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Protective role of medroxyprogesterone acetate on *N*-methyl-*N*-nitrosourea-induced lymphomas in BALB/c female mice

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Abstract

In a previous paper we reported the occurrence of a high incidence of lymphomas in *N*-methyl-*N*-nitrosourea (MNU)-treated mice, in the course of an experiment of combined chemical-hormonal carcinogenesis in mammary gland, in which we used medroxyprogesterone acetate (MPA) and MNU in different treatment protocols. In this report we have analyzed the action of MPA in the leukemogenic effects of MNU, by specifically selecting for the analysis experimental groups in which only few mammary carcinomas had developed. A high incidence of lymphomas (65%, median latency: 176 days) was registered in MNU-treated mice, and the administration of MPA was associated with a significant reduction in the incidence of lymphomas ($P < 0.001$) in all protocols.

Keywords: Progestins; Medroxyprogesterone acetate; Lymphoma; Mice; *N*-methyl-*N*-nitrosourea

1. Introduction

Medroxyprogesterone acetate (MPA) is a synthetic progestin with androgenic and glucocorticoid properties [1] which has been widely used in the treatment of mammary and endometrial adenocarcinomas [2] and as supportive therapy in anorexia/cachexia syndrome [3]. It has been reported that patients receiving chemotherapy and MPA display a better general clinic state and that this drug is an important aid towards improving the quality of life of patients with either hormone-sensitive or hormone-insensitive tumors. These observations suggested that the analgesic and anabolic actions of MPA could be responsible of these effects [2] and that MPA might be myeloprotective [4].

In previous papers we demonstrated that the administration of MPA induces mammary carcinomas in female BALB/c mice with a latency of about a year [5]. Shorter latency times are observed when MPA is used in combination with the chemical carcinogen *N*-methyl-*N*-nitrosourea (MNU) 50 mg/kg [6]. MPA + MNU-treated animals had significant higher weight values than MNU-treated mice. MNU alone is not a mammary carcinogen in mice but it has been reported to induce leukemias, lymphomas [6] and lung adenomas. Indeed, a high incidence of lymphomas was observed in MNU-treated mice in our experiments [7]. The object of this study was to evaluate the effect of MPA on the incidence of lymphomas using protocols in which only few mammary carcinomas are induced.

2. Materials and methods

2.1. Animals

All experiments were carried out using two month old virgin female BALB/c mice (National Academy of Medicine, Buenos Aires, Argentina), housed six per cage in air-conditioned rooms at 20 ± 2°C, kept under

Abbreviations: MNU, *N*-methyl-*N*-nitrosourea; MPA, medroxyprogesterone acetate.

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an automatic 12 h light/12 h darkness schedule and given pellets and tap water ad libitum.

2.2. Effect of MPA on MNU-induced leukemogenesis

The mice were injected with 40 mg of MPA depot (Medrosterona, Gador Laboratories, Buenos Aires, Argentina) subcutaneously (sc) every three months in the right flank. In the experiments in which MPA treatment had to be stopped, after two months, the animals were anaesthetized and the hormone depot removed. Successive vaginal smears were used to confirm the absence of progestin effect. MNU (Sigma, St. Louis, MO) was administered intraperitoneally (ip) in one dose of 50 mg/kg of body weight. The carcinogen was diluted in isotonic 8.7 mM sodium phosphate buffer to a final volume of 0.1 ml and used in the first 15 min after it has been prepared. The experimental groups were designed in order to evaluate if MPA was necessary to be present throughout the experiment and if MPA was effective before or after the carcinogenic insult. Short term versus long term MPA administration was also evaluated.

Treatment Protocols	Symbol	Lymphomas nt/n	Lymphomas %	Lung Tumors nt/n	Lung Tumors %
Group 1	▲	18/28	65	24/28	86
Group 2	□	0/30	0	0/30	0
Group 3	▼	7/35	19*	31/35	88
Group 4	◆	5/31	17*	28/31	89
Group 5	●	7/27	26*	23/27	85
Group 6	■	7/50	15*	44/50	89

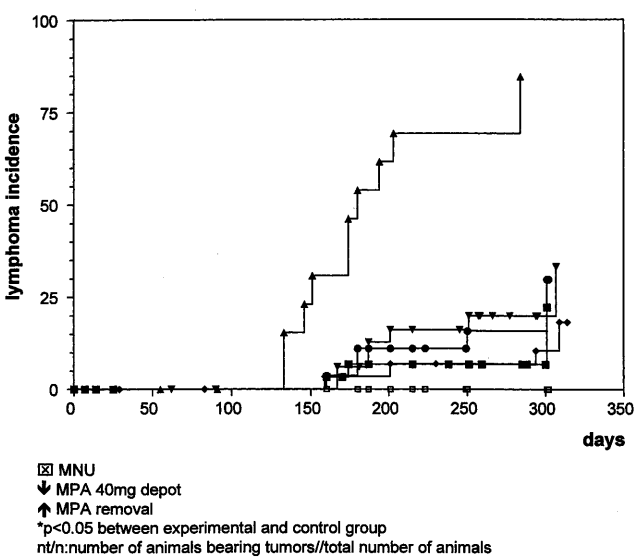

Fig. 1. Top: incidence of lymphomas and lung tumors in BALB/c mice treated with MNU (50 mg/kg) ip and/or MPA depot (40 mg sc) using different protocols. (*$P < 0.05$ between MPA-MNU treated and MNU-treated mice). Bottom: actuarial lymphoma incidence in the same groups.

The animals were divided into the following treatment groups (27–50/group): group 1, (control); MNU, single dose; group 2, MPA every 3 months; group 3, MNU 1 week after the first inoculum of MPA and MPA every three months; group 4, MPA for 2 months, 15 days after MPA removal, MNU; group 5, the same as group 4 but starting with the MNU dose and after 1 week MPA treatment; group 6, MNU and 1 week later MPA every 3 months. All animals sacrificed due to mammary tumors, lymphomas or at the end of the experiment (9 months from MNU inoculum) were autopsied. Samples of tumors and selected organs (lung, liver, uterus, spleen, and small intestine) were fixed in 10% buffered formalin, processed through graded alcohols and xylene, and embedded in paraffin. Five-micron sections were cut and stained with hematoxylin–eosin.

2.3. Statistical analysis

Fisher test was used to compare tumor incidence considering the initial number of mice in each group. Actuarial lymphoma incidence was calculated using the product limit estimate of the survival distribution of Kaplan–Meier and distribution equality was assessed with the log–rank test.

2.4. Immunocytochemistry

Immunocytochemical assays were performed on 5 μm slides from formalin-fixed, paraffin-embedded tissues, with the Elite ABC system (Vector Labs, Burlingame, CA), prepared according to the instructions of the manufacturer. The following antibodies were used: anti-Fc fragment, ATCC Rat Hybridoma 2.4G2 producing IgG 2B, anti-CD3 145-2C11 Hamster Monoclonal IgG, anti-MAC M1/70 Rat IgG 2B.

3. Results

A high incidence of lymphomas with a median latency value of 176 (133–270 days) was registered in MNU-treated mice (Fig. 1). The incidence of lymphomas was significantly lower in all MPA-MNU-treated animals as compared with mice treated with MNU alone ($P < 0.05$). In addition to Fisher test, the actuarial incidence of lymphomas was calculated to rule out a possible underestimation due to the interference of animals that had to be excluded because they developed mammary tumors (groups 3 and 6). This inhibitory effect was similar in all protocols where MPA was used: MPA for 2 months prior MNU (group 4), MPA for 2 months (group 5) or throughout (group 6) after MNU or MNU injected in MPA-treated mice (group 3). Only very few mammary tumors appeared in MNU-MPA treated animals of groups 3 and 4 and a

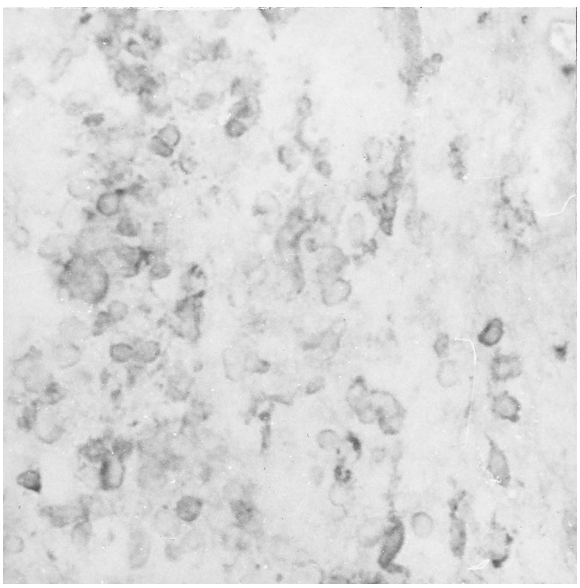

Fig. 2. The picture shows positive immunostaining for CD30 in kidney infiltrating lymphocytes from a malignant lymphoma (× 200).

higher incidence was obtained in groups 5 and 6 as reported previously [6]. Most neoplasias were either small cell or mixed small and large lymphocytic lymphomas with or without thymic involvement. Tumors with no thymus involvement were usually more invasive comprising liver, spleen, lung peritoneum, uterus and ovaries. Five tumors were evaluated with immunohistochemistry, three gave positive staining for CD3 (Fig. 2), a T cell marker and two stained positively for Fc receptor.

The incidence of lung tumors was similar in all groups treated with MNU regardless of the presence of MPA and their presence was not associated as a cause of death.

4. Discussion

The results reported herein demonstrate that MPA inhibits the development of MNU-induced lymphomas in mice. Similar findings were reported by Guzman et al. [8] in a series of experiments in BALB/c mice in which the progestational milieu was achieved by implanting hypophysis under the spleen. Under these conditions there is an increase in PRL and progesterone levels, the latter probably due to the increase in LH secretion. Our results clearly show that progestins alone are able to exert a protective leukemogenic effect. The data presented herein also indicate that MPA is probably preventing both the initiation and promotion of the target cell populations, since it was protective not only previous to MNU injection, but also when administered after MNU administration, when the initiating events may have already occurred. Clinical studies have shown that MPA reduces the bone marrow toxicity induced by antitumor drugs [4]. Studies are needed to discriminate whether it is the progestagenic or the glucocorticoid effects of MPA the one that is playing this protective role.

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