

## LEVAMISOLE IN A DOUBLE-BLIND STUDY: NO EFFECT ON WARTS

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**Abstract.** Levamisole is reported to restore delayed hypersensitivity, when this is depressed. On the assumption that multiple warts, resistant to local treatment, are indicative of some immune deficiency, we chose levamisole for the treatment of warts. The dose, 150 mg daily in 3 days every second week, was given to 49 patients who had common warts and to 50 patients who had condyloma (venereal warts). After 6 weeks of double-blind study, most of the patients underwent open levamisole treatment. However, the levamisole group failed to show enhanced wart regression compared with the control group. No inter-group difference could be detected as regards change in delayed hypersensitivity after levamisole treatment, when measured by the mantoux response.

**Key words:** Warts; Levamisole

Levamisole (5) is an anthelmintic drug (26) which is reported to stimulate an immune response in animals against certain bacterial (19,6) and viral (6) infections, as well as against some tumours (20). It seems to restore the capacity to develop cutaneous delayed hypersensitivity in individuals who have anergy associated with old age and malignant diseases (27). Levamisole is believed to act by stimulating macrophages to enhanced phagocytosis (11) and a direct effect on lymphocytes has also been observed (9). A possible clinical use for levamisole has been suggested after uncontrolled trials in various diseases (5). Levamisole has demonstrated a positive effect in controlled clinical studies on advanced human breast cancer (22), recurrent upper respiratory tract infections in children (28), and in rheumatoid arthritis (12).

Verrucae vulgaris (common warts) and condylomata acuminata (venereal warts) are both skin tumours caused by a polyoma virus, but it may be that they are caused by different viruses (1). Common warts usually respond to surgical or local chemical treatment. In immunodeficient patients,

however, warts tend to recur after local treatment (23). Histopathological features of regressing plane warts show a dermal lymphocytic infiltrate suggesting a cell-mediated immune response (24), but this histopathological change may be absent from regressing common warts (3). A positive response to wart antigen in a leukocyte migration inhibition test after clearance of the warts also points to the importance of delayed hypersensitivity in the spontaneous regression of warts (15).

Contact dermatitis induced by dinitrochlorobenzene (DNCB) at the site of recalcitrant warts can hasten their rejection (7). Humoral immunity may also be involved in the production of spontaneous wart regression. Matthews and Shirodaria (14), found wart virus specific IgM in all of the warts on patients whose warts were regressing; only 12% of non-regressing warts contained wart virus specific IgM. Pyrhönen and Penttinen (16) found that complement-fixing anti-wart virus IgG and IgM were of value in predicting wart regression. Of those patients who had high complement-fixing antibody titres, 94% were free from warts within 2 months.

On the assumption that resistant multiple warts might indicate some immune deficiency, patients resistant to conventional local treatment were treated with levamisole. The results of our pilot study looked promising (10). In order to establish whether this beneficial therapeutic effect of levamisole could be reproduced, we performed a controlled clinical study.

### MATERIALS AND METHODS

49 consecutive patients with verrucae vulgaris and 50 patients with condylomata acuminata, which were refractory to conventional treatment for more than 6 months received either levamisole or placebo for a period of 6

Table 1. Patients with verrucae vulgaris before levamisole treatment

	Sex		Age (years) Median (95% C.L.)	Duration of warts (months) Median (95% C.L.)	Warts			Localization	
	♀	♂			Common	Plane	Mosaic	Plantar	Other
Placebo	20	4	22 (15-26)	24 (18-36)	17	3	4	16	8
Levamisole	13	12	20 (17-22)	24 (18-48)	13	1	11	21	4
Test P-value	Fisher $p < 0.05$		Mann-Whitney N.S., $p > 0.1$	Mann-Whitney N.S., $p > 0.1$	Fisher N.S., $p > 0.05$			Fisher N.S., $p > 0.1$	

weeks. The placebo contained 65.7 mg of lactose, whereas the levamisole tablets contained 59 mg of levamisole hydrochloride and only 7 mg of lactose. All the other ingredients as well as the red coating were identical in the two tablets. 34 of the wart patients had multiple solitary warts. 15 had "mosaic warts". Patients who might have been pregnant, were pregnant or were nursing infants were excluded from the study. Random allocation to the treatment groups was done by Janssen Pharmaceutical (Beerse, Belgium) and was blind to the treating doctors. The patients were tested for the presence of delayed hypersensitivity by challenge with 2 i.u. of purified protein derivative of tuberculin (mantoux—R23 from The State Serum Institute, Copenhagen), the dosage (0.1 ml) was given intracutaneously in the middle third of the dorsal part of the forearm on day 0 and repeated 6 weeks later. The diameter of induration was measured 48 hours after challenge. Serum albumin,  $\gamma$ -globulin, measles, rubella and mumps complement-fixing antibody titres (performed by The State Serum Institute, Copenhagen) were measured on the two days when the mantoux test was performed.

Photographs of the warts were taken on day 0 and after 6, 12, and 18 weeks and the severity of the warts was ranked in three classes, depending on the number of warts. Class 1 = 1-5 warts, class 2 = 6-10 warts, and class 3 more than 10 warts. Levamisole or placebo treatment was started 1 week after the first tuberculin test (mantoux test). The dosage schedule was 150 mg a day for 3 consecutive days and was given every other week. At the conclusion of the 6 week double-blind period, most of the pa-

tients received open levamisole treatment until the symptoms disappeared, until side effects appeared, or until the patients left the study for other reasons. No patients received levamisole for more than 1 year. After 12 weeks the oral treatment was combined with the local application of 25% glutaraldehyde once daily on the warts and 10-20% podophyllin in alcohol once weekly on the condyloma.

## RESULTS

Table I describes the wart patients' age, sex, lesion duration, severity and location. Table II describes the same variables for the condyloma patients. The male/female ratio was unevenly distributed between the two treatment groups of wart patients. The diameter of induration of the mantoux response was smaller in the levamisole-treated group of wart patients. After the double-blind period the mantoux response was greater than the pretreatment value in both wart groups—although the increase was significant only in the levamisole-treated group (17) (Fig. 1). However, no significant difference in the magnitude of the change of mantoux response was found between the two wart groups. Neither of the condyloma groups changed their mantoux response (Fig. 2). No significant difference was found be-

Table II. Patients with condylomata acuminata before levamisole treatment

	Sex		Age (years) Median (95% C.L.)	Duration of the condyloma (months) Median (95% C.L.)	Localization			Ranking			Mantoux test day 0 Induration (MM) Median (95% C.L.)
	♀	♂			Anal	Genital	Both	+	++	+++	
	Placebo	12	13	23 (21-26)	10 (6-12)	6	11	7	0	15	10
Levamisole	11	14	24 (21-26)	6 (6-18)	3	14	5	2	14	9	18 (14-22)
Test P-value	Fisher $p > 0.2$		Mann-Whitney N.S., $p > 0.1$					Mann-Whitney N.S., $p > 0.1$			

Ranking			Mantoux test day 0 Diam. of induration (mm) Median (95% C.L.)
+	++	+++	
6	11	7	18 (15-22)
3	10	12	13 (9-18)
Mann-Whitney N.S., $p > 0.1$			Mann-Whitney $p < 0.05$

tween levamisole and control groups as regards number of regressions, change in class ranking, serum albumin,  $\gamma$ -globulin, rubella, measles or mumps titres (Tables III and IV). Among the two wart groups, the number of reported side effects was equally distributed (Table III). The condyloma patients receiving placebo tablets reported no side effects (Table IV). Of 25 condyloma patients who received levamisole 6 reported side effects. Results from the open levamisole treatment after 6 weeks of double-blind treatment are summarized in Table V (warts) and Table VI (condyloma). There were no more warts or condyloma regressions in the groups

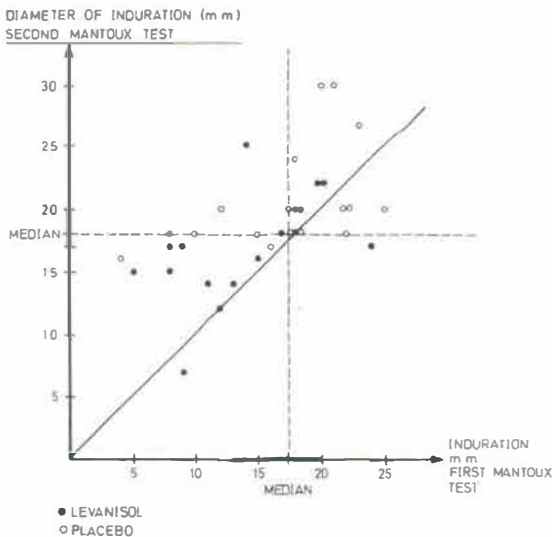


Fig. 1. *Verrucae vulgaris*. Only the levamisole-treated patients demonstrate a significant increase in mantoux response (Pratt test (3)  $p < 0.01$ ), but the difference between the two groups was not significant (Mann-Whitney U-test,  $p > 0.1$ ). 1st mantoux response below median: significant increase (Pratt test,  $p < 0.01$ ) 1st mantoux response above median: N.S.,  $p > 0.05$ .

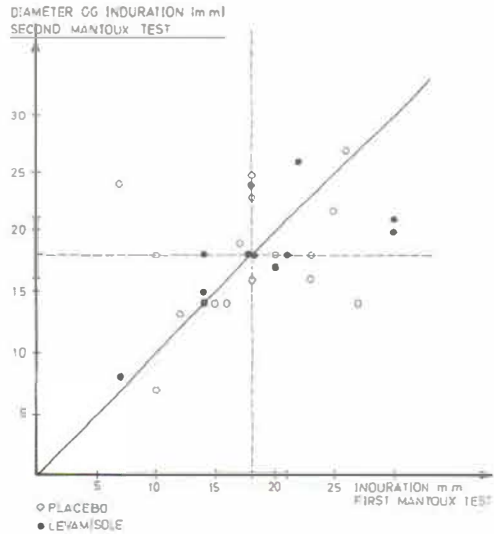


Fig. 2. *Condylomata acuminata*. No group demonstrates a significant increase in the mantoux response and no significant difference was found.

of patients who received levamisole from the start of the 6-week double-blind period than there were in the groups who received a placebo for the first 6 weeks of the study. Furthermore, the patients who received levamisole from the start actually required a total treatment period that was not statistically shorter than for the groups receiving the placebo.

## DISCUSSION

Levamisole had no clinical benefit in this controlled study of its use in treating condylomata acuminata and verrucae vulgaris. The duration of the double-blind treatment was fixed at 6 weeks because of our experience with the rapid resolution of common warts in children treated with levamisole (10). Perhaps if the controlled treatment period had been longer more patients would have benefitted. In the combined groups (levamisole + placebo) only 4 of 49 wart-patients regressed and only 11 improved their clinical ranking during the 6 weeks of double-blind treatment. Among the condyloma patients in the combined groups, 10 worsened, 17 improved, and 3 regressed.

Of the patients whose warts regressed during the open-treatment period, those who started with 6 weeks of placebo treatment regressed earlier. This earlier regression was not statistically significant (Table III).

Table III. The effect of levamisole on verrucae vulgaris (week 6 compared with week 0)<sup>a</sup>

C.F.ab=Complement-fixing antibody titer

	Ranking			No. of regressions	Serum albumin			$\gamma$ -Globulin			C.F.ab to mumps			C.F.ab to measles			Side effects	
	$\downarrow$	$\uparrow$	0		$\uparrow$	$\downarrow$	0	$\uparrow$	$\downarrow$	0	$\uparrow$	$\downarrow$	0	$\uparrow$	$\downarrow$	0	+	0
Placebo	6	1	17	3	6	8	(1)	7	6	(2)	2	0	(17)	4	2	(14)	6	20
Levamisole	5	0	20	1	7	9	(1)	5	10	(2)	5	7	(9)	2	7	(13)	6	21
Test	Fisher			Fisher	Fisher			Fisher			Fisher			Fisher			N.S.	
P-value	N.S., $p>0.2$			N.S., $p>0.2$	N.S., $p>0.2$			N.S., $p>0.2$			N.S., $p>0.2$			N.S., $p>0.2$			N.S.	

Table IV. The effect of levamisole on condylomata acuminata (week 6 compared with week 0)<sup>1</sup>

	Ranking			No. of regressions	Serum albumin			$\gamma$ -Globulin			C.F.ab to mumps			C.F.ab to morbilli			Side effects	
	$\downarrow$	$\uparrow$	0		$\uparrow$	$\downarrow$	0	$\uparrow$	$\downarrow$	0	$\uparrow$	$\downarrow$	0	$\uparrow$	$\downarrow$	0	+	0
Placebo	4	10	11	1	8	6	2	5	7	4	3	3	16	4	7	12	0	25
Levamisole	6	7	12	2	5	6	2	2	9	2	1	3	15	4	4	14	6	19
Test	Fisher			Fisher	Fisher			Fisher			Fisher			Fisher			Fisher	
P-value	N.S., $p>0.2$			N.S., $p>0.2$	N.S., $p>0.2$			N.S., $p>0.2$			N.S., $p>0.2$			N.S., $p>0.2$			$p<0.05$	

<sup>a</sup> Value of the parameters after 6 weeks of Levamisole treatment compared with the value of the parameters before treatment:

$\uparrow$ , post-treatment value greater than pretreatment value;  $\downarrow$ , post-treatment value lower than pretreatment value; 0, no change.

Levamisole modulates mouse immune responsiveness; the direction of change depends partly on the drug dosage (21). Irrespective of body weight, the patients received 150 mg levamisole daily on 3 consecutive days every second week. Thus we might have missed the optimal dosage for some of the patients. We chose this dosage schedule as Verhaegen et al. (29) found it to be more effective than continuous daily treatment in enhancing a DNCB response. In our pilot study a few condyloma patients received 150 mg levamisole daily on 3 consecutive days every week and this dosage schedule

was no better than the one we used. Multiple solitary warts often recur spontaneously, whereas "mosaic warts" (i.e. multiple confluent warts) seldom do so (18). Thus the confusing of several wart diagnoses might have obscured our study.

Levamisole is a low toxicity drug (26). Patients may report the same symptoms whether they are treated with a placebo or with levamisole (2). None of the condyloma patients who received the placebo complained of nausea or abdominalia, whereas 5 of the 25 patients who received levamisole did so. In the wart group the number of patients who had side

Table V. The effect of levamisole on verrucae vulgaris (open levamisole treatment included)

	Total no. of regressions	Duration of treatment of the patients whose warts regressed <sup>a</sup>	Duration of treatment (all patients) <sup>a</sup>
		Median (95% C.L.)	Median (95% C.L.)
Placebo first 6 weeks	8	15 (6-52)	19 (12-28)
Levamisole from day 0	10	30 (10-36)	32 (18-40)
Test	Fisher	Mann-Whitney	Mann-Whitney
P-value	N.S., $p>0.2$	N.S., $p>0.1$	N.S., $p>0.1$

Table VI. The effect of levamisole on condylomata acuminata (open levamisole treatment included)

	Total no. of regressions	Duration of treatment of the patients whose condyloma regressed <sup>a</sup> (Weeks) Median (95% C.L.)	Duration of treatment of all the patients <sup>a</sup> (weeks) Median (95% C.L.)
Placebo first 6 weeks	11	18 (11-34)	20 (14-29)
Levamisole from day 0	11	18 (6-28)	18 (12-26)
Test		Mann-Whitney	Mann-Whitney
P-value		N.S., $p > 0.1$	N.S., $p > 0.1$

<sup>a</sup> 6-week double-blind period included, but no conventional treatment prior to this.

effects was identical in the two treatments groups. Patients treated with the placebo never complained of the drug's sulfur smell, nor of urticaria. However, among all our patients we saw urticaria in 2 out of 50 who received levamisole and in none of the 49 who received the placebo. Because the 2 urticaria patients received no medication other than levamisole and had no infection, we felt that levamisole was the most likely cause of their urticaria. For ethical reasons we did not attempt to provoke their urticaria by re-administering the drug.

The condyloma and wart groups are drawn from a BCG-vaccinated population. We therefore felt that the mantoux test was an adequate indicator of ability to develop a cell-mediated immune response. No change in the induration after injection of tuberculin was found in the two condyloma groups. The induration increased in both wart groups in those patients whose initial mantoux response was low (less than the median). This might be a result of sensitization due to repeated mantoux testing (13, 8). K. Thestrup-Pedersen (25) only found an enhanced mantoux response on retesting those individuals who had a weak initial response. He found suppression of the mantoux reaction when retesting those individuals who had a strong positive reaction at their first test. We found no suppression on retesting. Both wart groups increased their mantoux response at their second test—although to a significant degree only in the group who received levamisole. A possible explanation is that the wart patients who received levamisole had a significantly lower mantoux response at their first (pretreatment) test than the patients who received the placebo. Depressed mantoux reactivity has been observed in children with common warts (4). This depressed

tuberculin sensitivity could be a predisposing factor or a result of the virus infection. Whether or not this depression changes after regression is not known. Our 4 patients (3 placebo and 1 levamisole) whose warts regressed during the double-blind period did not increase their cell-mediated immune response as measured with the mantoux test. Thus, if the wart virus depresses the cell-mediated immune response, it must occur early in the infection.

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