL-food is efficient against leprosy and apparently not very different from the control MDT in that respect.

Keywords: Amino acids – Multibacillary leprosy – Diet therapy – Multidrug-therapy – Tryptophan – Serotonin

Introduction

In spite of the availability of efficient drug therapy, the number of registered leprosy patients in the world appeared to be still increasing until recently (Noordeen and Bravo, 1986). Failure to reach systematically the leprosy patients, increasing incidence of *Mycobacterium leprae* drug resistance and relative expensiveness of the new drugs may be the main reasons for this.

The possible role of nutrition in the etiopathogenesis of leprosy has been reviewed by Foster et al. (1988). Dharmendra (1949) presented data showing how famine malnutrition may predispose to leprosy in humans. A possible link between lipids and leprosy is suggested by the epidemiology of leprosy. Thus, the prevalence of leprosy in Norway during the last century has been related to the intake of rancid fish oil, and Hutchinson (1906) found in different countries a high incidence of leprosy in people eating high amounts of partially decomposed fish. Bergel (1959) showed that a pro-oxidant diet increases the growth of *M. leprae* in the rat and made a reappraisal of Hutchinson's dietetic hypothesis in the light of the influence of pro-oxidant nutritional conditions (Bergel, 1960). Later, Bergel (1981) attributed the antileprosy effect of the classic drug Dapsone to its antioxidant properties and made a similar hypothesis about serotonin that is also a strong antioxidant (Bernheim et al., 1957).

A new light on the possible role of antioxidants in leprosy was shed by Prabhakaran (1972), who showed that *M. leprae* contains a specific phenolase that oxidizes L-Dopa, and that this L-Dopa oxidase plays an important metabolic role in the growth of *M. leprae*. The antioxidative compounds that inhibit this oxidase also stop the multiplication of the *M. leprae* bacillus. Although the role of L-Dopa utilization by *M. leprae* has been disputed (Kato et al. 1977), it was later confirmed that the incorporation and utilization of L-Dopa by *M. leprae* was indeed inhibited by a potent antioxidant, deoxyfructosyl-serotonin (DFS) (Jayaraman et al., 1980). At last, it was demonstrated that DFS, which can be considered a stabilized form of serotonin, showed, in fact, anti-leprosy activity in man (Antia et al., 1988). These newer developments initiated by L. Mester led one of us (J.M.) to envisage diet therapy as a new approach to the treatment of leprosy using tryptophan as main ingredient, in order to increase serotonin and possibly DFS levels in body fluids and tissues (Mauron and Mester, 1984).

The aim of this presentation is to expose the rationale of this dietary approach and to give a preliminary account of its effectiveness in treating leprosy patients.

Materials, patients and methods

Materials

1. The "anti-leprosy" food "NAL"

The term NAL stands for "*N*ourriture *A*nti-*L*èpre" in French. It was designed with the specific aim to increase the free tryptophan level in blood as a precursor of serotonin and, with the more general aim, of improving the nutritional status of the patients by providing about 50% of the minimum daily protein requirements and some vitamins.

Rationale: Tryptophan is given in the bound form in milk protein (25%) and free (75%). Niacin is added to inhibit tryptophan degradation in the liver by tryptophan pyrrolase.

Middle chain triglycerides ($C_8 + C_{10}$) (Miglyol) are included because the corresponding fatty acids compete with tryptophan on the same binding site on serum albumin thus increasing free tryptophan (Mac Menamy 1965). The unsaturated fatty acids present in corn oil have a similar effect.

Table 1. Composition of "*NAL*" (g/kg)

Wet Mix	Skimmed Milk (SNF) (as DM)	317.00
	Ca-Caseinate (as DM)	264.00
	Maltodextrin (MD-40) (as DM)	166.00
	Corn Oil	70.00
	Miglyol 812	75.00
	Lecithin	5.00
	Butylated hydroxyanisole (BHA)	0.052
Dry Mix	Cocoa "CAILLER" 10/12	50.00
	L-Tryptophan (Pharma quality)	18.00
	Na-saccharin	1.00
	Aspartame	0.50
	Vanillin	0.50
	Isoniazid (as marker)	0.70
	Vitamin A acetate (325,000 IU/g)	0.186
	Pyridoxine HCl	0.10
	DL-alpha-tocopherol, 50%	2.00
	Niacin	6.00

Table 2. Average analytical values for major ingredients in "NAL"

Protein (N × 6.32)	40%		
Fat	15%		
Carbohydrate	40%		
Minerals	3%		
Free tryptophan	1.8%		(=75%)
Bound tryptophan		0.6%	(=25%)
Total tryptophan		2.4%	

Antioxidants (vit. E, BHA) are added to preclude any fatty acid oxidation and enhance the antioxidant potential of the diet.

The ingredient composition and the average analytical values for the main nutrients in NAL are shown in Tables 1 and 2.

All the ingredients are food grade. The same batch of tryptophan was used throughout. It was produced in 1984 by Showa Denko, Japan, more than four years before the change in manufacturing procedure leading to the contamination of some batches in 1989, that resulted in the appearance of the *Eosinophilia myalgia* syndrome (EMS) in the U.S.A.

A water-soluble powder form of the NAL formula to prepare a tasty chocolate drink was developed by P. Hirsbrunner at the Nestlé Research Centre in Lausanne (Switzerland) and manufactured according to good manufacturing practice, including analytical conformity and bacteriological safety, at Alpura Koreco Ltd., Konolfingen (Switzerland). The NAL powder is delivered in 50 g aluminium bags that keep the powder intact for, at least, one year under tropical conditions.

The efficacy of the NAL-diet against leprosy was shown by the classic mouse foot-pad test in mice receiving 0.5 g of NAL (Balakrishnan et al., 1985). This treatment inhibited the multiplication of *M. leprae* in the mouse foot-pad and was as effective as 0.01% Dapsone (DDS) in the diet of the mouse. Since the mouse consumes about 5 g food (dry matter) per day, the effective dose of NAL can be estimated to correspond to 10% of the daily food intake (about 500 g for an adult man). The daily NAL-dose used for this clinical trial is therefore 50 g. It was very well accepted as a drink by the patients.

2. The MDT (multi-drug-therapy), regimen

The three drugs used according to WHO (1977) recommendations are:

Dapsone	100 mg/daily
Clofazimine	100 mg/daily (modified according to BLP experience)
Rifampicin	600 mg/28 days

Patients

Adults with multibacillary lepromatous (LL) or borderline-lepromatous (BL) leprosy showing a bacterial index (BI) of 3^+ or greater and a negative lepromin test are included in the trial. Informed consent for inclusion into the trial is obtained from the patients in an appropriate form. The protocol for this clinical trial conforms with the recommendations set out by WHO for lepromatous leprosy (WHO, Geneva 1977) and will not be detailed here. The clinical trial comprises three treatment groups:

1. The reference group (12 out-patients) with MDT regimen, for a duration of 24 months.
2. The test group (12-in-patients) with NAL only (50 g/day) for 6 months, followed by the classic MDT regimen (as out-patients) for 18 months.
3. An additional test group (12 patients) with NAL (50 g/day) + 1/2 MDT (half-dose of 1) for 24 months.

The patients are selected and assigned to the three treatment groups by Dr. R. Ganapati, Director of the Bombay Leprosy Project (BLP), who is responsible with his staff for the medical supervision of the patients, and the proper scientific management of the clinical assay according to the protocol. Most patients were untreated before. In the group 2 (NAL), one patient (Ch.M.) only had had monotherapy (Dapsone) stopped in 1985. In the groups 1 and 3 several patients had received some monotherapy or MDT either long time before the study or for a relatively short duration. At entry in the study, they all had a high BI.

In a preliminary trial with 3 patients originally classified as LL (lepromatous leprosy), Dr. Ganapati observed a definite regression in 2 out 3 patients upon ingestion of 50 g NAL daily for 3 months.

The MDT regimen used in the trial is derived from the WHO recommendations and has been used successfully for years at the BLP (see above).

The clinical trial is not yet completed but the fragmentary results for 32 patients after 6 months treatment are now available and presented here. (Other treatment length in curved brackets in the tables)

Methods

1. The clinical evaluation

a) The clinical examination is performed at BLP by Dr. Ganapati and his staff before treatment, and at 3 and 6 months intervals in the initial phase of the trial, later every 6 months. It includes the classic medical criteria for leprosy testing and will be described elsewhere, in the final paper after 24 months treatment.
b) The photographic assessment is made at BLP. Dr. Ganapati takes colour photographs of the main lesion sites in individual patients before and after 6 months treatment. In order to arrive at a semi-quantitative assessment, the photographs are coded and submitted to a representative sample of the medical and paramedical staff at BLP. In comparing pairs of the photographs before and after treatment, each examiner has to evaluate the difference giving following ratings: no change, deterioration, more than 25%, 50% or 75% improvement. The average of the ratings of the examiners is taken to characterize the evolution of the disease in each patient.
c) Clinical scoring is performed in addition in a blind manner, by an independent outside assessor and leprosy expert, Dr. Ramu, Sacred Heart Leprosy Centre, Kumbakonam.

For establishing his clinical score, Dr. Ramu divides the body of the patient in 7 sectors. Lesions in each sector are quantified, the score varying from 1 to 4. The scores of the 7 body sectors are added up to form the clinical score, which can, therefore, reach the maximal value of 28. In addition, Dr. Ramu's general appreciation is given; "I" meaning moderate to marked improvement, "Is" slight improvement. Details of this scoring method will be given in the complete publication.

2. The laboratory assessment

a) The usual methods of *clinical chemistry*, such as blood and urine analysis, differential leucocyte count and specific enzymes are done a the Jaslok Hospital, Bombay. No or very little abnormalities were found in the patients of this study. Never did the NAL-treatment induce *eosinophilia.*

The evolution of leprosy is followed by the routine determination of the bacterial index (BI) and morphological index (MI) in slit skin and nasal smears, histopathological examination of skin biopsy specimens and lepromin test. The data of these tests will be presented elsewhere and incorporated in the final evaluation. Determinations of Dapsone in random urine samples are frequently made (BLP) to ensure compliance with the prescribed regimen. So far, absence of Dapsone in the urine of the NAL patients was more than 98.5% (Tile tests).

b) *Mouse foot-pad* (MFP) studies are considered to furnish the most valid and objective data to judge the efficacy of a given treatment in multibacillary leprosy. In the mouse foot-pad procedure, materials obtained from clinically active lesions of the patient by biopsy are collected and suspensions with a standardized bacillary count are inoculated in albino mice. Harvests are commenced in the 5th month after inoculation, and afterwards every month until 1 year. The MFP tests are performed by Dr. Mahadevan, according to the method of Shepard (1960).

The number of bacilli per foot-pad is indicated at 6 months after inoculation, if not otherwise stated by a small note in square brackets in the tables. Presence of viable bacteria in the sample from the untreated patient and loss of viability in that from the treated one means a clear-cut positive response. This is termed "I" (= improvement) in the tables. If the comparison is not made at 6 months after (MFP) inoculation but at another time and needs some interpretation, the improvement is called "Im" (= marginal improvement).

c) *Serodiagnosis by ELISA-technique* is a new tool developed by Ranade and Mahadevan (1988) to detect lepromatous leprosy. A delipified component of the cell wall of *Mycobacterium leprae* (DCW) is used as antigen and the ELISA technique with sera from the patients serves to detect the antibodies. Bacteriologically positive lepromatous leprosy patients (LL) show a relative high antibody titer, the level of which diminishes already after 3–6 months of treatment. The test is, therefore, especially suitable for our purpose. The binding ability of DCW to antibodies in the sera of the patients is expressed as OD (optical density) recorded at 492 nm after ELISA. The OD readings vary from 1.8 (very active LL) to 0.15 (healthy or cured individuals). The sensitivity of the test is the better the higher the initial reading in the untreated sample is. A general appreciation is also given, "I" meaning clear-cut improvement, "Im" marginal improvement, total improvement being the sum of the two.

Results

1. Clinical evaluation

a) Since this paper is not for leprologists, only a few typical photographs of some lesions illustrated the morphological changes before and after treatment in the oral presentation. Some patients treated with NAL alone for 6 months showed good improvement observed generally with drug treatment only.

b) The quantified data for each patient (initials) are presented in tables 3 to 5 according to the 3 treatment groups (8 patients in MDT-group, 13 for NAL and 11 for NAL + 1/2 MDT). The results are those obtained up to May 1991 and are not yet complete for each patient, but sufficient for a provisional evaluation (complete data for a minimum of 6 patients in the MDT group).

c) For the *photographic assessment* (BLP) only positive changes are given in per cent. No appreciable change is notified as static. Deterioration is not quantified (only 1 case in 32 patients)
For the 6 patients in the MDT-group for whom the data are available, 2 are improved more than 50%, 3 more than 25% and 1 is static. The total improvement is thus 5. The corresponding figures for the NAL and the 1/2 MDT + NAL group are seen in the tables which should be self-explanatory.

d) For the *clinical score* (Ramu), an average improvement is calculated, i.e. the average without deterioration. Before treatment, this mean is 15.3 for the MDT-group, 15.1 for the NAL and 10.94 for the NAL + 1/2 MDT group. After the treatment, the corresponding values are: 11.64, 12.1 and 7.6 showing a rather similar improvement in the 3 groups. The diagnosis of Dr. Ramu is also indicated.

The clinical evaluation by photographic assessment (BLP) and by the independent assessor (Ramu) shows a similar trend for a majority of patients, but is often quantatively different. Some patients who are judged static by one method are rated slightly improved or, in 2 cases, slightly deteriorated in the other. A clear discrepancy is found in one patient only, patient M.A on NAL. At BLP he was rated slightly improved (>25%), by Dr. Ramu deteriorated. These differences may be attributed to the different methodologies that do not systematically involve the same lesions per patient, and to certain small variations in timing of the different evaluations. There are also a few data that are still missing. Nevertheless, the total number of cases improved upon treatment are found to be similar with both methods of clinical evaluation. 5 out of 8 patients are improved in the MDT-group with both methods, 10 out of 13 NAL patients are improved in the BLP evaluation and 8 in Dr. Ramu's assessment, 7 out of 11 patients in the 1/2 MDT + NAL group are improved in the first procedure and 6 in the second. The diagnosis "static" is more often made by Dr. Ramu than at BLP.

2. Laboratory findings

a) The mouse foot-pad (MFP) test shows that almost all patients were in a florid state of infection before treatment, since the inoculation with the initial biopsy samples displayed adequate viability of the bacteria. Low or no initial viability was found in 2 patients only (S.M.P. and R.J.). They had received Ayurvedic treatment before the study. It may be of interest to notice it, since in the few patients who had been given sporadic Dapsone or MDT treatment before the study, the viability of the bacteria of the initial inoculation was high. In the NAL-group, 3 initial mouse foot-pad inoculations were lost. There is little doubt that they would have shown vialability as all the other patients in this group.

Table 3. Experimental findings after 6 months treatment

MDT

Patients 8	Clinical Evaluation				Laboratory Findings						Overall Imp.
	BLP	Clinical Score		Diagnosis	Mouse foot-pad data			Serodiagnosis			
	pos. change %	Initial	6 m		Initial	6 m	Diagnosis	Initial	6 m	Diagnosis	
1. V.A.J.	static	12	9	Is	$6 \cdot 10^4$	no growth(ly)	Im	0.76	0.89	static	Pr
2. M.T.	>25	21	15	I	$1.05 \cdot 10^5$ [7 m]	no growth [7 m]	I	0.97	0.69	I	Df
3. J.P.B.	>50	9	7	Is	$1.8 \cdot 10^5$	no growth	I	0.84	0.48	I	Df
4. M.K.	>75	(15)	not cl.	not cl.	$3.75 \cdot 10^4$ [7 m]	no growth (7 m)	Im	1.35	1.10	I	Df
5. S.M.P.	>25	28	21	Is	low viabil.	no growth	not cl.	1.20	0.80	I	Pr
6. Mh.M.	>25	7	7	static	$2.4 \cdot 10^5$ [7 m]	no inocul.	not cl.	(0.69)	not done	not cl.	Mr
7. W.K.G.	not available	21	13.5	I	not avail.	not available	missing	1.02	0.79	I	Pr
8. G.S.M.	not available	9	9	static	not avail.	not available	missing	0.70	0.77	static	St
Average rating (I, Is, Im, static) (s = slight) (m = marginal)		15.3	11.6					0.977	0.788		
>25 %	3	Is		3		Im	2		Im	0	Df 3
>50 %	2	I		2		I	2		I	5	Pr 3
Total Impr.	5	Total Impr.		5		Total Impr.	4		Total Impr.	5	Mr 1
Static	1			2			0			2	St 1
Deterior.	0			0			0			0	
Not clear	0			1			2			1	
Missing	2			0			2			0	

Table 4. Experimental findings after 6 months treatment

NAL

	Clinical Evaluation				Laboratory Findings						Overall Imp.
	BLP	Clinical Score		Diagnosis	Mouse foot-pad data			Serodiagnosis			
Patients 13	pos. change %	Initial	6 m		Initial	6 m	Diagnosis	Initial	6 m	Diagnosis	
1. G.B.K.	>25	16	14	Is	$2.5 \cdot 10^5$	no growth	I	0.51	0.61	static	Pr
2. S.	>25	14	11	Is	not done	no growth (3 m)	not cl.	1.35	1.07	I	Pr
3. R.P.	>50	16	16	static	not done	no growth (3 m)	not cl.	(1.04)	not on time	not cl.	NC
4. B.S.	>25	28	18	I	$1.05 \cdot 10^5$	no growth	I	0.77	0.66	Im	Df
5. M.S.	>75	15	11 (3 m)	Is	$1.17 \cdot 10^6$	no growth (3 m)	I	1.27	0.49	I	Df
6. D.K.	Deter.	9.5	9.5 (4 m)	static	$6.75 \cdot 10^5$	no growth	I	0.94	0.42	I	Mr
7. G.K.	>25	out of date		not cl.	$1.35 \cdot 10^5$	no growth [7 m]	I	1.38	0.70	I	Df
8. R.B.	>25	14	8	I	not done	no growth [8 m]	not cl.	1.27	1.15	static	Mr
9. R.P.	>75	17	14	Is	$1.8 \cdot 10^5$	no growth	I	0.92	0.70	Im	Df
10. M.A.	>25	9	17	Deter.	$1.06 \cdot 10^6$	no growth	I	0.93	1.28	Deter.	NC
11. Ch.M.	>25	14	13	Is	$3.9 \cdot 10^5$	no growth	I	0.83	0.80	static	Pr
12. Mh.A.	not done	13	13	static	$5.1 \cdot 10^5$ [7 m]	no growth	Im	0.69	0.73	static	Mr
13. Y.Sh	not done	10	6	Is	not avail.	not available	missing	1.02	0.87	I	Pr
Average rating (I, Is, Im, static) (s = slight) (m = marginal)		15.1	12.1					0.995	0.745		
>25 %	7	Is		6		Im	1		Im	2	Df 4
>50 %	3	I		2		I	8		I	5	Pr 4
Total Impr.	10	Total Impr.		8		Total Impr.	9		Total Impr.	7	Mr 3
Static	0			3			0			4	NC 2
Deterior.	1			1			0			1	
Not clear	0			1			3			1	
Missing	2			0			1			0	

Table 5. Experimental findings after 6 months treatment

1/2 MDT + NAL

Patients 11	Clinical Evaluation				Laboratory Findings						Overall Imp.
	BLP	Clinical Score		Diagnosis	Mouse foot-pad data			Serodiagnosis			
	pos. change %	Initial	6 m		Initial	6 m	Diagnosis	Initial	6 m	Diagnosis	
1. J.G.P.	missing	12	8	I	not done	no growth	not cl.	0.47	0.32 (4 m)	Im	Pr
2. K.S.	missing	10	8	Is	not done	not done	missing	not done	not done	missing	Mr
3. D.M.	static	4	6	Deter.	$6.45 \cdot 10^5$	no growth	I	0.65	0.74 (7 m)	static	St
4. S.D.P.	>25	8	4	I	$1.25 \cdot 10^5$	no growth	I	not done	(0.59) (4 m)	not cl.	Pr
5. S.P.	>25	8.5	8.5	static	$4.8 \cdot 10^5$	no growth	I	0.95	0.41	I	Df
6. S.D.L.	>25	8	7	static	$7.5 \cdot 10^4$	no growth	I	1.10	0.67	I	Df
7. S.K.	missing	17	8	I	$2.1 \cdot 10^5$	no growth	I	0.77	0.58	Im	Df
8. D.G.	>50	7	4	Is	$1.57 \cdot 10^5$	no growth [6 m] growth [7; 8 m]	not cl.	0.96	1.07	static	Mr
9. R.J.	>25	14	14	static	no viability	no viability	not cl.	0.77	0.63	static	NC
10. M.A.	>25	14	7 (3 m)	I	$7.5 \cdot 10^4$ [7 m]	no growth	Im	0.69	0.46 (3m)	I	Df
11. R.K.D.	>25	(8)	not done	missing	$6 \cdot 10^4$ [7 m]	no growth (3 m)	Im	0.42	0.74 (3m)	Deter.	NC
Average rating (I, Is, Im, static) (s = slight) (m = marginal)		10.9	7.6					0.795	0.610		
>25 % 6		Is		2		Im	2		Im	2	Df 4
>50 % 1		I		4		I	5		I	3	Pr 2
Total Impr. 7		Total Impr.		6		Total Impr.	7		Total Impr.	5	Mr 2
Static 1				3			0			3	St 1
Deterior. 0				1			0			1	NC 2
Not clear 0				0			3			1	
Missing 3				1			1			1	

After treatment, 26 patients showed complete absence of viable bacteria in the MFP inoculum, for 3 patients the data were not yet available, for 1 patient the MFP inoculation was not done, for another it was not successful, and only in 1 patient (DG) was partial viability found, i.e. no growth at 6 months after MFP inoculation, growth at 7 and 8 months. Obviously, actual *loss* of viability can only be demonstrated for the patients whose initial samples display viable bacteria. This is designated total improvement in the tables. There are 4 for the MDT-group, 9 for NAL and 7 for 1/2 MDT + NAL. Were it not for several incomplete data, the success rate would still be higher. Nevertheless, these results demonstrate that each of the 3 treatments is able to kill and to clear bacilli after 6 months treatment, in other words that they have a "bactericidal" effect. (For a final conclusion, MI-values will have to be considered also).

b) The results obtained by serodiagnosis using the ELISA technique are similar to those obtained by the MFP-test, but less clear-cut. 17 patients are improved, 9 are static, 2 deteriorated (M.A. + R.K.D.), 3 cannot be evaluated and 1 is missing. The high proportion of static cases probably signifies that for serodiagnosis, the treatment period of 6 months is still too short for some patients to react. Indeed, in 5 of the static patients marked improvement was seen after 1 year treatment (MDT or 1/2 MDT + NAL) (not published here, since NAL treatment limited to 6 months).

For the ELISA ratings, an average improvement is calculated for each treatment group. It consist of the mean of the values for the patients classified "I", "Im" or static. Before treatment, this mean rating is 0.977 for the MDT-group, 0.995 for the NAL and 0.795 for the NAL + 1/2 MDT group. After treatment, the corresponding values decrease to 0.788, 0.745 and 0.610. The almost identical ratings for MDT and NAL before and after treatment should be noticed. The good correlation between these mean ELISA ratings and the mean clinical score is also remarkable, indicating that the clinical status is linked to the level of DCW antibody in the serum. The correlation does not hold for each patient, because in this short experimental period the immunological status and the clinical picture do not evolve in an identical manner for each patient. Nevertheless, in 15 patients the improvement by serodiagnosis is paralleled by a clinical improvement (either clinical score or BLP). In patient M.A. (NAL), the deterioration in ELISA rating is accompanied by a deterioration in clinical score, but not in BLP evaluation. In patient R.K.D. there is a discrepancy between deterioration in the ELISA rating and slight improvement by the BLP evaluation.

3. Comparison between treatment groups

In consulting the summary tables, it is relatively straightforward to make an appropriate comparison of the efficiency of the 3 treatments. At first sight, the differences are small. However, since the study is still going on and the number of patients as yet uneven, some bias may be introduced in looking at the absolute figures. Therefore, in table 6 the results are given in percentage improvement and the basis is not the number of patients, but only of those patients who have

Table 6. Percentage improvement after 6 months

Treatment Group	Clinical Evaluation			Laboratory Findings	
	BLP Photogr. Assessm.			Mouse foot-pad test	Serodiagnosis
	>25%	>50%	Clinical Score	Loss of viability	ELISA ratings
NAL	63.6	27.2	19.9	75	25.1
MDT	50	33	24.2	66.6	19.4
NAL + 1/2 MDT	75	12.5	30.4	70	23.3

completed the particular test. MDT ranks highest for the above 50% percent clinical improvement by photographic assessment (BLP), lowest in the MFP-test and in the ELISA rating. NAL performs best in the MFP-test and in the serodiagnostic rating and NAL + 1/2 MDT is best in the clinical score but, in the BLP evaluation, it shows the least proportion of patients with above 50% improvement. Altogether, however, the differences are very small and do not allow to distinguish the 3 groups.

Another way is to take the loss of bacillary viability in the mouse foot-pad method as a basis for the most objective and valid comparison. In this case, the NAL-group ranks clearly highest with 9 total improvements (out of 13 patients) of which only 1 is marginal. The MDT-group is lowest with only 4 improvements (out of 8 patients) of which 2 are marginal. However, the number of patients in the MDT-group is only 8, for 2 patients the MFP-data are not yet available and in 1 patient the viable, initial bacillary load was low. On the other hand, 3 initial biopses were lost in the NAL-group.

At last, we can try to make a comparison in integrating the clinical and laboratory findings. For this overall evaluation, we use 3 criteria as basis: 1) the clinical improvement, either by photographic assessment or clinical score, or both; 2) the loss of bacillary viability by MFP; 3) the improvement by serodiagnosis. A positive response upon treatment of all 3 criteria is defined as definite improvement (Df), of any 2 of 3 criteria, partial improvement (Pr) and a positive response of 1 of the 3 criteria only, marginal improvement (Mr). Df should not be understood as "cured" in the clinical sense, it means only positive response in the 3 criteria.

No change in any of the criteria or cancellation of a deteriation in one test by an improvement in another is called static response.

In the MDT-group, 3 out 8 patients are definitely improved, in the NAL-group 4 out of 13 and in the 1/2 MDT + NAL-group 4 out of 11. Thus, the 2 groups containing MDT have the same proportion of definite improvements (approx. 37%), the NAL-group slightly less (31%).

A few patients show deterioration for some criteria but never for the 3 together. In the MDT-group, no patient displays any deterioration. In the NAL-group, patient D.K. deteriorates clinically according to the photographic assessment, not by clinical score, and improves markedly in the laboratory findings. Patient M.A. deteriorates by the clinical score and by the serodiagnosis but improves slightly in the BLP evaluation, and definitely in the MFP-test. The overall reaction to the treatment remains thus not clear (NC). In the NAL + 1/2 MDT-group, patient D.M. deteriorates in the clinical score and patient R.K.D. in the ELISA-rating. In this study so far, deterioration which would be the normal course of events in untreated lepromatous leprosy, is very rare. It is found partially in 4 out of 32 patients and in 5 out of 128 measurements. That the only patient showing deterioration for 2 criteria is in the NAL-group may be taken as a hint that the antileprosy effect of NAL is slightly inferior to that of MDT.

The provisional results of this 6 month-study using a tryptophan-enriched food (NAL), to treat lepromatous leprosy patients strongly suggest that this dietary treatment is efficient in improving the course of the disease by the

3 criteria used (clinical, MFP, and serodiagnosis). The efficiency appears to be similar, or slightly inferior, to that of MDT during the short period of 6 months. In the presence of NAL, a 50% reduction in the MDT dose level does not impair the anti-leprosy effect.

Discussion

The efficacy of this particular dietary treatment of multibacillary lepromatous leprosy is probably due to several causes. The general amelioration of the nutritional status of the patient by the food supplement could enable him to reinforce his own defense mechanisms. Prevention of leprosy by dietary means has been suggested by several authors (Foster et al., 1988), but improvement of highly infected lepromatous patients by a food supplementation is unlikely. In addition, the patients of this study did not show any overt signs of malnutrition. At the most we could, therefore, admit an adjuvant effect of the food supplement, if any.

The specific nutrient of the NAL-food is tryptophan and NAL is designed to increase free tryptophan in serum as a precursor of serotonin and possibly DFS. In the original hypothesis of Mester and Garnier (1985), the active compound synthesized *in vivo* upon ingestion of NAL is DFS. The presence of DFS in sufficient amounts in serum upon ingestion of tryptophan or NAL has not been clearly demonstrated and confirmed. We consider, therefore, the active compound to be more likely serotonin itself. Like DFS, serotonin is a potent antioxidant that could inhibit L-Dopa oxidase and the utilization of L-Dopa by *M. leprae* slowing down the growth of the bacilli. At best, this mechanism would be bacteriostatic, but not "bactericidal" as is apparently the NAL-diet (loss of viability of the bacilli in the MFP-test). Inhibition of L-Dopa oxidase is, therefore, not sufficient as an explanation of the "NAL effect". The latter must involve the immunological defense mechanisms of the host, who becomes able to kill the bacilli. Now, Mahadevan et al. (1991) presented data to show that DFS is capable of reducing the viability of phagocytosed *M. leprae*, through activation of the host macrophage, that regains the ability to kill *M. leprae* through reactive oxygen intermediates. We suggest that serotonin acts in an identical way in activating the macrophage of the host and this could explain, in part, the "bactericidal" effect of the NAL-diet. However, serotonin would have to be more active than DFS in activating the macrophages to fully explain the "bactericidal" effect of NAL, since DFS in the clinical study was bactericidal in 4 out of 6 patients only (Antia et al., 1988).

Serotonin could also participate in the trapping of the bacilli at the site of infection in activating the reticuloendothelial response as shown in murine leprosy (Kato and Gözsy, 1982).

At last, tryptophan could be used directly to replenish the drained tryptophan stores of the patient. Indeed, the discovery of the extrahepatic pathway of tryptophan degradation via indoleamine-2,3-dioxygenase (IDO) (Shimizu et al., 1978, Ozaki et al., 1987) which is induced by cancer, viral infection and bacterial lipopolysaccharides could lead to a depletion of tryptophan in critical tissues such a macrophages. Werner et al. (1987) showed that human macrophages

degrade tryptophan upon induction by interferon-gamma. Evidence is accumulating that IDO, sometimes designated as immune tryptophan-2,3-dioxygenase, plays an important role in the cellular immunodefense, and that tryptophan might be an important immunomodulator. An explanation for the efficacy of the NAL-food in the treatment of leprosy should also be saught in this direction.

From the practical point of view, it is premature to speculate about the eventual use of the NAL-food in combatting leprosy. If the positive results obtained so far are confirmed, the dietary approach may be envisaged as complement to the drug treatment that could be shortened or reduced if the immunostimulating effect of tryptophan overcomes the immunosuppressive properties of Dapsone and Rifampicin (Anderson et al., 1981; Paunescu, 1980). The NAL-food may be even more useful in the *prevention* of leprosy in particular high risk population groups.

A more definite conclusion must await the completion of the two years study.

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Authors' address: Prof J. Mauron, Avenue de Blonay 2, CH-1800 Vevey, Switzerland.

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