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Effect of Medroxyprogesterone Acetate on DMBA-induced Rat Mammary Carcinoma and on Immunological Reactivity

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Abstract—*The antineoplastic activity of medroxyprogesterone acetate (MPA) was investigated in rats bearing DMBA-induced mammary carcinomas, a classical model of hormone-dependent tumor. Using a repeated injection schedule of relatively short duration, MPA was markedly effective (80% CR + PR) not only on relatively small (1–1.5 cm) tumors but also on advanced (4.5–5.5 cm) neoplasms. MPA effectiveness was comparable to that of a frankly toxic adriamycin regimen. In antitumorally effective schedules MPA was incapable of significantly affecting in either direction cellular and humoral immunological reactivities in rodents.*

INTRODUCTION

IN RECENT years a renewed interest has centered on the use of medroxyprogesterone acetate (MPA) in the treatment of various hormone-dependent human neoplasms and, notably, in breast cancer. In this condition high-dose MPA treatment has in fact been reported to produce 28–46% objective response rates in advanced disease [1]. The mechanism(s) of the antitumoral effect of MPA, a synthetic progesterone derivative possessing other hormonal activities in addition to high progestational capacity [2], is still a matter of discussion, as are various aspects of its clinical use (e.g., dose, schedule, employ before or after chemotherapy). Considering that available information on the antineoplastic effect of MPA in experimental conditions is still limited [2–4], it was of interest to explore its activity in a well-characterized rodent model of mammary carcinoma. We also investigated MPA effects on immunological reactivities, with the aim of obtaining a better characterization of its anticancer potential, mode of action and pharmacological properties.

MATERIALS AND METHODS

Animals

Female CD-COBS rats and $CD2F_1$ mice (Charles River Italy, Calco), 120–130 and 20–22 g respectively at the start of experiments, were used.

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Drugs

MPA, obtained from Farmitalia, Milan, was administered s.c. suspended in sesame oil using 0.3 ml per injection. Adriamycin (AM) (Farmitalia) was given i.v. freshly dissolved in sterile saline. Control animals were always given equal volumes of the appropriate vehicle. DTIC (dimethyltriazenoimidazole carboxamide, NSC 45388) was freshly dissolved in saline after admixture with an equal part (w/w) of citric acid.

Evaluation of antineoplastic activity

Mammary tumors were induced in rats by a single i.v. injection of 5 mg 7,12-dimethylbenz(a)anthracene (DMBA, Upjohn, Kalamazoo, MI, U.S.A.) administered as a 15% oil emulsion. Two catagories of tumor-bearing rats were investigated. In the 'small tumor' category (8–10 rats per experimental group) tumor diameters at treatment initiation (hereafter day 1) ranged between 1.0 and 1.5 cm. In this category neoplasms which grew to ≥ 3 cm and remained above this value were scored as progressions, whereas tumors whose mean diameter was maintained between 1 and 3 cm in the 120 days observation period were rated as stabilizations. Decreases in tumor diameter which were either incomplete (i.e. between 1.0 and 0.2 cm) and/or not maintained throughout the entire observation period were scored as partial regressions (PR); in the latter case, however, tumor regrowths had to be ≤ 3 cm to be considered PR. Complete regres-

sions (CR) were confirmed microscopically at autopsy.

The second category of tumor-bearing rats investigated consisted of a small series (4–6 rats per group) of extra animals with tumor diameters of 4.5–5.5 cm at treatment initiation. In this category tumors reaching diameters ≥6.5 cm were scored as progressions, whereas tumors remaining in the 3.5–6.5 cm range were rated as stabilizations. PR was considered as each decrease in tumor size that was either incomplete (i.e. ≥0.2 cm) or not definitive in the 120 days observation period, provided regrowths did not reach above 3.5 cm. The shortest and longest diameters were taken blindly by two independent observers at 3–4 days intervals on individually marked animals using Vernier calipers and results averaged. Animals with grossly ulcerated neoplasms were discarded from the experiment.

Immunological investigations

The antibody response to sheep erythrocytes (SRBC) was evaluated counting the number of hemolytic plaque-forming cells (PFC) in the spleen, as previously described [5], using inocula of 4×10^8 SRBC for mice and 0.5 ml of 10% washed suspension for rats and 8 animals per group. The *in vitro* response of splenocytes from drug-treated animals to the mitogens Concanavalin A (ConA, Calbiochem) and LPS (*E.coli* lipopolysaccharide, Difco) was evalutated measuring the uptake of [^{3}H]-thymidine, as detailed elsewhere [6], using triplicate cultures and 5 animals per group. The capacity of MPA to influence the response of compatible $CD2F_1$ hosts to tumor-associated antigens was evaluated using the highly immunogenic L1210 Ha subline maintained in isogenic DBA/2 mice by weekly i.p. transplants. Experimental details have been described previously [7]; briefly, *in vitro* X-irradiated (5,000 rads) L1210 Ha cells (10^6) were injected i.p. 10 days before the i.p. inoculation of graded numbers of live (>95% trypan blue excluding) cells from the same tumor. Ten animals per group were used.

RESULTS

Data obtained when the antineoplastic activity of MPA (administered 5 days per week for 4 consecutive weeks) was tested on small (1.0–1.5 cm) DMBA-induced rat mammary carcinomas are shown in Table 1, where the representative results of 1 of the 2 experiments performed with this agent are presented. It may be observed that in the control group a majority (6/10 and 5/9 in the 2 tests) of animals had progressive tumors, whereas in 4/10 and 4/9 rats neoplastic growth was classified as stable, i.e., with diameters which remained in the 1.0–3.0 cm range in the observation period. In these conditions MPA was markedly antitumorally effective since treatments with 100 or 50 mg/kg s.c. were associated not only with the absence of progressive neoplasms but also with 40–50% complete regressions in both experiments performed. No differences in the effectiveness of these MPA doses could be recognized in terms of the total proportion of CR or PR (9/19 and 7/19 for both doses respectively). However, in both tests conducted tumor regressions occurred in significantly shorter times with the 100 mg/kg dose. A clear antineoplastic activity was observed also with the 25 mg/kg MPA treatment, as shown by the reduction in progressive tumors (2/10 and 2/9 in the 2 experiments) in comparison to controls and the obtainment of approximately 40%

Table 1. *Effect of medroxyprogesterone acetate on 'small'* (1–1.5 cm) *DMBA-mammary carcinomas in rats*

Experimental group (mg/kg)	Regressions Complete	Partial	Stable	Progress	CR* on day	Death† on day
Control	0/10	0/10	4/10	6/10	—	81
MPA 100‡	5/10	3/10	2/10	0/10	9,15,23,32,38	—
MPA 50	4/10	4/10	2/10	0/10	26,34,49,72	—
MPA 25	2/10	2/10	4/10	2/10	14.16	116
AM 4§	6/10	1/10	3/10	0/10	7,9,11,26,30,42	26,34,63,83
AM + MPA 100	3/10	3/10	4/10	0/10	7,9,12	25,35,52,68,70,78,85
AM + MPA 50	3/8	2/8	3/8	0/8	23,26,28	31,32,45,66,81
AM + MPA 25	4/10	4/10	2/10	0/10	7,7,10,31	31,42,65,78,82

*CR = Complete regression.
†Observation period 120 days; day of treatment initiation taken as day 1.
‡MPA injected s.c. 5 days/wk, per 4 consecutive wks, starting on day 1.
§Adriamycin injected i.v. on days 1,8,15,22.

regressions (2CR + PR in both tests). In the second of such experiments chemotherapy with AM (4 mg/kg i.v. × 4 at weekly intervals, starting on day 1) was also tested. This treatment proved very active, as evidenced by the observation of 6/10 CR and 3/10 tumor stabilizations, i.e., results comparable with those seen with 100 mg/kg MPA in the same experiment. Table 1 also shows that the time to regression was comparable with AM and MPA 100 mg/kg. This AM schedule was, however, quite toxic, as shown by the fact that 6/10 rats succumbed before day 120, in contrast with only 4/76 deaths before this date observed collectively in the control and MPA-treated groups. When these AM and MPA treatments were combined evidence for an additive effect could not be observed in these experimental conditions, nor was AM toxicity apparently modified as judged on the basis of deaths before day 120.

When MPA antineoplastic capacity was assessed on 'large' (4.5–5.5 cm) tumors (Table 2), data obtained indicate that the compound exerted a clear activity also in these conditions. At variance with 6/6 progressive tumors in controls, 4/6 regressions were in fact seen after MPA, doses of 50 and 100 mg/kg giving similar results, which in turn were comparable with those seen with AM. Although the proportion of regressions observed combining MPA and AM was higher than using either agent alone, the very limited number of animals tested precludes the reaching of a conclusion on the comparative effectiveness of the combined treatment in these animals.

In subsequent studies it was investigated whether MPA treatments could modify, in either direction, immunological reactivities in rodents. When the primary humoral response to the T-dependent antigen SRBC was assessed counting the number of anti-SRBC hemolytic antibody-producing splenocytes, at peak response day (days 4 and 5 for mice and rats respectively) no significant changes were seen in mice or rats given various single or repeated MPA treatments over a range of doses (Table 3). No significant changes in comparison to vehicle-treated controls were observed, either, when the kinetics of this primary antibody response was followed counting PFC levels beyond peak-day, since values of day 7 and 10 were comparable to controls. In mice given 4 days before SRBC a single 75 mg/kg dose of DTIC as an immunodepressant [8], concurrent MPA administration (100 mg/kg from day −10 to +1) did not result in significant modifications in PFC levels measured either 4 or 7 days after antigen. Table 3 also shows that, as judged on the basis of [^{3}H]-thymidine uptake, the capacity of splenocytes from MPA-treated rodents to respond *in vitro* to the T and B mitogens, ConA and LPS respectively, was not modified in respect to controls after single or repeated drug administrations, cells being placed in culture 24 hr after treatment discontinuation. Lastly, the capacity of MPA given in conjunction with a priming inoculum of X-irradiated L1210 Ha leukemia cells to influence the survival of compatible $CD2F_1$ mice subsequently inoculated with graded numbers of live cells of the same tumor was studied in order to assess possible drug effects on host responsiveness to tumor-associated antigens.

Table 4 shows that the survival of mice also given MPA was not different from that of mice pretreated only with the tumor vaccine, survival in this group being in turn significantly longer than that of non-preimmunized animals.

DISCUSSION

Findings presented here show that MPA

Table 2. Effect of medroxyprogesterone acetate on 'large' (4.5–5.5 cm) DMBA-mammary carcinomas in rats

Experimental group (mg/kg)	Regressions Complete	Partial	Stable	Progress	CR* on day	Death† on day
Control	0/6		0/6	0/6	6/6	—
MPA 100‡	0/6		4/6	1/6	1/6	—
MPA 50	1/6		3/6	1/6	1/6	44
AM 4§	1/6		3/6	2/6	0/6	35
AM + MPA 100	0/4		4/4	0/4	0/4	—
AM + MPA 50	3/4		1/4	0/4	0/4	23,36,38

*CR = Complete regression.
†Observation period 120 days; day of treatment initiation taken as day 1.
‡MPA given s.c. 5 days/wk per 4 consecutive wks, starting on day 1.
§Adriamycin given i.v. on days 1,8,15,22.

Table 3. Effect of medroxyprogesterone acetate on the primary anti-SRBC response and on splenocyte mitogen responsiveness in rodents

Species	Experimental group	mg/kg	No. of injections	PFC/spleen*	S.I.† at ConA (μg/ml) 0.2	0.8	1.6	S.I.† at LPS (μg/ml) 0.5	50
Mouse	Control	—	—	46,875 (44,210–49,760)	2.18	7.44	5.75	1.57	3.77
	MPA	100	1	44,630 (42,505–47,080)	2.01	6.73	4.86	1.83	3.94
		500	1	45,310 (42,770–49,605)					
		1000	1	43,845 (41,980–46,550)	2.31	7.26	6.08	1.67	3.64
		100	5	46,625 (38,600–48,230)					
		500	5	48,090 (44,375–53,140)					
	Control	—		58,270 (54,100–62,765)	1.93	7.20	4.98	2.11	4.24
	MPA	100	10	53,635 (50,805–57,950)	2.47	6.69	5.07	1.87	3.78
	DTIC‡	75	1	22,480 (21,010–25,390)					
	DTIC+ MPA	75 100	1 10	23,265 (20,120–27,435)					
	MPA	50	20	63,400 (58,460–69,765)	1.87	7.68	5.35	1.72	4.55
		100	20	61,145 (57,890–66,365)	2.38	6.58	6.27	1.96	4.38
Rat	Control	—	—	36,470 (16,560–80,955)		6.81	5.98		
	MPA	100	1	33,905 (14,235–82,880)		5.57	7.06		
		100	10	45,260 (29,115–94,230)					
		100	20	40,175 (21,905–78,740)		7.44	7.21		

*PFC: plaque-forming cells.

†Stimulation Index, i.e., ratio of mean cpm of cultures with and without mitogens. Background cpm were in the 8665–11,400 and 9010–19,395 ranges for ConA and LPS respectively for mouse splenocytes, and 5615–8970 range for rat splenocytes.

‡DTIC was injected i.p. 4 days before antigen (4×10^8 SRBC i.p.).

In the assessment of anti-SRBC response, MPA treatment (5 days/wk) was stopped 1 day after SRBC inoculum and PFC measured after further 3 days in mice and 4 days in rats. For evaluating mitogen responsiveness, splenocytes were taken 24 hr after the last MPA treatment and [^{3}H]-thymidine uptake measured after 3 days in culture.

possesses a definite antineoplastic activity in rats bearing DMBA-induced mammary tumors, i.e., in a system which bears a number of resemblances with human breast cancer as regards responsiveness to chemotherapy [9, 10] and, especially, endocrine treatment [10, 11]. This result thus confirms and extends the data of Danguy *et al.* [3] in an analogous system and those of Di Marco and his group [2, 4] in another hormone-sensitive rat model, the 13762 mammary adenocarcinoma. In this experimental system MPA antitumoral activity was observed not only in relatively advanced (1–1.5 cm) tumors but was also clearly evident when drug treatment was firstly applied to very large (4.5–5.5 cm) tumors, i.e., a condition chosen to simulate advanced, metastatic clinical disease. In both conditions drug activity was revealed not only by the obtainment of arrests in tumor growth, but especially by the induction of definite partial and microscopically confirmed, complete neoplastic regressions, with PR + CR response rates of approximately 80% in the animals with lower initial tumor burden treated with 50–100 mg/kg MPA. In the conditions investigated MPA activity appeared to be dose-dependent, as shown not only by the lower effectiveness of the 25 mg/kg in comparison to 50 mg/kg dose but, additionally, by the significantly faster obtainment of CR seen with 100 mg/kg, although the total response rates found with the latter and the 50 mg/kg doses were comparable. These findings thus appear to parallel a series of clinical results [1],

Table 4. Effect of medroxyprogesterone acetate on the survival of preimmunized, L1210 Ha leukemia challenged CD2F$_1$ mice

10^6 X-rayed L1210 Ha cells i.p. on day −10	MPA (100 mg/kg s.c.) on days:	No. L1210 Ha cells i.p. on day 0	MST* (days)
—	—	10^6	7.3
—	—	10^5	9.5
+	–	10^6	12.7†
+	–	10^5	16.8†
+	−14→−5	10^6	12.2
+	+	10^5	17.4
+	−19→−10	10^6	13.5
+	+	10^5	16.0
+	−9→−1	10^6	11.9
+	+	10^5	17.6
+	−19→−1	10^6	13.1
+	+	10^5	16.4
–	−19→−1	10^6	7.1
–	+	10^5	9.6

*Median survival time.
†$P < 0.05$ vs untreated controls.

indicating that the recent practice of high-dose (≥500 mg/day) MPA therapy is significantly more effective than previously used, low-dose treatments. Our data cannot resolve, however, whether or not there is a high threshold dose beyond which no further therapeutic advantage is obtained. In recent clinical trials in breast cancer [12, 13] the effectiveness of 500 mg/day MPA doses was found to be comparable to those reported in previous studies using double or still higher dosages. As an index of MPA effectiveness in this model system, it is of note that the activity of the two higher MPA doses tested were equivalent to that of AM employed at clearly toxic doses; the effectiveness of this anthracycline in experimental and clinical breast cancer is well-established [14]. In the rats with smaller tumors no evidence for an additive effect was detected when MPA was combined with AM, whereas in the larger tumor group the limited number of animals precludes the reaching of any conclusion on this matter. Also, in view of the fact that only one type of AM and MPA treatment schedule was employed here, these results cannot be taken as evidence against the therapeutic potential of MPA-chemotherapy combinations. Indeed, additive effects for such combined approaches have been reported in another rat mammary carcinoma model less responsive to MPA than that investigated here [15], and results strongly indicative of a better efficacy of MPA when associated with poly-chemotherapy have been described in breast cancer patients by several groups (see [1]).

The antitumoral mode of action of MPA, a synthetic steroid which, in addition to its progestinic effect, possesses other hormonal activities [2], is still a matter for discussion. As recently reviewed [2], although experimental evidence supports the possibility that its inhibitory capacity on hormone-sensitive mammary tumors is related to a decrease in estrogen receptors directly exerted on tumor cells, or mediated through inhibition of prolactin secretion at the hypothalamic–hypophyseal level [3], androgenic or glucocorticoid-like mechanisms cannot be excluded. Non-receptor mediated mechanisms might also be operative [2]. Investigation of MPA immunological effects were thus of interest, in order to obtain further indications on its possible model of action and to better characterize this agent pharmacotoxicologically. Progestins in relatively high doses have been reported to cause immunodepression in animals [16–18] and drug-induced decreases in host resistance are regarded as an important determinant of the infectious complications frequently observed in cancer patients [19].

When administered to rodents over a range of doses and schedules, inclusive of those shown to be antitumorally effective in this and other studies, MPA did not significantly modify the humoral response to the T–B lymphocyte-dependent antigen SRBC, nor did it affect the responsiveness of MPA-splenocytes to T and B cell-specific mitogens.

The reactivity to non-specific stimulants such as ConA is widely regarded as a correlate of

cell-mediated reactivity. The antibody response of mice also treated with DTIC, a potent immunodepressant [8], was also not further aggravated by MPA injections. Furthermore, preliminary data did not reveal significant changes in the cytotoxic activity of macrophages from MPA-treated mice. Macrophages are currently credited with a front-line role in natural resistance mechanisms [20]. In this connection it has been shown that mice are more responsive than rats to the immunodepression induced by estro-progestins [16]. An additional indication of the lack of influence of MPA on immunity is the observation that its administration did not modify the increase in host resistance against challenges of the highly immunogenic L1210 Ha leukemia [21] given by previous specific immunization. In identical test conditions we have previously shown various immunostimulants to be active [5, 7, 22, 23]. Collectively, these findings support the contention that a modulation of host antitumor reactivity does not play a determinant role in the tumor inhibitory capacity of MPA. In addition, in antitumorally-effective regimens in animals the compound does not appear to be substantially immunodepressive. If confirmed also in humans, this conclusion would constitute a motive of further clinical interest in MPA whose use in patients has so far been associated with very limited side effects [1].

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