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Activity of 6-Methyl-8-substituted Ergolines against the 7,12-Dimethylbenz[a]anthracene-induced Mammary Carcinoma[1]

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SUMMARY

The ability of a series of 8β-carboxamido ergolines, 8-formamido ergolines, and 8-methyl ergolines to cause regressions of established dimethylbenz[a]anthracene-induced mammary carcinomas was compared to some ergot alkaloids. Although most of the ergoline derivatives depressed serum prolactin concentrations in rats, only a few had pronounced effects against the dimethylbenz[a]anthracene-induced mammary carcinoma in rats. Some derivatives from each of the three groups of substituted ergolines gave comparable activities against the dimethylbenz[a]anthracene-induced mammary carcinoma.

INTRODUCTION

Many postmenopausal women with metastatic breast carcinoma have a higher mean basal serum level of prolactin than do postmenopausal women without breast cancer (6). Although the dependency of normal breast development upon PL[2] has been known for many years, the dependency of some breast cancer on PL has been established recently (8, 9). Therefore, drugs that are capable of lowering serum PL concentrations may be useful in the treatment of PL-dependent breast cancer.

The 1st class of compounds that was found to inhibit PL was the ergot alkaloids (4, 5, 7). Studies by Nagasawa and Meites (7) and by Shaar and Clemens (11) showed the direct effect of ergocornine on serum PL in rats. A synthetic derivative of ergocryptine, 2-bromo-α-ergocryptine, also inhibited lactation and experimental mammary tumors (3).

A program of chemical modifications of the ergoline nucleus of the ergot alkaloids was begun to determine the effect of various chemical groups on the PL and tumor inhibition. Many of the derivatives were 9,10-dihydro derivatives, because the reduction of this double bond reduced or eliminated the vasoconstrictive properties of the alkaloids (1).

METHODS AND MATERIALS

Measurement of Antitumor Effect. The DMBA-induced mammary carcinoma in Sprague-Dawley rats was the experimental tumor used because it is PL dependent (7). The tumors were induced by a single p.o. dose of 20 mg of DMBA (Calbiochem, La Jolla, Calif.) in 1.0 ml of corn oil by gavage in 50- to 54-day-old female Sprague-Dawley rats weighing 100 ± 10 g. The 1st tumors were seen in 7 weeks.

Rats with tumor areas of 36 to 100 sq mm were put into groups of 5 or 10 for testing. Many rats had more than 1 tumor. Each tumor was identified by diagram and its area was measured by vernier calipers and recorded separately. Compounds were dissolved in corn oil and dosed s.c. at 3 mg/kg once daily for 13 days, and the tumors were measured on the 14th day. The following parameters were also included: the number of tumors that regressed and the percentage of change in the area, the number of tumors that grew and the percentage of change in the area, the number of complete remissions, and the number of new tumors that appeared during the treatment period. The volumes for dosing were 0.1 ml s.c., 0.5 ml p.o., 0.2 ml i.m., and 0.2 ml i.v. (water-soluble salts only).

RESULTS

Ergot Alkaloids

Ergocornine and several other related ergot alkaloids were used as standard references for PL depression and activity against the DMBA-induced mammary carcinoma. Ergocornine (I) was the most active of the ergot alkaloids tested. It depressed the PL serum levels by 76% and reduced the area of the DMBA tumors by 31%. Dihydroergocornine (II) caused a 56% lowering of serum PL but only prevented the DMBA tumors from enlarging. Ergonovine (III) and ergotamine (IV) were less active in both respects (Table 1).

8-Ergolines

Several known ergolines and the new 6-methyl-8-substituted derivatives were compared to ergocornine and the other ergots. The PL inhibition data in Table 1 are those of Clemens *et al.* (2).

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[2] The abbreviations used are: PL, prolactin; DMBA, 7,12-dimethylbenz-[a]anthracene; LT, lergotrile; LTM, lergotrile mesylate.

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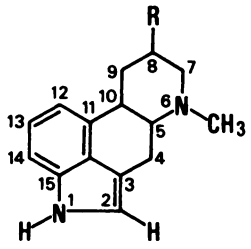
Table 1

Activity of ergoline derivatives (3 mg/kg s.c.; 13 days) against the DMBA-induced mammary carcinoma



6-Methylergoline nucleus

In these experiments 101 control rats were used. There were 20 partial and 5 complete remissions among the 121 tumors. The average area of the control tumors increased by 98.2%, and 81 new tumors appeared during the 14-day test. The average gain in body weight was 10.4 g.

Compound		R	Double-bond position	% prolactin inhibition at 1 hr	AWC[a] (g)	Partial remissions/ total tumors	Complete remissions/ total tumors	New tumors/ total rats	% growth (+) or regression (−) of tumor
Ergocornine	I[b]		9-10	76[c]	+2.2	9/12	2/12	5/10[d]	−31
Dihydroergo-cornine	II			47	+1.0	5/9	1/9	0/5	+3
Ergonovine	III		9-10	35	+0.8	2/5	0/5	1/5	+34
Ergotamine	IV		9-10	17	−0.8	2/7	0/7	2/5	+45
		8β-Carboxamides							
	V	$CONHCH_2CH_2CH(CH_3)OH$	9-10	60	−4.9	11/15	1/15	8/9	−32
	VI	CONH–C6H4–OH	9-10	58	−5.6	5/6	1/6	2/5	−35
	VII	$CONHCH_2C(CH_3)_3$	9-10	65	−0.4	1/6	1/6	1/5	+30
	VIII	CONH–C4H3S	9-10	57	−0.4	1/4	0/4	6/4	+68
	IX	CON–C4H8O	9-10	37	+0.2	1/5	0/5	1/5	+110
		8-Formamides							
	X	α-NHCHO	9-10	76	+6.8	6/6	5/6	2/5	−81
	XI	α-NHCHO-10β		70	+1.2	2/5	0/5	3/5	0
	XII	α-NHCHO-2,3-dihydro	9-10	41	+9.0	3/7	0/7	3/5	+36
	XIII	α-$NHCHOCH_3$	9-10	77	−14.0	4/7	2/7	0/5	+27
	XIV	β-NHCON–C4H4N		16	+4.6	2/10	0/10	0/5	+101
		8-Methylergolines							
Agroclavine	XV	CH_3	8-9	62	+0.2	1/6	0/6	1/5	+49
Elymoclavine	XVI	CH_2OH	8-9	71	−4.4	5/6	3/6	3/5	−41
Lysergol	XVII	CH_2OH	9-10	56	−0.5	11/18	0/18	5/10	−29
	XVIII	CH_2OH		59	+4.8	1/12	0/12	6/10	+66
	XIX	CH_2Cl		45	−1.8	3/6	1/6	6/5	+32
	XX	CH_2CN		85	−3.5	7/10	2/10	3/10	−38
	XXI	CH_2CN, 2-bromo		53	+9.9	7/10	1/10	3/10	−29
	XXII	CH_2CN, 2-chloro		63	−4.0	6/10	1/10	1/5	−30

[a] AWC, average weight change in rats during treatment.
[b] See Chart 1 for some of the structures of the ergot alkaloids.
[c] Data from paper of Clemens *et al.* (2).
[d] New tumors that appeared during treatment period.

Group 1, the 6-Methyl-8β-carboxamide Ergolines. The 8β-carboxamides, V to IX, caused 37 to 65% depression of serum PL in rats after 1 hr. Only 2 of the carboxamides, V and VI, had an effect against the DMBA tumor, with 32 and 35% remission, respectively.

Group 2, the 6-Methyl-8-formamides. The most active PL depressors in this group, X to XIV, were the unsubstituted formamides, X and XI, and the acetamide, XIII. However, only X was active in causing regression of the DMBA tumor. XI maintained the tumors at a zero growth

rate, while XIII retarded the growth of tumors (27%) compared to the control tumors, which grew by 96%.

Group 3, the 6-Methyl- 8-methyl Ergolines. Agroclavine (XV) the unsubstituted 8-methylergoline, depressed serum PL by 62% but it had little activity against the DMBA tumor (49% growth with 1 of 6 partial regressions). Monosubstituted 8β-methyl derivatives showed both PL depression and activity against the DMBA tumor. Lysergol (XVII), the 8β-hydroxymethylergoline, caused a 56% PL depression and a 29% regression of the DMBA tumor. Dihydrolysergol (XVIII), although as active as lysergol in depressing PL, was not effective against the DMBA tumor. The chloromethylergoline (XIX) was less active than agroclavine in prolactin inhibition.

The most active 8β-methylergoline derivative was the 8β-acetonitrile (XX) (10). It was also the most active of all the compounds in Table 1 in lowering PL serum levels in rats (85%) and was one of the best in causing an overall regression of the DMBA tumor (38%). Seven of 10 tumors partially regressed while 2 tumors regressed completely.

The substitution of halogens at position 2 in other ergolines is known to reduce toxicity. The 2-bromo-8β-acetonitrile (XXI) and the 2-chloro-8β-acetonitrile (XXII) showed less inhibition of PL release (53 and 63%, respectively). However, the 2-bromo-8β-acetonitrile and 2-chloro-8β-acetonitrile caused a number of regressions of the DMBA tumor, comparable to the regression seen with the parent compound.

There was little difference in the weight changes in the rats treated with either the acetonitrile or 2-chloroacetonitrile at 3 mg/kg. The 2-chloroacetonitrile derivative (XXII), LT, was favored for clinical trials. More extensive comparisons in rats with the acetonitrile showed that LT was less toxic and showed better activity than did 6-methylergoline-8β-acetonitrile at the lower doses against the DMBA tumor (Table 2).

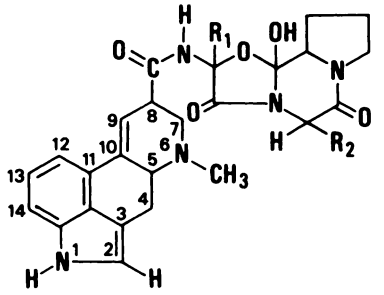
The methane sulfonate salt of LT, LTM, was water soluble and it was active only when given p.o. or i.v. (Table 3).

LTM was inactive at 3 and 6 mg/kg p.o. against the Ca755 mammary carcinoma, X5563 plasma cell myeloma, and the Mecca lymphosarcoma in mice. These tumors are not known to be PL dependent.

DISCUSSION

Previous studies have indicated that the ergot alkaloids can reduce the serum PL in animals (7, 11). The dependency of some breast tumors on pituitary hormones, and especially PL, has been focused on as an area for chemotherapy of breast carcinoma. The complex side chain of the ergot alkaloids complicates a synthetic approach to the availability of the ergots.

Several of the 6-methylergoline 8-derivatives showed ability to depress PL levels in serum and to cause a regression of the PL-dependent DMBA-induced mammary carcinoma. These compounds may have been able to depress serum PL levels for long periods of time, whereas compounds that were inactive against the tumor may not have been able to sustain the low PL levels. Therefore, those derivatives that would depress PL for 24 hr or longer would



$R_1 = CH(CH_3)_2$ $R_1 = CH_3$
$R_2 = CH(CH_3)_2$ $R_2 = CH_2C_6H_5$

I ERGOCORNINE IV ERGOTAMINE
[II 9-10, DIHYDROERGOCORNINE]

Chart 1. Structure of ergot alkaloid nucleus. Differences between some ergots are indicated.

Table 2
Activity of acetonitrile and LT against the DMBA mammary carcinoma in rats

Compound	Dose (mg/kg x doses s.c.)	AWC[a] (g)	New tumors	No. of tumors, increased area	No. of tumors, decreased area	No. of complete regressions	Av. % change in area
AN	7 × 13	−24.8	1[b]	0	5 (100)[c]	5	−100
AN	5 × 13	−17.8	1	3 (192)[d]	2 (58)	1	+65
AN	3 × 13	+0.4	2	6 (98)	1 (27)		+80
Control	0	+4.0	3	6 (106)	0		+106
LT	7 × 13	−1.4	1	1 (114)	4 (63)	1	−28
LT	5 × 13	−3.8	3	2 (47)	7 (39)	1	−20
LT	3 × 13	−4.0	1	1 (83)	9 (54)	1	−40
Control	0	+2.2	2	3 (219)	2 (48)		+25

[a] AWC, average weight change of the rats during treatment period, 5 rats/group; AN, acetonitrile.
[b] Number of new tumors that appeared during treatment.
[c] % decrease.
[d] % increase.

Table 3
Activity of LTM (3 mg/kg; 13 days) against the DMBA-induced mammary carcinoma
Five rats were used in each series; some had multiple tumors.

Route	New tumors	No. of tumors	No. of tumors	No. of complete regressions	Av. % change in tumor area
i.p.	2	4 (107)[a]	4 (69)[b]	2	+19
s.c.	1	3 (84)	1 (100)	1	+38
i.v.	0	0	7 (48)	1	−48
p.o.	0	0	6 (73)	3	−73

[a] % increase in size.
[b] % decrease in size.

have kept the PL low until the next daily dose of the ergoline.

The ergot alkaloids have an undesirable vasoconstrictive effect that has limited their use in long-term therapy. This effect can be eliminated by reduction of the double bond at positions 8 or 9. Dihydroergocornine lowers PL but is not a vasoconstrictor. Several of the ergolines studied in this report were dihydro derivatives. They were effective PL inhibitors and were active against the DMBA carcinoma.

One of the most effective ergoline derivatives, the 8-formamido (X), produced severe central nervous system toxicity in rats. The carboxamide series (V to IX) showed a dependency on the nature of the side chain.

LTM was selected as a clinical candidate and is now being evaluated against several PL-dependent disorders and in PL-dependent mammary carcinoma.

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