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Correlation between Inhibitory Effect on Prolactin Secretion and Antitumor Activity of New Ergoline Compounds on DMBA-induced Tumors in Rats

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Abstract—*Five recently synthesized (355/1057, 355/1000, 355/1101, 355/1138 and FCE 21336) and 4 well-known (bromocriptine, metergoline, 1-demethylmetergoline and pergolide) prolactin-lowering ergoline derivatives and 1 ergoline (nicergoline) without antiprolactin activity were tested against 7-12-dimethylbenzanthracene (DMBA)-induced mammary carcinomas in rats. Nicergoline did not show any activity, while the other compounds, tested at doses inhibiting prolactin secretion, proved active against established tumors and on the onset of new tumors. The activity of 3 of the new ergolines (355/1000, 355/1057 and FCE 21336) and of bromocriptine and pergolide was also tested at different oral doses and was correlated with serum prolactin levels 24 hr after the last dose. All the compounds proved highly effective, inducing 50–60% regression of the initial tumors. The inhibition of serum prolactin levels was dose-related and, for all the compounds tested except bromocriptine, a good correlation was found between doses administered and complete tumor remissions.*

INTRODUCTION

THE IMPORTANT role of prolactin (PRL) on the development of spontaneous mammary tumors in mice [1] and rats [2] and its importance in the induction [3] and growth [4–7] of DMBA-induced rat mammary carcinomas is well established. Both estrogens and PRL are essential for the growth of these tumors and interactions between these two hormones are present at the target cells [8]. However, some results suggest that PRL is more important than estrogens in maintaining the growth of DMBA-induced mammary tumors [9]. In this experimental system an increase in plasma PRL levels frequently promotes tumor growth [5] while a reduction in PRL levels is often followed by tumor regression, even though no clear correlation between PRL inhibition and effects on tumor growth has been established [6, 10].

To further investigate the relationship between PRL secretion-inhibiting activity and antitumor activity we used the DMBA-induced mammary tumor in rats to study 5 new antiprolactin ergoline derivatives, 355/1000 [11], 355/1101 [11], 355/1057 and 355/1138 (Bernadi *et al.*, in preparation) and FCE 21336 [12], and 3 well-known antiprolactin drugs, bromocriptine [13], pergolide [14] and metergoline [15]. The antitumor activity of 1-demethylmetergoline, an active metabolite of metergoline [16, 17], and of nicergoline, an α-adrenergic blocking ergoline derivative [18] without antiprolactin activity, was also studied.

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MATERIALS AND METHODS

Drugs

The ergoline derivatives 355/1000, 355/1101, 355/1057, 355/1138, FCE 21336, metergoline, 1-demethylmetergoline and nicergoline were provided by Farmitalia Carlo Erba, Milan, Italy. Bromocriptine was supplied by Sandoz, Milan, Italy and pergolide was supplied by Eli Lilly, Rome, Italy. Their chemical names are reported in Table 1, together with the vehicles used for the preparation of the solutions. The doses used are reported as free base.

Table 1. Compounds

Laboratory code or INN	Chemical name	Solvents* s.c.	p.o./i.p.
355/1000	8β-benzoyloxyacetyl-6-methyl-ergoline	MA+H_2O	GA
355/1101	8β-benzoyloxyacetyl-2-chloro-6-methyl-ergoline	GA	GA
355/1057	2-cyano-3-(6-methylergolin-8β-yl)propionamide	MA+H_2O	GA
355/1138	2-cyano-3-(6-methylergolin-8β-yl)-*N*-ethylpropionamide	MA+H_2O	GA
FCE 21336	1-ethyl-3-(3'-dimethylaminopropyl)-3-(6'-allylergoline-8'β-carbonyl)-urea diphosphate	H_2O	H_2O
Metergoline	8β-carbobenzyloxyaminomethyl-1,6-dimethyl-ergoline	MA+H_2O	GA
1-Demethylmetergoline	8β-carbobenzyloxyaminomethyl-6-methyl-ergoline	MA+H_2O	GA
Nicergoline	10-methoxy-1,6-dimethylergoline-8β-methanol 5-bromo-3-pyridine carboxylate (ester)	TA+H_2O	GA
Bromocriptine	2-Br-α-ergocryptine mesylate	E+H_2O	GA
Pergolide	(8β)-8-((methylthio)methyl)-6-propylergolinemesylate	E+MA+H_2O	E+MA+H_2O

*MA: 0.1 M methanesulfonic acid (equimolar); GA: 5% gum arabic; TA: 0.1 M tartaric acid (equimolar); E: ethanol.

Nidation inhibition in rats

The egg nidation inhibition test in rats was made [19] to assess the prolactin secretion inhibitory activity of the ergoline derivatives. Female Sprague–Dawley rats (200–250 g body wt) were caged with fertile males the evening of the day of proestrus. The presence of spermatozoa in the vaginal smear taken the following morning was used as an indication of impregnation (day 1 of pregnancy). The pregnant rats were treated p.o. or s.c. on the morning of day 5, killed on day 14 and the uteri were examined for the presence of implantation sites.

The compounds were tested at different doses (8–10 animals per group) and the ED_{50} values were calculated by probit analysis. The nidation-inhibiting ED_{50} of a drug is similar to the dose needed to induce 50% suppression of serum prolactin 16–24 hr later in adult male rats [20].

Mammary tumor induction

Tumors were induced by 7,12-dimethylbenzanthracene (DMBA) given i.v. or orally. Female Sprague–Dawley (CD) rats (Charles River, Calco, Italy), 50–55 days old, were given a single oral dose of 20mg DMBA dissolved in 1 ml of sesame oil or a single i.v. injection of a lipid emulsion containing 5 mg DMBA. The DMBA used for the oral route was obtained from Sigma Chemical Co., St. Louis, MA. The DMBA lipid emulsion used by i.v. route was a gift from Dr. Paul Schurr, Upjohn Co., Kalamazoo, MI.

Antitumor activity evaluation

Animals were housed in standard laboratory conditions and assigned to experimental groups when each rat had at least 1 tumor measuring more than 1 cm in average diameter. Animals with tumors arising more than 4 months after induction were discarded. Drugs were administered once daily s.c., i.p. or p.o. on 5 consecutive days a week for 4 weeks; in one experiment animals were treated 6 days a week for 4 weeks. Control rats were treated by the same route and with the same solvent used for test drugs. All treatments were given at 10 a.m. Tumor diameters were measured by calipers at the beginning of therapy and twice weekly thereafter. Tumor weight was calculated from tumor diameters according to Geran *et al.* [21]. At the end of the treatment period the following parameters were recorded: the number of tumors that regressed completely (CR), the number of tumors which decreased to less than 50% of the initial weight (PR) and the number of new tumors that appeared during the treatment period.

Prolactin assay in tumor-bearing rats

Blood samples for PRL determination were collected via the eye plexus 24 hr after the last dose. The samples were kept on ice for 1 hr and centrifuged at +4°C. Serum was removed and stored at -20°C until analyzed. PRL levels were assayed by a double antibody radioimmunoassay according to Niswender *et al.* [22] using the kit supplied by the National Hormone and Pituitary Program, NIADDK, National Institute of Health, Bethesda, MD. The results were converted to percentage differences from control values.

RESULTS

Nidation inhibition in rats

Table 2 sets out the nidation inhibitory ED_{50} values (as an index of antiprolactin activity) of the new ergoline compounds and of nicergoline compared with known PRL-lowering ergoline drugs such as bromocriptine, metergoline, 1-demethylmetergoline and pergolide, given s.c. and p.o. Nicergoline did not inhibit nidation in rats at the maximal dose tested of 100 mg/kg, but

Table 2. Nidation inhibition in rat

Compound*	ED_{50} (mg/kg)† (s.c.)	(p.o.)
355/1000	1.000	0.500
355/1101	0.700	1.500
355/1057	n.d.‡	10.700
355/1138	0.700	0.400
FCE 21336	0.009	0.025
Metergoline	79.200	20.800
1-Demethylmetergoline	15.800	4.400
Nicergoline	>100.000	>100.000
Bromocriptine	0.900	5.700
Pergolide	0.020	0.017

*Compounds were administered once on day 5 after insemination.

†Dose inhibiting egg nidation in 50% of the animals (3–4 doses per compound; at least 8 animals per dose).

‡n.d.: not determined.

all the other compounds were effective on this PRL-dependent process. Some of the new compounds proved very potent, giving an oral ED_{50} at very low doses: 0.025, 0.4 and 0.5 mg/kg respectively for FCE 21336, 355/1138 and 355/1000. 1-Demethylmetergoline, the main metabolite of metergoline in the rat [17], was 5 times more potent than metergoline; both compounds were more potent (4 times) by the oral than the subcutaneous route, as previously reported [16]. Bromocriptine was much more effective (about 6 times) when given s.c. compared with the oral route. Pergolide and FCE 21336 were the most potent compounds.

Antitumor activity

Tumor responses to the compounds, tested at doses higher than those active on nidation inhibition and administered 5 days a week for 4 weeks, are reported in Table 3. All the new ergoline compounds, bromocriptine and 1-demethylmetergoline when given s.c. were effective on established tumors since treated animals showed 35–75% tumor regressions (CR + PR). In addition, fewer new tumors developed in all the treated animals compared to controls. Metergoline, administered orally at 4 mg/kg, a dose well tolerated in normal rats, was toxic on DMBA-treated rats. This dose, about 1/5 of the nidation inhibitory ED_{50}, was not effective on either existing tumors or new tumors.

When metergoline was administered i.p. (8 mg/ kg) it was not toxic and caused 44% tumor regressions. Nicergoline was not active at all.

The antitumor activity of some compounds (355/1000, 355/1057, FCE 21336, bromocriptine and pergolide) was more extensively investigated and correlated with PRL secretion inhibition. The compounds were administered at different oral doses (5 or 6 days a week for 4 weeks) and serum PRL levels were assayed 24 hr after the last dose. Results are reported in Table 4.

All the compounds were active, causing 50–60% tumor regression. FCE 21336 was highly effective, with a high percentage of complete regressions (40–54%); the compound proved more active when administered according to a 6-day schedule with a 1-day interval each week rather than with the 5-day schedule. All the compounds tested, except bromocriptine, showed increasing activity as the dose rose. Development of new tumors during the treatment period was also markedly reduced in animals treated with all the compounds compared with controls. Inhibition of serum PRL levels 24 hr after the last dose was dose-related for all the compounds tested; at the highest doses tested 355/1000 and FCE 21336 caused almost complete PRL inhibition (90%), lasting until 24 hr after treatment.

DISCUSSION

This study shows that repeated treatment with the new ergoline compounds, tested at doses and

Table 3. Activity against DMBA-induced tumors in rats

Compound	Dose* (mg/kg)	Route	% CR	% PR	% CR+PR	New tumors/ rat	Deaths
355/1000	4	s.c.	45	8	53	0.2	0/28
355/1101	4	s.c.	58	17	75	0.1	0/8
355/1057	16	s.c.	33	11	44	0.6	0/8
355/1138	4	s.c.	21	14	35	0.1	0/8
Metergoline	4	p.o.	8	0	8	1.0	2/9
	8	i.p.	44	0	44	0.4	0/8
1-Demethylmetergoline	8	s.c.	23	15	38	0.4	0/10
Nicergoline	10	s.c.	8	8	16	0.9	0/10
Bromocriptine	4	s.c.	33	27	60	0.1	0/25

Tumor induction by oral route. CR = complete remission; PR = partial remission. In these experiments 42 control (vehicle-treated) rats were used with 58 initial tumors. At the end of the 4-week treatment period there were 3% CR (2/58), 14% PR (8/58) and 0.95 new tumors per rat (40 tumors/42 rats).

*5 days/week for 4 weeks.

Table 4. Activity against DMBA-induced tumors after oral treatment

Compound	Dose† (mg/kg)	No. of rats	No. of tumors	% CR		% PR		% CR+PR		New tumors/ rat		% PRL change‡	
355/1000*	0.125	21	31	12	27	0	0	12	27	0.7	0.60	-33	0
	1.000	24	30	13	13	19	7	32	20	0.4	0.10	-84	-79
	8.000	22	32	31	37	31	25	62	62	0.1	0.20	-87	-95
355/1057	1.250	10	10	10		20		30		0.3		+ 9	
	10.000	11	15	27		13		40		0.4		+24	
	80.000	9	11	36		27		63		0.8		-78	
FCE 21336*	0.001	18	27		7		29		36		0.70		+69
	0.010	25	32	20§	9	30§	23	50§	32	0.1§	0.40	-57§	+ 7
	0.100	26	45	54§	23	0§	18	54§	41	0.2§	0.05	-84§	-90
	1.000	26	29	45§	40	11§	15	56§	55	0.2§	0.00	-92§	-89
Bromocriptine*	4.000	8	11	0		18		18		0.5		- 6	
	10.000	10	11		36		18		54		0.20		-30
	32.000	19	31	6	12	31	19	37	31	0.5	0.30	-82	-74
Pergolide	0.001	18	27	4		14		18		0.4		+33	
	0.010	18	22	8		23		31		0.3		-14	
	0.100	18	23	22		30		52		0.2		-70	

Tumor induction by i.v. route. CR = complete remission; PR = partial remission. In these experiments 48 control (vehicle-treated) rats were used with 74 initial tumors. At the end of the 4-week treatment period there were 3% CR (2/74), 9% PR (7/74) and 0.96 new tumors per rat (46 tumors/48 rats).

*Two experiments were performed; values in each column refer to single experiments.

†5 days/week for 4 weeks.

‡24 hr after the last dose.

§In this experiment animals were treated 6 days/week for 4 weeks.

by routes of administration known to inhibit a PRL-dependent process like egg nidation in rats, are effective on DMBA-induced tumors. That these tumors are dependent on PRL has been well documented [3–5, 9], but no clear correlation between PRL inhibition and effects on tumor growth has yet been established. Previous studies have shown that ergoline compounds which reduce serum PRL levels are often, but not always, active on DMBA-induced tumors [6, 10], and some studies have reported that not all the compounds which depress serum PRL levels cause regression of these tumors [10]. One of the reasons for the lack of antitumor activity of some of these compounds might be that they are not able to keep PRL levels low for long enough since their effect on PRL has been checked only 1 hr after treatment [10].

It seems that the antitumor activity of this class of compounds in the experimental system employed here is correlated with their anti-prolactin activity. In fact, nicergoline, an ergoline derivative with α-adrenolytic activity [18] which does not inhibit nidation in rats, had no effect on this tumor. Metergoline, too, which lowers serum prolactin both in clinical studies and in rats [23–25], did not show any antitumor effect at oral doses much lower than the nidation inhibitory ED_{50}.

In contrast, when metergoline was given i.p., a route by which it is more effective on PRL inhibition [15], it was active both on existing and new tumors. All the other compounds, selected as very effective PRL inhibitors, were active against these tumors.

The correlation between PRL inhibition and antitumor activity of this class of compounds is confirmed by the results of experiments in which different doses of the drugs were tested and PRL inhibition was evaluated 24 hr after the last dose. Correlation analysis of the data referring to all the drugs showed that PRL inhibition was significantly related to the total number of remissions ($r = 0.62$, $P = 0.003$) and the onset of new tumors ($r = 0.52$, $P = 0.017$). For some compounds, such as FCE 21336, given 6 days a week, and pergolide, the trend of correlation with remissions is even better ($r = 0.99$).

As regards the remissions evaluated, the number of complete remissions correlated with PRL inhibition ($r = 0.62$, $P = 0.003$) while the number of partial remissions did not. Perhaps this latter response is due to tumors in which only some cells are sensitive to PRL; it must also be considered that treatment lasted only 4 weeks and a longer treatment period might result in some partial remissions becoming complete. The lack of correlation for oral bromocriptine between PRL inhibition and antitumor effect may be due to the fact that the higher dose tested (32 mg/kg) was slightly toxic since all the treated rats lost 10–15 g during the first week of treatment, compared with weight loss of 0–5 g in control rats and rats treated with the other drugs.

Controversial results have been reported about the effect of bromocriptine on DMBA-induced tumors. Some authors, giving the drugs s.c., found good activity on established tumors and no effect on new tumors [7], while others [6] at a similar dose and by the same route reported little anititumor effect and good inhibition of the onset of new tumors. It must be pointed out that not all DMBA-induced mammary carcinomas are PRL-dependent [9] and as the experimental system is heterogeneous, large numbers of animals must be used to permit conclusions on the activity of antiprolactin drugs on these tumors. Since the antitumor effect of ergot compounds on breast cancer depends on their inhibition of PRL secretion, ergoline derivatives like these recently synthesized, which are very effective and potent on PRL inhibition, might be useful not only on PRL-dependent disorders in man but also in pituitary and breast cancer. The usefulness of antiprolactin drugs on pituitary tumors has been recently pointed out [25] and reduction of serum PRL has been associated with a decrease of pituitary tumor size and correction of the physiological defects associated with this tumor [26].

The role of PRL in human breast cancer is still controversial. Clinical and biochemical evidence suggests that this hormone may indeed play an important role in the natural history of breast cancer [27], but the first clinical trials with PRL-suppressing drugs such as bromocriptine and L-dopa have yielded conflicting results [28–30]. Recently it was reported that plasma PRL levels were higher in postmenopausal breast cancer patients than in normal postmenopausal women [31, 32]. It has also been found that hyperprolactinemia is an indicator of poor prognosis in metastatic breast cancer and that in hyperprolactinemic patients refractory to chemotherapy, normalization of PRL by bromocriptine increases chemotherapy remission rates [33].

Much recent work deals with detection of PRL receptors in human breast cancer and their correlation with histology, menopausal status, recurrency rates and presence of other hormone receptors [34–37]. Since a correlation has been reported between the number of PRL receptors and the responsiveness of DMBA-tumors to endocrine manipulations [38], investigation of the PRL receptor status of human mammary tumors considered for treatment with ergoline compounds might prove helpful in assessing the biological effectiveness of these drugs.

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