6-Methyl-8-β-ergoline-acetonitrile-Induced Inhibition of Mammary Hyperplastic Alveolar Nodular Development and Growth in C3H/HeJ Female Mice[1] (37178)

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Ergocornine and 2-bromo-α-ergocryptine (CB-154), two chemically similar alkaloids extracted from ergot, have been shown to be effective suppressors of pituitary prolactin secretion in murine species (1, 2) as well as in man (3, 4). The effect of these alkaloids in murine mammary tumorigenesis has recently received considerable attention, since prolactin is considered to be an important, perhaps essential, hormone in this neoplastic process (5, 6). Specifically, ergocornine and/or CB-154, the only ergot alkaloids so far shown to markedly impede murine mammary tumorigenesis, have been reported to suppress the development (7) and growth (7, 8) of hyperplastic alveolar nodules in the mammary glands of mice and to promote the regression (9) and reduce the incidence (10) of carcinogen-induced rat mammary tumors. In addition, these alkaloids have been shown to induce regression of spontaneous rat mammary tumors (11) and suppress the appearance of mammary tumors in mice bearing pituitary isografts (12).

A nontoxic, synthetic alkaloid, 6-methyl-8-β-ergoline-acetonitrile (MEA), which can easily be prepared from lysergic acid, has been shown to exhibit prolactin-suppressing activity similar to the ergot alkaloids. Recently it was demonstrated by radioimmunoassay that MEA is an effective suppressor of prolactin secretion in the rat (Clemens, J. A., unpublished data) and the drug also has been reported to interfere with pseudopregnancy and pregnancy in mice (13) and to prevent pregnancy in the rat (14). This alkaloid, however, has not been adequately evaluated for its effect on murine mammary tumors. This report describes the effect of MEA on (a) development and (b) growth of hyperplastic alveolar nodules in C3H/HeJ female mice.

Materials and Methods. All animals used in this study were C3H/HeJ female mice obtained from the Jackson Laboratories, Bar Harbor, ME. They were housed in a temperature (75 ± 1°F)- and light controlled (14 hr/day) room and provided a diet of Wayne Lab Blox (Allied Mills Inc., Chicago, IL) and water *ad libitum*.

Treatment of young nulliparous mice with MEA. Twenty 5-wk-old mice were injected sc daily for 9 mo with 0.1 mg MEA suspended in saline. The MEA suspension (1 mg/ml) was prepared by initially dissolving the drug in a minimal amount of ethanol and diluting to volume with saline. Ethanol constituted 1% or less of the final suspension. Ten 5-wk-old mice, injected sc daily for 9 mo with the saline–ethanol solution, served as controls. Estrous cycles were determined on the MEA-treated and control mice approximately 8 mo after the onset of treatment.

All mice were sacrificed 24 hr after their last injection, the ovaries were excised, weighed and fixed in Bouin's fluid for histological evaluation. Inguinal mammary glands were excised, spread flat on cork, fixed in 15% formalin and stained for wholemount evaluation by a standard procedure (15).

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TABLE I. Effect of 9 mo of Treatment of C3H/HeJ Nulliparous Mice with 6-Methyl-8-β-ergoline-acetonitrile (MEA) on Degree of Mammary Gland Development and Number of Mammary Hyperplastic Alveolar Nodules.

Group	Treatment[c]	No. of mice	Mean[d] inguinal mammary gland development	Mean[d] no. of hyperplastic alveolar nodules inguinal mammary glands	No. of mice free of hyperplastic alveolar nodules inguinal mammary glands
I	Controls	10	3.8 ± 0.2^a	1.6 ± 0.4^a	2
II	MEA[e]	20	2.2 ± 0.1^b	0.4 ± 0.1^b	13

[a]/[b] $p < .001$.
[c] All mice were 5 wk of age at the beginning of treatment.
[d] Mean ± standard error.
[e] MEA, 0.1 mg/mouse/day.

Mammary glands were rated for development according to the following: 1 = few ducts, few or no end buds; 2 = moderate duct growth, moderate number of end buds; 3 = numerous ducts and branches, many end buds; 4 = numerous ducts and branches, minimum lobulo-alveolar growth; 5 = numerous ducts and branches, moderate lobulo-alveolar growth; 6 = numerous ducts and branches, dense lobulo-alveolar growth as in late pregnancy. The number of hyperplastic alveolar nodules (HAN) were counted in the wholemount preparation. Only hyperplastic nodular outgrowths equal to or greater than 0.5 mm in diameter were recorded for computation. The wholemount preparations were examined under tenfold magnification and coded prior to grading. Mean differences between organ weight, number of HAN and length of estrous cycle were evaluated statistically by Student's *t* test. Mean differences between mammary gland development were evaluated statistically by the nonparametric Wilcoxin rank procedure test.

Treatment of mature multiparous mice with MEA. Twenty 6-mo-old mice were injected sc daily for 30 days with 0.1 mg MEA. Forty 6-mo-old mice served as controls; 20 were sacrificed at Day 0 and 20 at Day 30. The controls (Day 30) were injected daily with the diluent (saline–ethanol) only. Twenty-four hours after the last injection, the mice were sacrificed and ovarian, uterine and pituitaries were excised and weighed. Ovaries were fixed in Bouin's fluid for histological evaluation. Mammary glands were excised and evaluated for development and number of HAN. Twenty mice (controls, Day 0) were sacrificed at the beginning of the treatments for the purpose of determining the number of existing HAN in the mammary glands of the mice at the onset of treatment.

Results. Daily administration of MEA for 9 mo to young nulliparous mice resulted in a highly significant ($p < 0.001$) reduction in the incidence of HAN (Table I). Sixty-five percent of the MEA-treated mice were totally free of these hyperplasias in their inguinal mammary glands. Mammary gland development was significantly suppressed in the MEA-treated mice whereas ovarian weight was slightly increased in these animals (Table II). The length of the estrous cycle was not significantly altered in the MEA-treated mice although some of the animals showed a tendency of repeated days of estrus. MEA treatment had no significant effect on body weight gains during the treatment periods nor was there any apparent effect on ovarian histology. The drug appears to be considerably less toxic than the ergot alkaloids previously tested (7) as *all* treated mice tolerated the dosage without any observable side effects.

Daily administration of MEA for 30 days to mature multiparous mice induced a highly significant ($p < 0.001$) diminution in number of HAN (Table III). Significant atrophy of the mammary glands was also observed in these animals. No significant effect of the treatment was observed on body, uterine or pituitary weight (Table IV). Although

TABLE II. Effect of 9 mo of Treatment of C3H/HeJ Nulliparous Mice with 6-Methyl-8-β-ergoline-acetonitrile (MEA) on Ovarian Weight and Length of Estrous Cycle.

Group	Treatment[c]	No. of mice	Mean[d] ovarian wt (mg)	Mean[d] length of estrous cycle (days)
I	Controls	10	10.7 ± 0.6[a]	5.4 ± 0.1
II	MEA[e]	20	13.0 ± 0.5[b]	4.9 ± 0.3

[a]/[b] $p < .01$.

[c] All mice were 5 wk of age at the beginning of treatment. Estrous cycles were determined 8 mo after the onset of treatment.

[d] Mean ± standard error.

[e] MEA, 0.1 mg/mouse/day.

ovarian weight was not significantly altered in the MEA-treated mice, a number of the ovaries in these animals contained a large number of very prominent corpora lutea.

Discussion. The daily administration of MEA to either nulliparous or multiparous C3H/HeJ mice markedly inhibited the development and growth of HAN. These hyperplasias have been described by a number of investigators and established by the Berkeley group (16) as the precursors of mammary tumors in the mouse. HAN are considered precancerous primarily because tumors arise from them much more frequently and in less time than from normal tissue. The hormonal dependency of these hyperplasias is shown in studies which demonstrate that hypophysectomy or ovariectomy of mammary-tumor-susceptible mice suppresses their development and growth (17). Recently, it was reported that prolactin appears to be the principal hormone in the maintenance of these nodular outgrowths (18, 19).

MEA has been shown to be a potent suppressor of prolactin secretion in the rat, the most efficacious prolactin-inhibiting alkaloid thus far tested in this species (Clemens, J. A., unpublished data). The alkaloid also appears to effectively inhibit prolactin secretion in the mouse, as administration of the drug to this species has been reported to prevent pseudopregnancy and pregnancy. This effect can be overcome by concurrent administration of prolactin (13). Significant mammary gland atrophy, unaltered estrous cycles and a lack of an effect

TABLE III. Effect of 30 Days of Treatment of C3H/HeJ Multiparous Mice with 6-Methyl-8-β-ergoline-acetonitrile (MEA) on Degree of Mammary Gland Development and Number of Mammary Hyperplastic Alveolar Nodules.

Group	Treatment[d]	No. of mice	Mean[e] inguinal mammary gland development	Mean[e] no. of hyperplastic alveolar nodules inguinal mammary glands	No. of mice free of hyperplastic alveolar nodules inguinal mammary glands
I	Controls, Day 0	20	3.7 ± 0.1[a]	3.9 ± 0.6[c]	2
II	Controls, Day 30	20	3.9 ± 0.1[a]	4.8 ± 0.6[a]	0
III	MEA,[f] Day 30	20	2.6 ± 0.1[b]	2.2 ± 0.3[b]	5

[a]/[b] $p < .001$.

[c]/[b] $p < .02$.

[d] All mice were 6 months of age at the beginning of treatment.

[e] Mean ± standard error.

[f] MEA, 0.1 mg/mouse/day.

TABLE IV. Effect of 30 Days of Treatment of C3H/HeJ Multiparous Mice with 6-Methyl-8-β-ergoline-acetonitrile (MEA) on Body, Ovary, Uterus and Pituitary Weight.

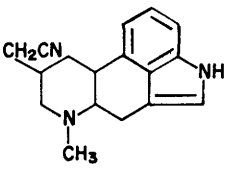
Group	Treatment[c]	No. of mice	Mean body wt (g)	Mean wt (mg)[d]		
				Ovary	Uterus	Pituitary
I	Controls	20	26.6	15.9 ± 0.8[a]	124.0 ± 9.1[a]	3.3 ± 0.2[a]
II	MEA[e]	20	28.0	17.1 ± 0.7[b]	108.3 ± 5.9[b]	2.9 ± 0.2[b]

[a/b] Not significantly different.
[c] All mice were 6 months of age at the beginning of treatment.
[d] Mean ± standard error.
[e] MEA 0.1 mg/mouse/day.

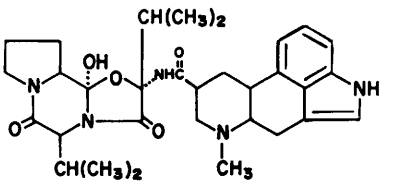
on uterine weight in MEA-treated mice, as observed in our study, provide further evidence that the drug acts by suppressing the secretion of this hormone. Furthermore, MEA appears to be relatively specific for prolactin as is the case with ergocornine and CB-154 in rats (20) and mice (8), as it does not appreciably influence the secretion of other pituitary hormones with the possible exception of LH. Although the site of action of MEA has not been determined, it is probable that it acts at the pituitary and/or hypothalamic level as do ergocornine and CB-154 (2, 20), because of structural similarities (Fig. 1).

Although MEA has been shown in this study to be highly effective in suppressing the developmental stages of murine mammary tumorigenesis, it remains to be determined whether or not this activity will result in a subsequent reduction in the incidence of mammary tumors. We have previously reported that the daily administration of CB-154 to young nulliparous C3H/HeJ mice markedly inhibits the development of HAN (7) and also reduces the incidence of mammary tumors (Welsch, C. W., unpublished data). In comparing the two alkaloids, CB-154 appears to be more effective in suppressing normal mammary gland and HAN development in the mouse than MEA, but the natural ergot has the disadvantage of being considerably more toxic, at the same dose level, than MEA. It is conceivable that the dose of MEA could be substantially increased, without significant toxicity, resulting in suppression of HAN development equal to or exceeding that observed with CB-154.

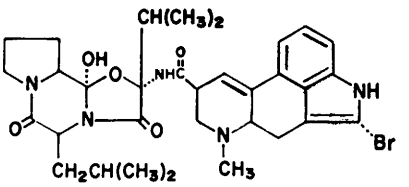
Established spontaneous mouse mammary tumors are almost exclusively independent of hormonal influences (21), therefore the hormonal control of these neoplasms is only feasible at the earlier, hormone-sensitive, developmental stages. Sixty–70% of advanced disseminated human breast cancers are also nonresponsive to hormone treatment (22) and



6-Methyl-8-β-Ergoline Acetonitrile



ERGOCORNINE



2-Br-α-ERGOCRYPTINE

FIG. 1. Structural formulas of MEA (top), ergocornine (middle) and CB-154 (bottom).

it can be presumed that only the developmental stages of these neoplasms are sensitive to the hormonal environment, perhaps because they are derived from and still closely akin to hormone responsive tissue. HAN-like structures have recently been identified in the breasts of women (23) although the growth response of these hyperplasias to various hormonal environments is totally unknown. Substantial evidence is now available in the human demonstrating the existence of prolactin as an identifiable hormonal substance (24), but the role it plays in human breast cancer is open to conjecture. There is a real need to define the hormonal requirements of normal and neoplastic human mammary tissue. Results of the present study suggest that, once these requirements are known, specific hormonal suppression by drug intervention may prove of great value in prophylaxis and early treatment.

Summary. Daily administration of 6-methyl-8-β-ergoline-acetonitrile (MEA) for 9 mo to young nulliparous C3H/HeJ mice or for 30 days to mature multiparous C3H/HeJ mice resulted in a significant reduction in incidence and diminution in number, respectively, of hyperplastic alveolar nodules (HAN). The alkaloid is known to suppress the secretion of pituitary prolactin. The results of this study provide impetus for continued investigation into the utilization of specific hormone suppression in the prophylaxis of mammary tumorigenesis.

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