SHORT COMMUNICATION

Promoter effect of medroxyprogesterone acetate (MPA) in N-methyl-N-nitroso urea (MNU) induced mammary tumors in BALB/c mice

Patricia Pazos, Claudia Lanari, Patricia Elizalde, Fernanda Montecchia, Eduardo H. Charreau and Alfredo A. Molinolo

Instituto de Biología y Medicina Experimental (IBYME) and Instituto de Investigaciones Hematológicas, Academia Nacional de Medicina, Vuelta de Obligado 2490, 1428 Buenos Aires, Argentina

The promoter effect of medroxyprogesterone acetate (MPA) in mammary carcinogenesis in female BALB/c mice was investigated using methyl nitrosourea (MNU) as initiator. Nine out of 43 animals developed mammary carcinomas in the group treated with MNU (50 mg/kg) and MPA (administration of 40 mg every 3 months) starting 1 week after MNU administration. No tumors appeared in controls receiving only MNU or MPA during the time course of the experiment (9 months). The tumors were lobular adenocarcinomas showing different degrees of squamous differentiation with low or undetectable estrogen and progesterone receptors, and expressing epidermal growth factor receptors. These results support the hypothesis that MPA promotes the growth of MNU induced lesions.

The role of progestins in mammary gland tumorigenesis remains a controversial issue; many reports suggest they play a protective role, while other evidence points towards a possible active contribution of these hormones to the carcinogenic process (1).

In a previous paper we described a model of carcinogenesis in BALB/c female mice in which the co-administration of methyl nitrosourea (MNU*) and medroxyprogesterone acetate (MPA) induced mammary adenocarcinomas (2). This project had been undertaken as an attempt to reduce the latency period of the neoplasms originated using an earlier tumor model, also designed in our laboratory, in which MPA alone was used to induce ductal adenocarcinomas in virgin BALB/c female mice (3,4). In the present report we present experimental evidence regarding the tumor promoter and permissive roles of MPA in this experimental model of chemical-hormonal carcinogenesis. Experiments were carried out using 2-month-old virgin female BALB/c mice raised at the colony of the National Academy of Medicine, Buenos Aires, Argentina. They were housed at six per cage in air-conditioned rooms at 20 ± 2°C, kept under an automatic 12-h light/dark schedule and given pellets and tap water ad libitum.

The mice were injected with 40 mg of MPA depot (Medrosterona, Dr Gador Laboratories, Buenos Aires, Argentina) s.c. every 3 months in the right flank. In the experiment in which MPA treatment had to be stopped, after 2 months, the animals were anaesthetized and the hormone removed. Successive vaginal smears were used to confirm the absence of progestagen effect. MNU (Sigma Co., St Louis, MO) was administered i.p. in one dose of 50 mg/kg of body wt. The carcinogen was diluted in isotonic 8.7 mM sodium phosphate buffer to a final vol. of 0.1 ml and it was used within 15 min of preparation. The animals were divided into the following treatment groups: group 1 (n = 43) MNU, single dose; group 2 (n = 22) MPA (40 mg) every 3 months; group 3 (n = 44) MPA, and then MNU as a single dose 1 week after; group 4 (n = 43) MNU one dose, MPA (40 mg) starting 1 week later, and then every 3 months until the end of the experiment; and group 5 (n = 42) MNU, and MPA (40 mg) inoculated 1 week after MNU injection and then stopped 2 months later.

The mean latency of mammary tumors induced by MPA alone was 52 weeks (3). To avoid overlap between those tumors originating from MPA alone and tumors originating from MPA–MNU treatment, the MNU/MPA experiment was terminated at 9 months. Because at the end of the experiment there was a large number of surviving animals in the group inoculated with a single dose of MNU (group 1), we decided to corroborate the effectiveness of a single dose of MNU to induce the initiation process and to verify the promoter activity of MPA. Fourteen randomly chosen mice that belonged to this treatment group were inoculated with 40 mg MPA; five mice remained untreated as controls.

All mice were observed weekly and when the tumors began to appear, their growth was closely observed. When the tumors reached ~100 mm², the animals were killed and histological studies and syngeneic passages were conducted. Tumor latency refers to the day the tumors first became palpable. Autopsies were performed in every animal. Samples of tumors and selected organs (lung, liver, uterus, spleen, small intestine) were fixed in 10% buffered formalin, processed through graded alcohols and xylene, and embedded in paraffin. Slides (5 µm) were cut and stained with hematoxylin and eosin. Individual tumor samples were processed as previously described (5) to obtain the cytosol fraction. Estrogen receptors (ER) and progesterone receptors (PR) were measured with the charcoal technique; the details of the incubation have been reported previously (4). Epidermal growth factor receptors (EGF-R) were measured using a radioreceptor assay (4).

No mammary tumors appeared during the time course of the experiment in mice treated with a single MNU dose or in the group treated with MPA alone (Figure 1). A high incidence of mammary tumors was registered in the experimental groups in which MPA treatment was started before or 1 week after MNU inoculation (P < 0.05). The fact that tumor incidence seemed to be even higher in animals treated with MPA before MNU suggests that, besides its promoter effect, MPA may favor the initiation event acting as a permissive agent. In this case the difference in actuarial incidence between these groups is not significant, but becomes significant if the total number

© Oxford University Press 529
of tumors is considered. The fact that tumor incidence is low when animals were treated for only 2 months with MPA (group 5) suggests that this promoter effect requires a constant hormonal stimuli. After the experiment was terminated at month 9, we decided to inoculate the surviving animals with MPA, to ascertain the presence of initiated mammary cell populations in mice that had not developed mammary tumors. Mammary tumors developed in 4/14 mice treated with 40 mg of MPA with a latency of $378 \pm 50$ days, while none were detected in the untreated control group. Although only a small number of animals remained and the differences between the groups was not statistically significant, these results also favor the promoter effect of MPA.

All tumors were classified as lobular adenocarcinomas with varying histological patterns and different degrees of squamous differentiation. Most of the tumors were composed of lobular structures with or without glandular differentiation. Squamous metaplasia were observed in a significant number of tumors ranging from isolated squamous differentiation to extensive metaplasia, some of them with ‘ghost’ and ‘shadow’ cells, resembling the histology of the human pilomatrixoma (Figure 2) (6). These tumors showed a progestin-independent (PI) growth pattern in syngeneic passages, had no detectable levels of ER and PR, and expressed EGF-R (8.33 fmol/mg protein).

The results reported herein demonstrate a promoter effect for MPA in carcinogenesis and suggest that this hormone may also be playing a permissive role (7). In previous experiments we have demonstrated that MPA, at higher doses and longer exposition times, behaves as a complete mammary carcinogen in BALB/c mice (3,4). We also demonstrated that three 50 mg/kg wt doses of MNU could induce mammary tumors in the same strain, with an incidence of 10–15%, and a significantly shorter latency than those induced with MPA alone. If MPA was continuously administered throughout the experiment, tumor incidence reached almost 80% (2). In the experiments reported herein, in order to be able to dissect the initiating effect of MNU from the putative promoter action of MPA, we treated the animals with a single 50 mg/kg MNU injection, which is a dose at which MNU has been demonstrated to behave as an initiator only (8). When the animals were treated with MNU alone, no mammary tumors developed. Nevertheless, mammary tumors developed when, almost a year after the MNU injection, the surviving animals were treated with MPA, demonstrating that MNU had initiated mammary cell populations at 50 mg/kg and that MPA was an effective tumor promoter in this system.

The fact that MNU behaves as an initiator has been demonstrated in several experimental models (9) and its ability to induce ras mutations has been correlated to its initiating role. Hormones have already been demonstrated to play a promotional role in MNU carcinogenesis, as shown by Sukumar et al. (10) for estrogens in the rat. The possible role of progestins in tumor promotion is, however, far less clear, although it has been demonstrated that a progesterational environment modulates tumor incidence (11–13). Aldaz et al. (14) have recently demonstrated that MPA treatment increases the incidence of DMBA induced mammary tumors in virgin CD2F1 mice, and they suggested that MPA probably increases the number of target cells for the chemical carcinogen and, by possibly increasing the rate of tumor development, acts as a permissive agent. The promoter effect of progesterone was described by Huggins and Young (15), who found that this hormone increased both incidence and growth rate of DMBA-induced mammary cancers in rats with intact ovaries, whereas estrogens delayed the appearance. MPA is a complete carcinogen by itself since it is able to induce mammary tumors in BALB/c mice without need of any chemical carcinogen. Nevertheless, most MPA-induced tumors are ductal progestin-dependent mammary adenocarcinomas expressing high levels of hormone receptors (16), and nearly all MNU–MPA induced
tumors were lobular PI mammary adenocarcinomas suggesting that both diseases are essentially different.

The presence of squamous metaplasia has also been reported in mice treated with MNU in vitro and then inoculated to BALB/c mice (17,18). We have never seen this lesion in tumors induced by MNU and it is not frequently found in tumors that originate from MPA/MNU-treated animals in which MNU was inoculated 1 week after MPA. These tumors, although different from MPA-induced tumors, may be indistinguishable from those induced with progesterone alone (16). Nevertheless, an increase in squamous metaplasia is observed in tumors that arise in mice in which MNU is administered 2 months after MPA inoculation, or in three doses. Although an accurate interpretation of these results is difficult at this time, it seems plausible that the hormonal environment at the time of administration of the carcinogen, is somehow involved in determining the histological type of the tumor.

Acknowledgements

We thank Mrs M.T.Santarelli for statistical assistance and Mr J.Portaluppi and Mr A.Morales for efficient technical assistance. This work was supported by Fundación Sales, CONICET, International Center for Genetic Engineering and Biotechnology, Trieste (ICGEB) and Laboratorios Gador, Buenos Aires.

References


Received on March 25, 1997; revised on October 15, 1997; accepted on October 24, 1997.