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Combined and Sequential Treatment using FCE 21336, a New Prolactin-lowering Drug, and Medroxyprogesterone Acetate (MPA) in DMBA-induced Tumors in Rats

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Abstract—*The effect of the new, prolactin-lowering ergoline derivative FCE 21336 and medroxyprogesterone acetate (MPA) given alone and in combination was tested on DMBA-induced mammary tumors in rats. FCE 21336 (0.05 and 0.4 mg/kg p.o.) and MPA (25 and 50 mg/kg s.c.), administered 5 days/week for 4 weeks, inhibited the growth of established tumors and reduced serum prolactin levels. Combined treatment inhibited tumor growth more than single treatment. These results were confirmed in a second experiment: the antitumor effect of the combination of FCE 21336 (0.1 mg/kg p.o.) and MPA (50 mg/kg s.c.) was greater than that of the single treatment and was similar to the effect of ovariectomy. In this experiment rats with tumors that did not respond to 4 weeks' treatment with FCE 21336 (0.1 mg/kg p.o.) were treated during the next 4 weeks with MPA (50 mg/kg s.c.). MPA was effective on FCE 21336-unresponsive tumors. These data indicate that combined FCE 21336 and MPA treatment is more effective than single treatment and that MPA is effective on tumors not sensitive to the prolactin-lowering drug.*

INTRODUCTION

PROLACTIN (PRL) is known to be essential for growth of 7,12-dimethylbenzanthracene (DMBA)-induced mammary tumors in rats. Ergot alkaloids and ergoline derivatives inhibit the release of PRL from the pituitary and produce about 50% of mammary tumor regressions [1–3]. In this tumor model different types of mammary carcinoma arise with different sensitivities to PRL deprivation. Some tumors require PRL, others need PRL and estrogen during their growth period and a small number are not influenced by hormones [4]. Because of this heterogeneous hormone responsiveness we studied the effect of a combined endocrine treatment using medroxyprogesterone acetate (MPA) and a new PRL-lowering drug FCE 21336 [1-ethyl-3-(3′-dimethyl-aminopropyl)-3-(6′-allylergoline-8′β-carbonyl)-urea diphosphate]. The new ergoline derivative has a potent, long-lasting effect on PRL secretion in rats [5] and in previous studies it was highly effective on DMBA-induced tumors [6]. MPA, at high doses, is active on DMBA-induced tumors [7, 8]. In this study we investigated the antitumor activity of the two compounds given in combination, comparing the effect of the endocrine treatment with the effect of ovariectomy, and assessed the activity of MPA on FCE 21336-resistant tumors.

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MATERIALS AND METHODS

Animals

Female Sprague–Dawley rats were supplied by Charles River, Italy. The animals were housed 4–5 per cage and maintained in an air-conditioned, temperature (23°C)- and light (12 hr of light from 7 a.m. to 7 p.m.)-controlled room.

Mammary tumor model

At 50 days of age rats were treated intragastrically with 15–20 mg of DMBA (Sigma Chemical Co. Saint Louis, MI) dissolved in sesame oil (1

ml/rat). Animals were examined weekly by palpation starting 40 days after DMBA treatment; they were put sequentially into experimental groups when at least one tumor 1 cm in diameter was found. Animals without tumors by day 150 were discarded. The two perpendicular tumor axes were measured with calipers twice weekly during treatment. Tumor weight was calculated according to Geran *et al.* [9]. Tumor response to the drug was designated as CR (complete remission, disappearance of the tumor), PR (partial remission, more than 50% reduction of tumor weight), NC (no change, less than 50% increase or decrease) and P (progression, more than 50% increase).

Drugs

FCE 21336 was dissolved in H_2O and administered orally in a volume of 5 ml/kg body weight. The doses are reported as free base. MPA was suspended in sesame oil and injected s.c. in a volume of 2 ml/kg body weight. Controls were treated with both vehicles. Treatments were always given at 10 a.m.

Experimental design

Experiment I. FCE 21336 was administered orally at doses of 0.05 and 0.40 mg/kg; MPA was injected s.c. at doses of 25 and 50 mg/kg. Combined treatment using these doses was given to four groups of animals. Animals were treated 5 days/week for 4 weeks. At the end of treatment (24 hr after the last drug dose) blood samples for PRL assay were collected from the eye plexus. Samples were kept at 4°C for 1 hr, centrifuged and serum samples were stored at −20°C until analyzed.

Experiment II. Rats were treated 5 days/week for 4 weeks with FCE 21336 0.1 mg/kg p.o. and MPA 50 mg/kg s.c., given alone and in combination. One group of animals was ovariectomized, under ether anesthesia, on day 1 of treatment. Rats with tumors not responding (classified as NC or P) to the 4 weeks of treatment with FCE 21336 were randomized in two groups. Three days after the last FCE 21336 dose one group of animals was ovariectomized and the other was treated with MPA 50 mg/kg s.c. 5 days/week for 4 weeks.

Prolactin assay

Serum PRL was analyzed by a double antibody radioimmunoassay according to Niswender *et al.* [10], using the kit supplied by the NIAMDD Rat Pituitary Hormone Distribution Program, National Institutes of Health, Bethesda, MD. The results are expressed in ng/ml in terms of NIAMDD rat PRL RP-1.

Statistical analysis

Experiment I was carried out according to a 3×3 factorial design. A binary response variable was obtained by considering CR and PR as positive responses and NC and P as negative responses. This type of response was analyzed according to a logistic model [11] including the linear and quadratic terms of regressions for each treatment and their interactions. These analyses were made using the GLIM package [12]. PRL data of treated groups were compared with those of controls by Dunnett's *t* test. Data of experiment II were analyzed by Fisher's exact test.

RESULTS

Experiment I: antitumor and PRL-lowering activity of FCE 21336 and MPA

Tumor responses to the different treatment regimens are presented in Table 1. The percentage of tumor regressions (CR + PR) in MPA-treated groups were 64% at 25 mg/kg and 75% at 50

Table 1. Response of DMBA-induced mammary tumors to treatment with FCE 21336 and MPA

Treatment (mg/kg)*				Effect at the end of treatment No. (%) of:†					Prolactin‡
FCE 21336 (p.o.)	MPA (s.c.)	No. of rats	No. of tumors	CR	PR	NC	P	New tumors/ rat	(ng/ml) mean ± S.E.
0	0	11	14	0 (0)	1 (7)	7 (50)	6 (43)	1	24.1 ± 3.4
0	25	11	11	5 (46)	2 (18)	1 (9)	3 (27)	0.2	5.9 ± 1.4§
0	50	11	12	6 (50)	3 (25)	2 (17)	1 (8)	0.3	6.7 ± 2.5§
0.05	0	10	12	2 (17)	5 (41)	3 (25)	2 (17)	0	12.3 ± 5.4§
0.05	25	10	10	7 (70)	3 (30)	0 (0)	0 (0)	0.2	2.0 ± 0.3§
0.05	50	10	11	10 (91)	0 (0)	0 (0)	1 (9)	0	2.1 ± 0.2§
0.40	0	10	11	4 (36)	0 (0)	5 (45)	2 (19)	0	1.7 ± 0.2§
0.40	25	10	15	14 (93)	0 (0)	0 (0)	1 (7)	0	1.0 ± 0.1§
0.40	50	11	14	10 (72)	2 (14)	1 (7)	1 (7)	0	1.4 ± 0.2§

*Compounds were administered daily, 5 times a week for 4 weeks.

†CR = complete remission; PR = partial remission; NC = no change; P = progression.

‡24 hr after the last dose.

§$P < 0.01$ vs controls (Dunnett's *t* test).

mg/kg. The incidence of newly appearing tumors was low at both doses. FCE 21336 at the dose of 0.05 mg/kg induced 58% regressions and at the dose of 0.40 mg/kg 36% regressions. No new tumors appeared in either group. Combined treatment inhibited tumor growth more than single treatment, the percentage of regressions ranging from 86 to 100%. Development of new tumors was also reduced or completely inhibited.

Statistical analysis, as reported in Materials and Methods, showed that the combined treatment with FCE 21336 plus MPA 25 mg/kg or MPA 50 mg/kg gave on average a parallel and significantly higher response than FCE 21336 alone. The combined treatment with MPA and FCE 21336 at the dose of 0.05 mg/kg but not at the dose of 0.40 mg/kg gave on average a parallel and significantly higher response than MPA alone. As regards the PRL-lowering activity, FCE 21336 caused a significant, dose-related decrease of serum PRL; mean serum PRL was reduced by 49 and 93%. MPA reduced serum PRL at both doses tested: the percentage of inhibition was 75 and 72%. In the rats given the combined treatment serum PRL was lowered 91–96%.

Experiment II: comparison of combined treatment and ovariectomy; activity of MPA on FCE 21336-resistant tumors

The effect of the combined treatment using FCE 21336 0.1 mg/kg plus MPA 50 mg/kg was compared with the effect of ovariectomy. Results reported in Table 2 show that ovariectomy was highly effective on DMBA-induced tumors (100% tumor regressions). The frequency of tumor regressions in the ovariectomized group was significantly higher than in the groups treated with FCE 21336 alone ($P < 0.001$) and MPA alone ($P = 0.017$) but was not statistically different from the frequency in the group given the combination of FCE 21336 and MPA ($P = 0.26$).

In this experiment a large number of rats were treated with FCE 21336 in order to select animals with tumors not responding to the 4-week antiprolactin treatment. The animals with FCE 21336-unresponsive tumors were divided in two groups: 3 days after the last FCE 21336 dose they were either ovariectomized or treated with MPA 50 mg/kg s.c. for 4 weeks. Both treatments induced regressions of FCE 21336-unresponsive tumors (Table 3). Ovariectomy (90% regressions) was more effective ($P = 0.036$) than MPA (61% regressions). MPA activity on FCE 21336-pretreated tumors was similar to that on non-pretreated tumors observed in the previous experiment (Tables 2 and 3).

DISCUSSION

Prolactin and estrogen are essential for the development and growth of DMBA-induced mammary tumors [13]. When circulating levels of these two hormones were altered by drug treatment

Table 2. Response of DMBA-induced mammary tumors to treatment with FCE 21336 and MPA or to ovariectomy

Treatment (mg/kg)*				Effect at the end of treatment				
FCE 21336 (p.o.)	MPA (s.c.)	No. of rats	No. of tumors	No. (%) of:† CR	PR	NC	P	New tumors/ rat
0	0	11	19	1 (5)	1 (5)	7 (37)	10 (53)	1.4
0.1	0	64	101	41 (40)	19 (19)	20 (20)	21 (21)	0.2
0	50	12	18	8 (44)	5 (28)	3 (17)	2 (11)	0
0.1	50	12	21	17 (81)	2 (9)	1 (5)	1 (5)	0.05
Ovariectomy		12	20	16 (80)	4 (20)	0 (0)	0 (0)	0

*Compounds were administered daily, 5 times a week for 4 weeks. Ovariectomy was performed on day 1 of treatment and its effect was evaluated at the end of the 4 weeks.

†CR = complete remission; PR = partial remission; NC = no change; P = progression.

Table 3. Effect of MPA (50 mg/kg s.c.) or ovariectomy on DMBA-induced mammary tumors unresponsive to a previous 4-week treatment with FCE 21336 (0.1 mg/kg p.o.)

				No. of tumors†			
	No. of	Start of 2nd treatment		End of 2nd treatment			
2nd treatment*	rats	Total	(NC + P)	CR	PR	NC	P
MPA	15	18	(9 + 9)	7 (39)‡	4 (22)	3 (17)	4 (22)
Ovariectomy	15	21	(11 + 10)	13 (62)	6 (28)	1 (5)	1 (5)

*Three days after the last dose of FCE 21336 rats were treated with MPA daily, 5 times a week for 4 weeks, or ovariectomized.

†CR = complete remission; PR = partial remission; NC = no change; P = progression.

‡(Percentage.)

or surgical manipulations development and growth of mammary tumors were profoundly affected.

The findings reported here indicate that combined treatment using MPA and FCE 21336, a new PRL-lowering drug, resulted in a larger percentage of regressions in the DMBA-induced tumor model than either treatment singly. The activity of the combined treatment was comparable to that of ovariectomy which, in our experiment, resulted in 100% regression of tumors.

FCE 21336 is a potent, long-acting inhibitor of both basal secretion and pharmacological and physiological hypersecretion of PRL in rats [5]. In rats bearing DMBA-induced tumors FCE 21336 reduced serum PRL levels, and a correlation between lowering of serum PRL and antitumor activity has already been described [6]. FCE 21336 does not influence LH secretion [14], it has no uterotrophic or anti-uterotrophic activity in rats and it does not display antiprogestinic activity in rabbits [Di Salle *et al.*, unpublished data]. In addition, FCE 21336 has no direct cytotoxic activity *in vitro* on HeLa cells [Geroni *et al.*, unpublished data]. Therefore lowering of PRL is probably the main mechanism of the antitumor activity of this compound.

The antitumor and prolactin-lowering activity of MPA in DMBA-induced mammary tumor model has been described [7]. In the present investigation (experiment I) MPA reduced serum PRL at both doses tested. The antitumor activity of MPA in this experimental model in rats can only partially be attributed to its PRL-lowering activity. In fact we observed (experiment II) that MPA was also effective on tumors unresponsive to previous therapy with the hypoprolactinemic drug FCE 21336.

Other hormonal activities of MPA besides its antigonadotrophic effect [15] help explain its effect on hormone-sensitive tumors: interaction with steroid receptors in the tumor tissue [16], lowering of estradiol levels through increased catabolism [17] and decreased androgen–estrogen conversion [18], inhibition of DNA synthesis [19].

Even though it has been shown that PRL plays a prominent role in the maintenance of growth of DMBA-induced rat mammary carcinoma [20], the role of PRL in human breast cancer remains controversial [21]. Combined treatment using high-dose MPA and bromocriptine has already been tested in advanced breast cancer patients and the high percentage of positive responses was correlated with the estrogen and progesterone receptor content of the tumors [22]. The findings reported here suggest that a similar combination of PRL-lowering drug and high-dose MPA may be more beneficial for treating hormone-responsive human breast carcinomas than single endocrine therapy.

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