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Inhibitory Effect of Levamisole and Adriamycin on Rat Mammary Cancer Induced by 7,12-Dimethylbenz(a)anthracene

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Abstract—*Effects of levamisole (LMS) and adriamycin (ADR) on the growth of rat mammary cancer induced by 7,12-DMBA were studied. Both LMS and ADR, when administered alone, significantly inhibited growth of small tumors. When the tumor was large, however, LMS alone or ADR alone was not effective while concurrent administration of LMS and ADR resulted in inhibition of tumor growth.*

INTRODUCTION

LEVAMISOLE had been used as an anthelmintic for animals and man. Since Renoux *et al.* [1] reported in 1971 the effectiveness of levamisole against *Brucella abortus* infection, this product has been studied from an immunological viewpoint. It has been demonstrated step by step that levamisole restores immunological competence and enhances defense mechanism of the organism by improving delayed hypersensitivity reactions to PPD and DNCB [2] and stimulating macrophages [3]. However, data on the effectiveness of levamisole against animal experimental tumor are controversial: levamisole has antitumor effect [4–6] or levamisole is not effective [7–10].

The immunological competence of a cancer patient is generally depressed and is deteriorated further by surgical operation and radiotherapy. This is also the case with chemotherapy [11]. We are prescribing a postoperative adjuvant chemotherapy to Stage III and a chemotherapy to advanced mammary cancer patients using adriamycin and cyclophosphamide [12]. We find it interesting to know whether levamisole improves immunological competence depressed by the chemotherapy and whether levamisole is effective against experimental mammary tumor. In this paper, effects of levamisole and adriamycin on the growth of rat mammary cancer induced by 7.12-dimethylbenz(a)anthracene are reported.

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MATERIALS AND METHODS

Female Sprague–Dawley rats of 8 weeks of age (weighing 180–200 g) were fed, in a single dose, 20 mg of 7,12-DMBA [7,12-dimethylbenz(a)anthracene] dissolved in 1 ml of olive oil and those whose tumor grew to 0.5–1.5 cm in major axis 8–12 weeks after the feeding were admitted to the experiment. Some rats had two or more tumors. Histological examination after experiment confirmed diagnosis of adenocarcinoma for all the tumors tested. The size of a tumor was expressed as the product of the major axis (cm) and the axis making a right angle with the former. Measurement of tumors was performed once a week.

In accordance with the report of Sampson *et al.* [6] levamisole (LMS, supplied by Kyowa Hakko Kogyo Co., Ltd.) was administered by means of a stomach tube in a daily dose of 4 mg/ml H_2O/kg 6 days a week for 3–11 weeks. Adriamycin (ADR, supplied by Montedison Pharmaceuticals K.K.) was injected into the tail vein in three doses of 2 mg/2 ml physiological saline/kg at 2-week intervals between doses.

There were nine experimental groups marked with alphabets A to I. Group A was a non-treated control group with mammary tumors of about 0.25 cm^2 in size (12 animals). Group B received LMS continuously by oral route after the mammary tumor had grown to 0.25 cm^2 (14 animals). Group C received three doses of ADR through the tail vein at 2-week intervals between doses after the mam-

mary tumor had grown to about 0.25 cm^2 (4 animals). Group D, having tumors of about 0.25 cm^2 in size, received daily LMS for 3 consecutive weeks and then three doses of ADR administered through the tail vein at 2-week intervals between doses (4 animals). Group E, having tumors of about 0.25 cm^2 in size, received three doses of ADR at 2-week intervals between doses and then LMS continuously (8 animals). Group F was a non-treated control group with tumors of about 1.5 cm^2 in size (8 animals). Group G received LMS for 3 consecutive weeks after the tumor had grown to about 1.5 cm^2 (5 animals). Group H received three doses of ADR at 2-week intervals between doses after the tumor had grown to about 4.0 cm^2 (3 animals). Group I was subjected to a concurrent administration of ADR given three times at 2-week intervals and LMS given for 3 consecutive weeks, after the tumor had grown to about 1.5 cm^2 (5 animals). In these groups, LMS and ADR were evaluated for their inhibitory effect on mammary gland tumor induced by 7,12-DMBA.

RESULTS

As shown in Fig. 1, there was a statistically significant difference ($P<0.05$) in the 4th week of experiment between group A in which tumors continued to grow and group B in which tumors did not show any growth even 10 weeks after the start of LMS. The difference became more marked thereafter.

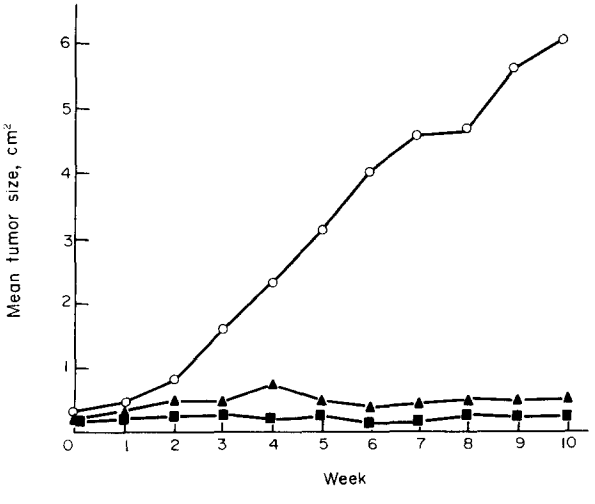

Fig. 1. Growth inhibition by levamisole and adriamycin in small tumors. Group A (○—○): control (17 tumors). Group B (▲—▲): LMS (levamisole), consecutive days (24 *tumors). Group C (■—■): ADR (adriamycin), in* weeks 0, 2 *and* 4 (6 *tumors).*

Tumors in group C did not show growth even 10 weeks after the start of ADR and the difference between these tumors and those in group A became significant ($P<0.05$) in the 6th week.

As shown in Fig. 2, the regimen consisting of 3-week LMS followed by three doses of ADR and another regimen consisting of ADR followed by LMS proved to be antitumoral; in other words, both groups D and E were significantly different ($P<0.05$) from group A as early as in the 4th week.

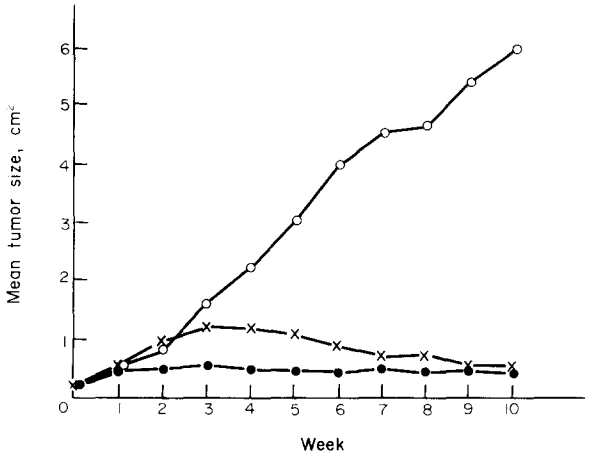

Fig. 2. Inhibition of tumor growth by levamisole preceded or followed by adriamycin. Group A (○—○): control (17 tumors). Group D (×—×): LMS→ADR, LMS for 3 consecutive weeks *followed by ADR in* weeks 3, 5 *and* 7 (5 *tumors). Group E (●—●): ADR→LMS, ADR in* weeks 0, 2 *and* 4 *followed by LMS on consecutive days* (15 *tumors).*

As to experimental groups receiving treatment after the tumor had grown to about 1.5 cm^2, there was no significant difference between groups F and G and between groups F and H (Fig. 3).

Concurrent administration of ADR and LMS exhibited antitumor effect in and after the 4th week and the difference between groups F and I became significant ($P<0.05$) in the 7th week.

The above findings can be summarized as follows: (1) Both LMS and ADR, when administered alone, significantly inhibited growth of a small tumor of 0.25 cm^2 in size. (2) When the tumor was as small as in (1), similar antitumor effect was noted with a combination of ADR and LMS whether ADR was preceded or followed by LMS. (3) Against a tumor exceeding 1.5 cm^2 in size, LMS alone or ADR alone was not effective while concurrent administration of ADR and LMS was effective.

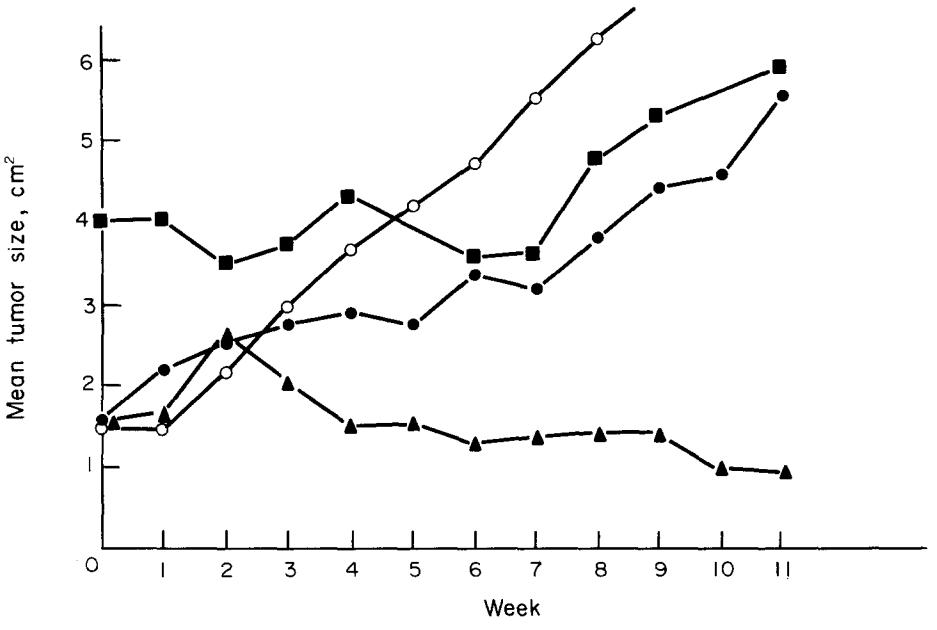

Fig. 3. Growth by levamisole and adriamycin in relatively large tumors. Group F (○—○); control (17 tumors). Group G (●—●): LMS, consecutive days (11 tumors). Group H (■—■): ADR, in weeks 0, 2 *and* 4 *(3 tumors). Group I (▲—▲): LMS+ADR, LMS on consecutive days and ADR in* weeks 0, 2 *and* 4 *(6 tumors).*

DISCUSSION

To get the full benefit of various cancer therapies, it is of importance to restore depressed immune functions of the host in a positive fashion. An immunostimulator is indispensable for this purpose.

In the experiments reported here, effects of LMS and ADR on the growth of rat mammary cancer induced by 7,12-DMBA were determined. Both LMS and ADR, when administered alone, inhibited growth of small tumors. When the tumor was large, however, LMS alone or ADR alone was not effective while concurrent administration of LMS and ADR resulted in inhibition of tumor growth.

The effectiveness of LMS in transplantable experimental tumor systems is variable according to the type of tumor, mode of administration, dose and other factors.

Renoux *et al.* [4] transplanted Lewis lung tumor (5×10^5 cells) into C57BL/6 mice s.c. and gave them LMS at the 0.5 mg/kg level 24 hr later. The treatment with LMS was thereafter repeated six times on and after the 7th day of transplantation at 2-day intervals. The animals were sacrificed in the 3rd week of inoculation and were macroscopically examined for pulmonary metastases. The authors reported that LMS prevented the growth of primary tumor and inhibited the development of pulmonary metastases.

Yamagata *et al.* [5], using LMS in 1 and 10 mg/kg levels by i.p. route, suggested a relationship of the tumor system to the effectiveness of LMS, since LMS proved effective in the sarcoma 180 system but not in the Ehrlich system. They insisted on the necessity for establishing an optimal timing of administration for the combination of LMS and antitumor agent(s) that had been effective in increasing lifespan in some of the animals.

In the experiments by Okabe *et al.* [13], LMS used alone by i.p. route was ineffective against sarcoma 180, leukemia P-388 and Lewis lung tumor, while LMS combined with mitomycin C was significantly effective in prolonging survival and in reducing pulmonary metastasis in the Lewis lung tumor system.

According to Potter *et al.* [8], s.c. administration of LMS had no effect on the growth of the following transplantable syngeneic tumors of hyperantigenicity: adenovirus-induced tumor of CBA mice, virus-induced tumor of hamsters, Moloney virus-induced lymphoma of BALB/c mice and chemically induced neoplasm of inbred rats.

Johnson *et al.* [10] found that i.p. administration of LMS was without influence on tumor growth, metastasis or survival of mice bearing L1210 leukemia, P-388 leukemia, B16 melanoma, Madison 109 lung cancer and

Lewis lung cancer, although tetramisole suppressed primary tumor development or exerted antitumor activity in Moloney virus-induced rhabdomyosarcoma of BALB/c mice and in mice inoculated with Moloney virus.

Hopper *et al.* [9] tested LMS in rats transplanted with highly antigenic methylcholanthrene-induced rat sarcoma or with weakly antigenic spontaneous rat epithelioma SpI. They demonstrated that 6 injections at 3-day intervals or 9 injections at 2-day intervals of LMS at 5 mg/kg by i.p. or s.c. route failed to suppress growth of tumor transplants, pulmonary metastases and recurrences after surgical removal of tumor.

Sampson *et al.* [6] treated SD rats bearing 7,12-DMBA-induced mammary tumor with daily oral administration of LMS at 2, 4 and 8 mg/kg. After 6 weeks, the tumor sizes of the animals receiving LMS, 2 or 4 mg/kg, were significantly smaller than those of controls. But in the animals receiving 8 mg/kg, tumor regression did not occur; the tumor growth tended to be rather accelerated as compared to untreated animals. The authors concluded that LMS could cause regression of DMBA-induced rat mammary cancer but that the effect was dependent on the dose.

Chirigos *et al.* [7] reported that in Moloney virus-induced mouse lymphoid leukemia, BCNU (30 mg/kg s.c.) given at 9 days after tumor inoculation in combination with LMS (5 mg/kg i.p.) given at 12 days after inoculation resulted in a more extended survival period than BCNU alone, LMS alone or other combination schedules did.

According to Amery *et al.* [14], LMS was unable to suppress growth of transplanted tumor of the animals of nearly normal immune status but it could restore cellular immunity in animals and man that were anergic. They expected that LMS combined with cytoreductive chemotherapy would increase lifespan.

It is evident from the authors cited that the antitumor activity of LMS is dependent on such variables as tumor system, time of administration and dose level. Further studies may be needed to form an optimal way of administration of LMS.

In consideration of experimental studies by other authors and of the present study, tests are in progress in which 150 mg of LMS is used for three consecutive days every fortnight in combination with chemotherapeutic regimens including ADR in patients with recurrent or advanced breast cancer. The objective is to prevent possible immunological impairment due to chemotherapy by the administration of LMS. It appears that LMS can be an excellent immunostimulator to be used in cancer immunochemotherapy.

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