Acta Oncologica Vol. 30 No. 3 1991

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LEVAMISOLE IN THE TREATMENT OF ADVANCED BREAST CANCER

A ten-year follow-up of a randomized study

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Abstract

A total of 101 patients with advanced breast cancer were treated during the years 1976 to 1978 with doxorubicin, vincristine and cyclophosphamide, and randomized to receive either levamisole or placebo in a double-blind fashion. The chemotherapy cycles were repeated every four weeks, and levamisole, 2.5 mg/kg, was given on two consecutive days every week except on the days chemotherapy was given. The patients treated with levamisole exhibited higher response rates (63%) than patients given placebo (47%). The survival rate was also significantly higher in the levamisole group. The results correlated with potentiation of intracutaneous PPD test. In the lymphocyte blast transformation tests, the suppression of T-cell response to mitogens two weeks after the start of chemotherapy was markedly diminished by levamisole. In contrast to some negative reports, the results of the present study are encouraging for further evaluation of levamisole and other biological response modifiers in breast cancer.

Key words: Breast cancer, advanced, levamisole, randomized trial.

In the 1970s, several approaches to active, non-specific immunotherapy were made in cancer treatment since some studies had suggested a correlation between immunological parameters and survival and response to antineoplastic therapies (1–3). Levamisole, due to its oral administration, had practical advantages as an immunopotentiator compared to bacterial vaccines. Clinical studies of levamisole in advanced breast cancer were initiated as some studies had suggested that this drug improves the response rate after cytotoxic chemotherapy. The first published clinical studies in advanced breast cancer were encouraging (4–7), also suggesting a correlation between the potentiation of intracutaneous delayed hypersensitivity reactions and the response rate. Some reports with negative results of levamisole in breast cancer have since been published (8, 9). With this background our preliminary study of levamisole in stage IV breast cancer (7) has now been reanalyzed after ten years of follow-up. Changes in tests for lymphocyte blast transformation to various mitogens have also now been analyzed in part of the patients.

Patient selection. One hundred and one female patients with disseminated breast cancer were entered into a randomized, double-blind study to receive either levamisole or placebo in addition to polychemotherapy during the years 1976 to 1978. Forty-eight patients received levamisole and 53 patients placebo. Female patients with metastatic or recurrent breast cancer were eligible if they 1) were younger than 75 years, 2) had not received previously chemotherapy or immunotherapy, 3) had no other malignancies in their history, 4) were mentally cooperative, and 5) were expected to live at least 2 months (to the third cycle of chemotherapy).

Treatment schedule. The patients received cyclic VAC polychemotherapy (vincristine 1 mg and doxorubicin (Adriamycin) 40 mg/m^2 on day 1, cyclophosphamide 150 mg/m^2 on days 2–6) which was repeated every 4 weeks. Doxorubicin was discontinued if any signs of cardiotoxicity appeared. The total dose of doxorubicin was limited to 500 mg/m^2. The patients received levamisole 2.5 mg/kg/day or placebo on two consecutive days a week, on days 8, 9, 15, 16, 22 and 23 of the 28-day cycle. The plan was to give at least 6 courses of chemotherapy and to continue levamisole for a total of one year, if response to the therapy was achieved. Up to nine courses were, however, usually given in patients with measurable disease. There were no differences in the planned number of VAC

Submitted 9 April 1990.
Accepted for publication 19 June 1990.

courses between the immunotherapy and control groups because of the randomization method. If no response was achieved after 3 courses of chemotherapy, the treatment was changed into CVMF (cyclophosphamide, vincristine, methotrexate and 5-fluorouracil) and hormones.

Immunocompetence tests. The immunocompetence of the patients was followed by intracutaneous tuberculin tests and micromitogen stimulation tests. Skin tests were performed with a protein purified derivative of BCG (PPD) as recall antigen. PPD was injected intradermally in doses of 0.1 and 1.0 TU (tuberculin unit). No primary immunization was applied, as tuberculosis used to be a common disease in Finland, and most Finns have been vaccinated with bacillus Calmette-Guerin (BCG). The reaction was measured on the third day after intradermal injection. The tests were made before the first course of chemotherapy and on the fourth week after the sixth course of the last course of chemotherapy.

The immunocompetence of the last 25 patients in the study was followed by lymphocyte blast transformation tests, as modified by Hall & Gordon (10). These tests were performed on days 1 (before chemotherapy), 15 and 28 just before the second course and repeated again before the fourth course of chemotherapy. Eighteen patients (9 in the levamisole and 9 in the placebo group) were tested as planned after completed three courses. Blood samples of 8 healthy laboratory workers aged 20 to 38 years were used as controls. The mitogens used were PHA (phythemagglutinin), PWM (pokeweed mitogen), and ConA (concanavallin A).

Response definitions. The response criteria were those defined by UICC. The study included 16 patients (8 patients in each group) who had no measurable tumors at the beginning of the treatment. These patients had recurrent disease in soft tissues or bones, and were treated with either surgery or radiotherapy before the start of chemotherapy. Survival was determined from the start of chemotherapy.

Statistical methods. Survival curves were constructed according to the life-table method using the BMDP computer program 1L (BMDP-1983). The program also evaluated the difference in survival between the groups using the log-rank or Mantel-Haenszel test. Fisher's exact test was used for frequency tables and Student's t-test for immune competence tests.

Clinical features of the study groups. The patient characteristics were comparable in the two groups in terms of age, number of pre- and postmenopausals, number of patients with previous hormone treatments, and sites of metastases (Table 1).

Results

Clinical results

The response rate was significantly higher ($p = 0.03$) in the VAC plus levamisole group than in the VAC plus placebo group (Table 2) when remissions were compared to disease progression. In the levamisole group, 63% of patients responded, compared to 48% in the placebo group. The number of responding patients in the levamisole group seemed to be higher when metastases occurred in the viscera and soft tissues (Table 3). The response rate for bone metastases was not high, and there was no apparent difference in response rate between levamisole and control patients. The survival curves (Figs 1 and 2), however, showed superior survival in the levamisole groups independent of sites of metastases ($p < 0.04$ and 0.05).

Survival curves for the total groups are presented in Fig. 3. The median survival was 33 months in the levamisole group and 19 months in the placebo group. The difference between the groups is highly significant ($p < 0.005$).

Fig. 1 shows survival of patients with bone metastases or local recurrences. The median survival was 43 months in the levamisole group, compared to 23 months in the

Table 1

Clinical features of patients in the different treatment groups

	VAC plus levamisole	VAC control
Total number of patients	48	53
Median age (range) in years	56 (19–69)	53 (37–74)
Median disease-free interval (range) in months	23 (0–112)	16 (0–109)
No. of premenopausal patients at diagnosis	18 (38%)	19 (39%)
No. of patients with previous hormone treatment	19 (40%)	23 (43%)
Site of metastases (No. of patients)		
Lymph nodes	6	2
Skin	4	6
Bones	18	20
Lung + pleura	16	16
Liver	7	10
NED*	8	8

*NED = no measurable lesions at the start of treatment.

Table 2

Response rates to treatment

	Number of patients (%) treated with	
	VAC plus levamisole	VAC control
Remission	25 (63%)	21 (47%)
Complete	11 (28%)	5 (11%)
Partial	14 (35%)	16 (36%)
Stabilization	10 (25%)	9 (20%)
Progression	5 (12%)	15 (33%)

Table 3

Response rate according to site of metastases

	VAC plus levamisole	VAC control
Lungs	14/16 (88%)	10/16 (63%)
Liver	5/7 (71%)	6/10 (60%)
Bones	10/18 (56%)	10/20 (50%)
Local	6/10 (60%)	1/8 (13%)

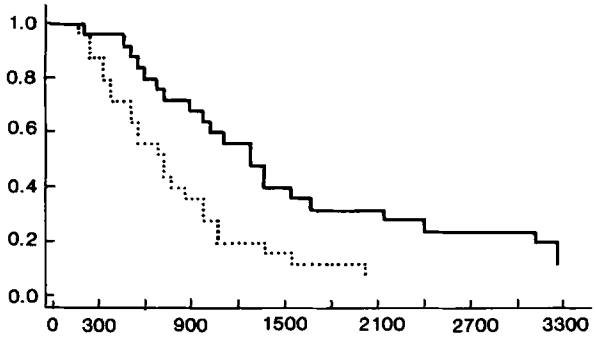

Fig. 1. Survival (days) of patients with bone or local metastases treated with VAC plus levamisole and VAC alone ($p = 0.04$). —— = Lavamisole ($n = 25$); · · · · = Control ($n = 23$).

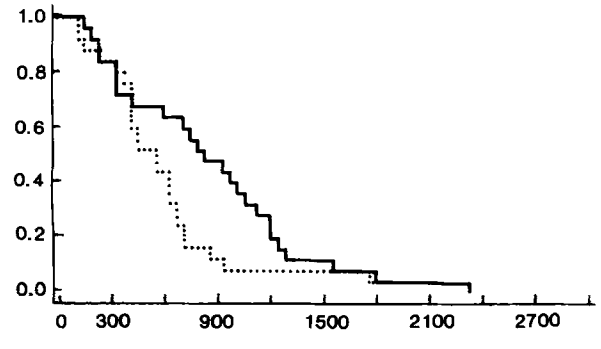

Fig. 2. Survival (days) of patients with visceral metastases treated with VAC plus levamisole and VAC alone ($p = 0.05$). —— = Levamisole ($n = 23$); · · · · = Control ($n = 28$).

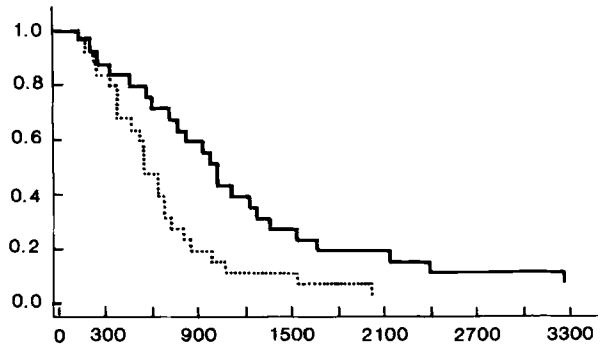

Fig. 3. Survival (days) of patients treated with VAC plus levamisole and VAC alone ($p = 0.005$). —— = Levamisole ($n = 48$); · · · · = Control ($n = 51$).

control group ($p < 0.04$). Fig. 2 shows survival of patients with visceral metastases. The median survival in the levamisole group was 27 months compared to 18 months in the control group (nearly significant, $p = 0.06$).

The median disease-free interval in the levamisole group was slightly longer (130 weeks) than in the control group (107 weeks), but the difference was not significant ($p = 0.28$). In the regression analysis (Cox's proportional hazard model) the site of metastases, the response, and the levamisole treatment showed significance. The influence of the disease-free interval did not reach significance.

Immunocompetence

A positive correlation was observed between immunocompetence measured by PPD skin tests and remissions achieved, as published earlier (7). In the levamisole group, with exclusion of the two patients who were positive to 0.1 TU before treatment, (11 patients), potentiation of PPD skin tests occurred in all patients with complete remission (Table 4). Potentiation of skin test to tuberculin was seen in 5 of 14 patients with partial remission and 3 of 8 patients with no evidence of measurable disease. In patients with stable or progressive disease or in those not receiving levamisole, no similar potentiation of skin tests was observed.

Table 4

Potentiation of skin reactions to PPD according to treatment responses

	VAC plus levamisole	VAC control
Remission	14/25 (56%)	3/21 (14%)
Complete	9/11 (92%)	1/5 (20%)
Partial	5/14 (36%)	2/16 (13%)
Stabilization	1/10 (10%)	2/9 (22%)
Progression	1/5 (20%)	-/11 (-)
No evidence of disease	3/8 (38%)	-/8 (-)

Table 5

Mitogen stimulation levels of the patients during the first VAC course and just before the fourth. Results from whole-blood microassays expressed as percentages of the median of normal controls ± SD

	Day 1	Day 15	Day 28	Day 28/3rd cycle
LMS	21 ± 8	18 ± 16	33 ± 9	26 ± 20 PHA
Placebo	14 ± 6	5 ± 3	26 ± 15	19 ± 16
LMS	30 ± 16	19 ± 16	25 ± 5	25 ± 16 PWM
Placebo	16 ± 18	6 ± 2	42 ± 20	22 ± 14
LMS	22 ± 7	17 ± 6	26 ± 8	21 ± 12 ConA
Placebo	12 ± 9	3 ± 2	26 ± 14	19 ± 14

Pretreatment mitogen levels of breast cancer patients and healthy controls (DPM ± SD).

	Bg	PHA	PWM	ConA
Patients	597	10906	2067	5706
(n = 25)	±546	±12903	±3656	6186
Controls	451	49430	7997	27093
	±219	±19700	±4716	±11878

Changes in lymphocyte blast transformation tests

The changes in response to various mitogens are presented in Table 5. Before the start of chemotherapy the responses of lymphocytes of the 25 breast cancer patients to the mitogens were significantly ($p < 0.01$) weaker than those of the healthy control persons. In the levamisole group, the original response levels were higher but they showed markedly smaller decrease on day 15, during the chemotherapy-induced bone marrow depression compared to the placebo group. There was, however, a higher subsequent rise in the placebo group during the recovery from the depression than in the levamisole group. There were also differences in the responses of the different lymphocyte subpopulations to the mitogens; in the placebo group, the PWM (helper) subpool was predominant after recovery, whereas the PHA subpool predominated in the levamisole group. There seemed to be a slight tachyphylaxis to boost reactions in the placebo group in the response of lymphocytes to mitogens before the fourth VAC cycle, and the responses were again slightly higher in patients treated with levamisole. In the levamisole group the responses to PHA remained the most active. In the placebo group a later rise in response to ConA could be seen, but response to PWM remained the highest.

Side-effects

The side-effects of the chemotherapy were moderately well tolerated. Transient alopecia occurred in most patients as well as nausea, vomiting and bone marrow depression, but the toxicity was limited to grade I or II. One patient had to stop the doxorubicin treatment due to cardiotoxicity.

Agranulocytosis was encountered unexpectedly often in the levamisole group, in which six patients developed agranulocytosis. The diagnostic criterion for agranulocytosis was that the leukocyte count did not recover after chemotherapy before levamisole treatment was interrupted. Agranulocytosis without any depression of other blood cells was then found both in peripheral blood and bone marrow. After cessation of levamisole treatment, the bone marrow recovered during the following 2–3 weeks without fatal complications. Similarly as in our other studies on levamisole in different stages of breast cancer, the development of agranulocytosis correlated positively with disease-free and overall survival (11, 12). One patient died after 18 months from the start of chemotherapy. Five patients survived more than 3 years, and three of them more than 9 years.

Seven additional patients had to stop levamisole, 4 due to granulocytopenia, two because of allergic skin reactions and one because of gastric pain. One patient in the levamisole group developed, and later died from, cerebral atrophy without signs of recurrent disease.

Discussion

Levamisole has no direct cytotoxic effect on tumor cells in therapeutic concentrations. Recently beneficial effects of levamisole and 5-fluorouracil were reported in Duke C colon cancer (13). However, levamisole was not shown to potentiate the cytotoxicity of 5-fluorouracil on colon cancer cells in vitro by Grem & Allegra (14). Levamisole has been shown to possess many different effects on immunity. It has proved to be an antianergic drug, and its effectivity is probably in part based on the active-SH group of its metabolite OMPI. Levamisole has also been shown to restore natural killer cell activity in hepatoma patients, but not, unlike interferon, to augment the action of normal NK-cells (15). Levamisole furthermore inhibits soluble immune response suppressors induced by interferon (16). According to findings by Obriri et al. (17) levamisole does not enhance the synthesis of interleukin-2 in lymphocytes, but augments its effectivity and prohibits its toxic effects (18). In a study by Han et al. (19) levamisole inhibited the effect of suppressor monocytes on T-lymphocyte response. Levamisole augmented the immune competence of spleen cells (20) in immunosuppressed C57BL mice when given 5 to 8 days after BCNU, but remained ineffective when given 1 day before or simultaneously with BCNU.

As an immunomodifier levamisole can be expected to be of benefit when given 1) at an early stage to prevent metastases in immunocompromised hosts, 2) as an adjuvant to effective chemotherapy to prevent drug-related immunosuppression, 3) properly timed, i.e. simultaneously with or immediately before cytostatic drugs (20, 21).

The present study suggested that levamisole improved both the response rate and survival. The potentiation of skin tests correlated positively with the therapeutic results, as found also by Rojas et al. (4) and Aboud et al. (22). Levamisole also seemed to be immunoprotective through earlier restoration of responsiveness to different mitogens after chemotherapy. In the control group the PWM (helper) subpool was predominant after recovery, while the PHA subpool predominated in the levamisole group. This is interesting, since in the studies by Rich et al. (23) suppressor-induced cells promoted T-suppressor cell growth following stimulation of peripheral blood mononuclear cells with pokeweed mitogen. Another interesting phenomenon was the correlation of levamisole-induced agranulocytosis with a better prognosis. In favourably reacting cases levamisole treatment has been found to induce a serum factor which itself causes immunostimulation in other individuals and in vitro (24). A serum factor which causes the degradation of granulocytes has also been found in patients developing agranulocytosis (25). The studies of levamisole in stage IV breast cancer by the SWOG (9) and SEG (8) gave different results. In the SWOG study the disease-free survival was unexpectedly short, only 4 months, in patients treated with levamisole. The chemotherapy program was CMFP including corticosteroids. In the SEG trial, the regimen used was FAC (fluorouracil, doxorubicin, and cyclophosphamide) given in 21-day cycles. Levamisole was given on days 4, 5, 11, 12, 18, 19 of each cycle. The patients with bone metastases only reacted poorly to chemotherapy. In the patients with soft tissue metastases the response rate in the levamisole group was 47% and in the placebo group 39%, which difference was not statistically significant. The median survival of patients in the SEG study was 20 months in the levamisole group and 18 months in the placebo group. In the current study, the median survival in patients with bone and/or local metastases was 42 months in the levamisole group and 23 months in the placebo group. In the SEG and the current study the median survival of patients given placebo seems to be the same. The chemotherapy regimens (FAC/VAC) were only slightly dissimilar. In the current study, levamisole did, however, markedly increase the median survival. The SEG study reported no changes in immunocompetence of patients or increased rates of levamisole-related agranulocytosis. There was a minor difference in timing of levamisole in these studies, levamisole in the SEG study preceding the courses of chemotherapy by two days only.

The beneficial survival effects of levamisole in combination with fluorouracil in Duke C colon cancer are similar to the results of our studies in breast cancer. There seems to exist a subgroup of breast cancer and colon cancer patients who benefit from levamisole treatment. When in addition levamisole seems to increase the efficacy of interferons or interleukin 2, further clinical research on levamisole and levamisole in combination with other biological response modifiers in breast and colon cancer is warranted.

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