



EFFECT OF LEVAMISOLE ON POTENTIATION OF CELL MEDIATED
IMMUNITY IN RHEUMATOID ARTHRITIS

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SUMMARY

The leukocyte migration test (LMT) in the presence of native IgG, and, levamisole were studied in 9 patients suffering from classical rheumatoid arthritis before and after treatment with levamisole.

Fifteen healthy blood donors were similarly investigated as controls.

T- lymphocyte sub-populations (Total and active) and B-cells were also measured in both groups simultaneously by E and EAC-rosette formation

The results show a significantly diminished leukocyte inhibitory factor (LIF) in the presence of native IgG after treatment with levamisole and a significant increase of active T-cells in this group of patients.

In addition after treatment the migration index show an increase in the presence of levamisole.

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We have also observed a good correlation between the clinical response of levamisole and our immunological data.

INTRODUCTION

Recently, several authors have demonstrated that levamisole an antihelmintic drug exerts some modulatory effect on the potentiation of the cell-mediated immune (CMI) response in anergic diseases (Biniaminov et al., 1975, Dan et al., 1976, Ramot et al., 1976, Symones et al., 1977) and it has been shown to enhance cellular immune responses to animals (Renoux et al., 1976, Renoux et al., 1976, Chirigos et al., 1973, Spreafico et al., 1975) and to restore cutaneous delayed hypersensitivity in individuals with cancer and Hodgkins' disease (Tripodi et al., 1973, Levo et al., 1973). Its mode of action remains obscure in spite of the increasing amount of experimental work and experience.

Among other clinical conditions in which levamisole has been beneficially applied, are some rheumatic diseases such as rheumatoid arthritis (RA), systemic lupus erythematosus, ankylosing spondylitis, and Reiter's Syndrome (Gordon et al., 1935, Basch et al., 1975, Huskisson et al., 1976, Ippen et al., 1976, Rosenthal et al., 1976, Rosenthal et al., 1976, Schuermans et al., 1976).

The in-vitro effect of levamisole on cells from normal individuals is uncertain, some authors having found it to be stimulatory (Pabst et al., 1975, Woods et al., 1975); whilst others have not demonstrated significant immunopotentialiation (Copeland et al., 1974,

Golding et al., 1976, Ramot et al., 1976).

The objective of this study was to assess whether levamisole could restore the in-vitro competence of non responsive lymphocytes to antigen (Immunoglobulin G.IgG) as measured by leukocyte migration test (LMT) and simultaneously to assess the different lymphocyte sub-populations in patients with RA, before and 6 months after administration of levamisole to determine to what extent lymphocyte counts can be used as a possible immunological parameter for the therapeutic effect of this drug.

MATERIALS AND METHODS

Nine patients (2 males and 7 females with the mean ages of 47 years) with classical rheumatoid arthritis (RA) were studied. The diagnosis was established according to diagnostic criteria of the American Rheumatism Association (1959). All patients showed active disease with demonstrable joint tenderness, effusions and elevated erythrocyte sedimentation rate (ESR). The following parameters were studied before and 6 months after treatment by levamisole: morning stiffness, number of inflamed joints, ESR (measured by westigren technique) and rheumatoid factor (measured by latex test). All the anti-rheumatic drugs were stopped during the study.

Fifteen healthy blood donors (10 males and 5 females with the mean ages of 42 years) were selected as normal controls.

Levamisole (Janssen pharmaceutica,Beerse, Belgium)

was administered at a dose of 150 mg/per day for 3 months and then thrice weekly for 3 months.

Native IgG (Pr. Ropartz, Centre de transfusion sanguine, Rouen, France) was used in concentrations of 50, 100 and 200 $\mu\text{g}/\text{ml}$ in TC 199 medium (Difco-Lab).

-Detection of T-lymphocytes (including total and active) and B cells:

This was done by using peripheral blood lymphocytes obtained by Ficoll-Hypaque gradient separation (Boyum, 1968).

Measurement of T-cell subpopulations was performed by the technique of Jondal et al., (1972) and Wybran (1976).

Briefly, for the active T-cells test, 0.1 ml of the lymphocyte suspension containing 5×10^6 cells/ml in HBSS (Hank's Balanced Salt Solution) was mixed with 0.1 ml of a 5% washed sheep erythrocyte suspension in HBSS at room temperature. The mixture was centrifuged at 200g for 5 min. The cell button was then gently resuspended with a pasteur pipette and the percentage of rosette-forming cells was determined microscopically in a haemocytometer. At least 300 lymphocytes were counted in triplicate and only rosettes possessing three or more adherent erythrocytes were counted.

The test for total-T cells, performed entirely at room temperature, differed in that 5×10^6 lymphocytes were added to tubes containing 0.3 ml of FCS (Foetal Calf Serum) and 2% sheep erythrocytes. The tubes were spun for 5 min. at 400g, then kept vertically, overnight in a rack at 20°C , and the cells were counted in a haemocytometer the following day.

-Measurement of Erythrocyte-Antibody-Complement-

dependent cells (EAC or B-lymphocytes) was carried out as follows: Mendes et al 1974). 0.1 ml of the lymphocyte suspension containing 2×10^6 cells/ml was mixed, at room temperature, with equal volumes of human erythrocytes sensitized by heterophilic antibodies and complement (HEAC) in triplicate glass tubes. The tubes were then immediately centrifuged at 200g for 5 min. at room temperature. After centrifugation, the cell bottom was gently resuspended and the rosette-forming cells determined as described previously.

-LMT was performed by the technique of Soborg and Bendixen (1967) as modified by Rosenberg and David (1970) and described earlier (Massoud et al, 1976). The supernatant, containing leukocytes from 20 ml. of heparinized venous blood, was centrifuged at 200g. for 10 min, and the cell pellet washed three times in HBSS. The washed leukocytes were resuspended in TC 199 medium (Difco-Lab.), aspirated into capillary tubes, sealed with Seal-Ease (Hynz Company, Germany) and centrifuged for 5 min. at 300g. The tubes were cut at the cell-fluid interface and that portion of the capillaries which contained the leukocytes was placed in migration chambers. The tests were set up in triplicate. The chambers were filled with TC 199 medium + 10% FCS (Difco-Lab.) containing Ag (IgG or levamisole) at different concentrations. They were incubated for 18 hr at 37°C . The migration pattern was projected, traced and the area of migration was measured by planimetry. Inhibition of migration was considered significant when the migration index (MI) in the presence of Ag was less than 80% of the control figure without Ag. MI was expressed as:

$$MI = \frac{\text{Area of migration in the presence of Ag} \times 100}{\text{Area of migration in control chambers}}$$

RESULTS:

1- Clinical and Biological Parameters

Morning stiffness of our patients was between 30 to 240 minutes. After six months of treatment by levamisole this symptom has disappeared in 5 patients and we saw a considerable decrease in 4 other patients (Table 1).

The extent of inflammation was measured by assigning points to inflamed joints in the following manner: one point was given for each joint except hand and feet.

Prior to treatment the total points given to inflamed joints in all patients ranged between 6 to 11, whereas after treatment there were no inflamed joints in 3 cases (numbered 1, 6, 7) and the total points for inflamed joints were considerably reduced in the remaining patients (Table 1).

Prior to treatment ESR was found to range between 40 to 110 for the first hour in all patients except one (case 9), who showed normal ESR. However, after treatment, ESR was reduced in all patients (Table 1).

Rheumatoid factor was also found to decrease in all cases and become negative in one case (Table 1).

2- Distribution of lymphocyte subpopulations in normal controls and RA (Table 2).

A significant reduction of total-T cells was observed in RA patients before treatment with levamisole as compared to the normal controls ($P < 0.005$). After treat-

ment, a slight restoration of total and active T-cells appeared in comparison to normal controls, but this increase is not highly significant as compared to the finding prior to treatment.

The percentage of EAC lymphocytes (B-cells) did not differ after levamisole therapy in the RA patients.

3- Inhibition of Migration in the presence of native IgG (Table 3).

Migration Index (MI) was positive in 77.7% of RA patients as detected by LMT in the presence of 100 ug/ml native IgG. After treatment, percentage of MI was reduced significantly ($P < 0.005$) and this diminution was correlated with remission of disease.

4- Leukocyte Migration Test in the presence of levamisole (Table 3).

55.5 % of patients showed inhibition in the presence of 60 mg/ml prior to treatment, whilst 20% inhibition in the normal controls. This inhibition becomes 100% after treatment ($P < 0.005$) as compared to the finding prior to, treatment.

DISCUSSION

In the peripheral blood of R.A. patients a significant decrease of total T-cells ($P < 0.005$) was demonstrated before treatment with levamisole as compared to normal controls (Table 2), but the percentage of active T-cells and B lymphocytes remained relatively unchanged (Table 2).

A considerable correlation was shown between the biological and clinical remission and increase of total and active T-cells in patients numbered 2,3,4,6, and 8 after

Table 1: Clinical and biological parameters in 9 patients suffering from RA

NO.	Patients		Morning stiff		Number of inflam				ESR		RF		Tt		Ta	
	B	A	B	A	A	B	A	B	A	B	A	B	A	B	A	B
1	MOS.	240	15	8	0	67	17	1/40	-	60	38	36	28			
2	SHA.	120	65	10	4	110	70	1/320	1/80	30	65*	10	58*			
3	HAG.	60	0	11	4	62	16	-	-	31	59*	18	38*			
4	MOM.	30	0	6	3	45	35	1/320	1/80	30	57*	28	39X			
5	MOI.	60	0	7	7	40	34	1/80	1/40	51	48	50	44			
6	ETE.	180	0	10	0	80	6	1/320	1/20	54	69X	38	52X			
7	IAF.	180	0	6	0	55	30	1/160	1/80	44	53	26	37			
8	HOS.	120	30	10	1	75	16	1/320	1/20	38	61*	15	45*			
9	KEY.	60	5	8	1	5	8	-	-	41	39	15	22			

Mean ± SD

421% 543% 262% 403%
 10.4 10.4 12.4 10.5

ESR = Erythrocyte sedimentation rate

RF = Rheumatoid factor

Tt = Total T-cells

Ta = Active T-cells

B = Before treatment

A = After treatment

*P 0.005

xP 0.05

Table 2: Relative distribution of peripheral blood lymphocytes (Tt, Ta and B cells) among normal controls and RA patients.

Test	Cells	Normal Controls	Before	Rheumatoid Arthritis After
% of Tr, Ta and B	Tt	$73.9\% \pm 11.35$	$42.1\% \pm 10.4$	$54.3\% \pm 10.4$
cells detected by	Ta	$30.4\% \pm 9.43$	$26.2\% \pm 12.4$	$40.3\% \pm 10.5$
Rosette Formation with Mean \pm SD	B	$20.2\% \pm 4.45$	$20.8\% \pm 4.4$	$17.75\% \pm 3$

Tt = Total T Cells

Ta = Active T Cells

B = B Lymphocytes

Table 3: Percentage positivity of LMT in the presence of native IgG and levamisole, among, normal controls and RA patients.

Ag	Normal Controls	RA	
		Prior	After
IgG 100 µg/ml	0	77.7	33.3
Levamisole 60 mg/ml	20	55.5	100

LMT = Leukocyte Migration Test

Ag = Antigen

IgG = Immunoglobulin G

RA = Rheumatoid Arthritis

treatment as compared to finding prior to treatment (Table 1). However in patients numbered 7 and 9 correlation of clinical remission occurred with elevation of both total and active T-cells in former and elevation of active T-cells only in the latter case (Table 1). In contrast, there was no correlation between the clinical remission and restoration of lymphocytic subpopulations numbered 1 and 5.

It was previously shown that the inhibition of leukocyte migration in the presence of native and denatured IgG was a manifestation of the disease in R.A. (Massoud et al., 1976).

In the present study, 77.7% of R.A. showed a positive MI in the presence of native IgG before treatment with levamisole (Table 3), but the inhibition of leukocyte migration became negative in patients numbered 2,3,4 and 8 after treatment with levamisole (Table 4). Concurrent with clinical remission (Table 2). Therefore a cl-

Table 4: Migration index in the presence of 100 ug native IgG/ml or 60 ug levamisole/ml before, and after treatment with levamisole among 9RA patients.

No.	Patients	100ug IgG/ml		60ug levamisole/ml	
		B	A	B	A
1	MOS.	0.70	0.61	0.42	0.55
2	SHA.	0.54	0.92	1.05	0.61
3	HAG.	0.68	0.81	1.34	0.64
4	MOM.	0.76	0.87	1.50	0.60
5	MOI.	0.54	0.84	0.36	0.78
6	ETE.	0.98	0.88	1.13	0.69
7	JAF.	0.68	0.75	0.71	0.48
8	HOS.	0.65	0.85	0.60	0.78
9	HEY.	1.01	0.75	0.60	0.55

IgG = Immunoglobulin G

B = Before Treatment

A = After Treatment

ose correlation between the clinical and immunological aspects of the disease was postulated, and this correlation suggested that treatment by levamisole in R.A. could restore some aspect of CMI status to normal.

Because the exact mode of action of levamisole remains unknown, it was felt that it would be of interest to determine whether the drug was acting directly on the lymphocytes or not (Table 3). It appeared that the sensitivity of the lymphocytes was augmented after treatment with levamisole in patients numbered 2,3,4 and 6 (Table 4). These results are similar to those of Chan and Symones; (1975) who found that prior incubation of patient's lymphocytes with levamisole for 18 hr increased phytohemagglutinin (PHA) induced protein synthesis in 69% of hyporesponsive patients. Our results are also in agreement with those of Symones and Rosenthal (1977) and Witcomb et al (1976). It would appear therefore that in-vitro levamisole incubation of unresponsive lymphocytes not only increase their response to PHA but also restricts uncontrolled release of lymphocyte products. Kantor (1975) studied anergic patients with various infections and suggested that feedback suppression by lymphocyte products was responsible for the impaired CMI observed (Sampson et al; 1976). In our experience T-cell function, E-rosette formation and leukocyte migration inhibition was restored in some cases and a considerable correlation was shown between the biological, immunological and clinical remission in the cases mentioned above.

Attention: Due to the side effects of Levamisole,
caution should be taken in its administration.

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