Prophylaxis of Early Preneoplastic Lesions of the Mammary Gland

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Summary

Daily treatment (for 12 to 14 months) of 2-month-old nulliparous or 8-month-old multiparous C3H/HeJ mice with 0.1 mg of 2-bromo-α-ergocryptine (CB-154) or 6-methyl-8-β-ergoline-acetonitrile, efficacious inhibitors of prolactin secretion, markedly reduced the incidence of spontaneous mammary hyperplastic nodules and mammary tumors. CB-154 appeared to be more effective than 6-methyl-8-β-ergoline-acetonitrile in suppressing the incidence of mammary tumors; the ergot virtually prevented the appearance of mammary tumors in nulliparous mice. Daily treatment of 5-month-old estrogen-treated, ovarietomized-hysterectomized C3H/HeJ mice for 12 months with CB-154 also significantly reduced the incidence of hyperplastic nodules and mammary tumors when compared with ovarietomized-hysterectomized mice treated with the steroid alone. Daily treatment of multiparous C3H/HeJ mammary tumor-bearing mice with CB-154 or 6-methyl-8-β-ergoline-acetonitrile generally failed, however, to promote regression of the mammary tumors. Thus significant prophylaxis of early preneoplastic lesions by drug-induced hormone (prolactin) suppression, resulting in a marked reduction in mammary tumor incidence, has been demonstrated in this study.

Introduction

Hyperplastic alveolar nodules of the mammary gland have been described by a number of investigators and affirmed by the Berkeley group (4) to be the precursors of many of the mammary tumors that occur in the mouse. These hyperplasias are considered precancerous 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 that demonstrate that hypophysectomy or ovarietomy of mammary tumor-susceptible mice suppresses their development and growth (9). The purpose of this report is to provide evidence that pituitary prolactin is critically important in the developmental and growth phases of these precancerous outgrowths in the mouse. The feasibility of such a study is derived from the recent availability of drugs that are potent inhibitors of prolactin secretion. These drugs, CB-154 and MEA, are members of a series of ergot alkaloids and ergoline derivatives, respectively, which effectively reduce prolactin secretion, not only in rodents (2, 3) but in domestic animals and humans as well (7, 8).

Materials and Methods

The mice used in these studies were C3H/HeJ female mice obtained from The Jackson Laboratory, Bar Harbor, Maine. All mice were housed in a temperature- (24 ± 1°) and light-controlled (14 hr/day) room and provided a diet of Wayne Lab Blox (Allied Mills, Inc., Chicago, Ill.) and water ad libitum.

Treatment of Young Nulliparous C3H/HeJ Mice for 12 to 14 Months with CB-154 or MEA. Ninety 2-month-old mice were given s.c. injections (daily for 1 year) of 0.1 mg CB-154 suspended in 0.9% NaCl solution. The CB-154 suspension (1 mg/ml) was prepared by dissolving the drug initially in a minimal amount of ethanol and diluting to volume with 0.9% NaCl solution. In a 2nd group, one hundred 2-month-old mice were given s.c. injections daily for 14 months of 0.1 mg MEA suspended in 0.9% NaCl solution. The MEA suspension was prepared similarly to the CB-154 preparation. A 3rd group of ninety 2-month-old mice given s.c. injections of the diluent only (daily for 1 year) served as controls. One year after the initiation of treatment and 24 hr after the last injection, a number of mice from each group were sacrificed. Inguinal mammary glands were excised, spread flat on cork, fixed in 15% formalin, and stained for whole-mount evaluation by a standard procedure. Mammary glands were rated for development, and the number of HN were counted. Only HN outgrowths equal to or greater than 0.5 mm in diameter were recorded for computation. The remaining mice were observed for an additional 8- to 10-month period, i.e., until the age of 2 years, during which time they received no treatment. All mice were examined for mammary tumors at monthly intervals from onset of treatment. At 2 years of age, all surviving mice were sacrificed. Inguinal mammary glands were excised and prepared for whole-mount evaluation. Mean differences between the number of HN were evaluated statistically by Student’s t test. Mean differences between the levels of mammary gland development were evaluated statistically by the nonparametric Wilcoxon rank procedure test. Differences between mammary tumor incidence were evaluated by χ² analysis.

Treatment of Mature Multiparous C3H/HeJ Mice for 12 Months with CB-154 or MEA. Seventy 8-month-old mice (retired breeders), free of palpable mammary tumors, were
given daily s.c. injections of 0.1 mg CB-154 for 30-day periods, at bimonthly intervals (alternate months), for 1 year. In a 2nd group, seventy 8-month-old mice (retired breeders) free of palpable mammary tumors, were given daily s.c. injections of 0.1 mg MEA for 30-day periods, at bimonthly intervals, for 1 year. Seventy 8-month-old mice similarly treated, but with the diluent only, served as controls.

All mice were examined for mammary tumors at monthly intervals from the onset of treatment. At 19 months of age and 24 hr after the last 30-day treatment period, all surviving mice were sacrificed and inguinal mammary glands were excised and evaluated for development and number of HN.

Treatment of Mature Ovariectomized Nulliparous C3H/HeJ Mice for 1 Year with CB-154 and Estradiol. One hundred fourteen 5-month-old mice were ovariectomized-hysterectomized and divided into 2 groups. Group 1, controls, received 17β-estradiol via drinking water and daily s.c. injections of 0.9% NaCl solution. Group 2, CB-154-treated, received 17β-estradiol via drinking water and daily s.c. injections of 0.1 mg CB-154. It was determined, on the basis of water consumption, that each mouse ingested approximately 2.1 μg of the steroid per day. All mice were examined once weekly for mammary tumors. One year after the onset of treatment, all surviving mice were sacrificed. Inguinal mammary glands were excised and prepared for whole-mount evaluation.

Treatment of Mature Multiparous Mammary Tumor Bearing C3H/HeJ Mice for 4 Weeks with CB-154 or MEA. Eight mice, each bearing a single palpable spontaneous mammary tumor, minimum diameter, 1 cm, were given s.c. injections of CB-154 (4 mice) and MEA (4 mice) daily for 4 weeks. The dose of CB-154 and MEA during the 1st week was 0.1 mg/mouse/day and, during the 2nd through 4th weeks, was 0.2 mg/mouse/day. Mammary tumors were measured at their largest diameter with a vernier caliper once weekly.

Results

Daily treatment of young nulliparous mice for 12 to 14 months with CB-154 or MEA significantly suppressed mammary gland development, inhibited HN formation, and sharply suppressed the appearance of mammary tumors (Table 1). Mammary glands of the CB-154-treated mice were conspicuously atrophic, consisting of a minimal number of bare ducts at the end of the treatment period (14 months of age) (Figs. 1 to 4). Ten months after cessation of treatment, mammary gland development did not differ significantly from that in the controls. Seventy % of the mice were totally free of HN in their inguinal mammary glands immediately after CB-154 treatment. Mammary tumor incidence was 27% in the controls (24 of 90), whereas only 1 of 90 mice in the CB-154-treated group developed mammary tumors. Mammary tumor incidence and number of HN in the MEA-treated mice were less than one-third that observed in the controls. CB-154 treatment appeared to be slightly superior to MEA treatment in reducing mammary tumor incidence and promoting mammary hypoplasia. Neither drug significantly altered body weight gains or markedly influenced the estrous cycle. Rate of death (non-tumor related) among controls and drug-treated mice was similar; therefore, the number of mice at risk in each group throughout the study was comparable.

Daily treatment of mature multiparous mice with CB-154 or MEA at bimonthly intervals for 1 year significantly suppressed mammary gland development, inhibited HN formation, and reduced the incidence of mammary tumors (Table 2). The mammary glands of the drug-treated animals were relatively hypoplastic but still contained a large number of HN, although the number of HN was significantly less than that observed in the controls. Fifty-one % (36 of 70) of the controls developed mammary tumors in contrast to only 13% (9 of 70) of the CB-154-treated mice and 33% (23 of 70) of the MEA-treated mice. Neither drug altered body weight gains, compared to controls, nor influenced rate of death (non-tumor related).

Daily treatment of estrogen-treated, ovariectomized-hysterectomized mice with CB-154 significantly suppressed mammary gland development, inhibited HN development, and reduced the number of mammary tumors (Table 3). Mammary gland development in the estrogen-treated mice consisted of an extensive ductal system and numerous alveoli, in contrast to moderate ductal growth and a conspicuous hypoplasia. Mammary tumor incidence and number of HN in the MEA-treated mice were less than one-third that observed in the controls. CB-154 treatment appeared to be slightly superior to MEA treatment in reducing mammary tumor incidence and promoting mammary hypoplasia. Neither drug significantly altered body weight gains, compared to controls, nor influenced rate of death (non-tumor related).

Table 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of mice at beginning of study</th>
<th>Mean inguinal mammary gland development at:</th>
<th>Mean no. of hyperplastic nodules in inguinal mammary gland at:</th>
<th>No. of mice with mammary tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>14-16 mos.</td>
<td>24 mos.</td>
<td>14-16 mos.</td>
</tr>
<tr>
<td>Controls</td>
<td>90</td>
<td>3.6 (a)</td>
<td>3.8</td>
<td>3.0 ± 0.5 (a)</td>
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<tr>
<td>CB-154</td>
<td>90</td>
<td>1.0 (b)</td>
<td>3.3</td>
<td>0.4 ± 0.2 (b)</td>
</tr>
<tr>
<td>MEA</td>
<td>100</td>
<td>2.2 (b)</td>
<td>3.4</td>
<td>0.4 ± 0.1 (b)</td>
</tr>
</tbody>
</table>

a p < 0.001, a/b.

b Mean ± S.E.
Effects of treatment of mature multiparous C3H/HeJ mice with CB-154 and MEA on degree of mammary gland development, number of mammary HN, and mammary tumor incidence

All mice were treated daily for 1 year for 30-day periods at bimonthly intervals beginning at 8 months and terminating at 19 months of age. All surviving mice were sacrificed at 19 months of age. Data were from Welsch et al. (12) and Welsch and Gribler (11).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of mice at beginning of study</th>
<th>Mean inguinal mammary gland development at 19 mos.</th>
<th>Mean no. of HN in inguinal mammary gland at 19 mos.</th>
<th>No. of mice with mammary tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>70</td>
<td>4.1 (a)</td>
<td>15.9 ± 1.5 (a)</td>
<td>36 (a)</td>
</tr>
<tr>
<td>CB-154</td>
<td>70</td>
<td>1.8 (b)</td>
<td>10.0 ± 1.4 (b)</td>
<td>9 (b)</td>
</tr>
<tr>
<td>MEA</td>
<td>70</td>
<td>2.0 (b)</td>
<td>7.0 ± 1.0 (b)</td>
<td>23 (b)</td>
</tr>
</tbody>
</table>

*p < 0.01, a/b.
Mean ± S.E.

Effects of CB-154 treatment of estrogen-stimulated mature ovariectomized C3H/HeJ mice on degree of mammary gland development, number of mammary HN and mammary tumor incidence

Each mouse was ovariectomized-hysterectomized at 5 months of age. Between 5 months and 17 months of age, each mouse received 17β-estradiol via drinking water. Approximately one-half of the mice during this period of estrogen treatment were also treated daily with CB-154. All surviving mice were sacrificed at 17 months of age. Data were from Brooks and Welsch (1).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of mice at beginning of study</th>
<th>Mean inguinal mammary gland development at 17 mo.</th>
<th>Mean no. of HN in inguinal mammary gland at 17 mos.</th>
<th>No. of mice with mammary tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estradiol</td>
<td>55</td>
<td>4.3 (a)</td>
<td>19.3 ± 2.0 (a)</td>
<td>25 (c)</td>
</tr>
<tr>
<td>Estradiol plus</td>
<td>59</td>
<td>1.6 (b)</td>
<td>3.5 ± 0.6 (b)</td>
<td>19 (d)</td>
</tr>
<tr>
<td>CB-154</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*p < 0.001, a/b; p < 0.05, c/d.
Mean ± S.E.

Discusson

The results of these studies demonstrate that chronic suppression of pituitary prolactin secretion in C3H mice markedly inhibits the development of mammary HN and sharply reduces the incidence of mammary tumors. These results were particularly striking when prolactin secretion was suppressed in young nulliparous mice, as only 1 in 90 of the CB-154-treated mice developed a mammary tumor, and this tumor was first detected 9 months after cessation of drug treatment. In contrast, spontaneous mammary tu-
mors developed in over 25% (24 of 90) of the nulliparous controls, all of which eventually succumbed to the rapid tumor growth that is characteristic of these neoplasms. The nearly total prevention of mammary tumorigenesis in the prolactin-suppressed, nulliparous mice suggests that this hormone may be essential for neoplastic transformation of the C3H/HeJ mouse mammary gland. It is conceivable that limited availability of prolactin during the time period in which the conversion or progression to neoplasia would ordinarily occur either (a) renders existing mammary cells metabolically refractory to the neoplastic process or (b) interferes with the development of progenitive cells, whose progeny would be susceptible to neoplastic transformation.

Treatment of multiparous mice with either the ergoline derivative or the ergot alkaloid reduced the number of HN and incidence of spontaneous mammary tumors, although an appreciable number of these mice still developed mammary tumors. Multiparous mice already had a number of HN in their mammas at the onset of drug treatment. It is probable that, in these mice, some fraction of the hyperplasias was unaffected by prolactin suppression; i.e., the lesions were prolactin-independent HN, and at least a proportion of these lesions eventually developed into a palpable mammary tumor. These results demonstrate that certain populations of spontaneously developing hyperplastic (preneoplastic) mammary cells can grow in a hormonal environment deficient in prolactin. This is further supported by the observation that suppression of prolactin secretion was ineffective in inducing mammary tumor regression in 7 of 8 of the multiparous mice already bearing mammary tumors at the onset of the treatment. Since inhibition of mammary tumorigenesis by prolactin suppression is much more apparent in young nulliparous mice, less striking in mature multiparous mice, and essentially ineffective in older mice already bearing mammary tumors, gradual progression toward prolactin independence has been clearly demonstrated in these studies. In effect, failure to demonstrate prolactin dependence of an advanced mammary neoplasm does not rule out the possibility of a critical role for this hormone in the earlier developmental stages of this disease.

It has been long known that chronic administration of a number of estrogens to rodents causes significant mammary dysplasia characterized most commonly by an increase in hyperplastic and neoplastic mammary development (6). The results of this study support the findings of these earlier investigations, as 45% of the ovariectomized-hysterectomized mice developed mammary tumors after 1 year of estrogen treatment. This is a tumor incidence approximately 8 times greater than that observed in intact nontreated nulliparous mice of similar age and strain (11). Concurrent suppression of prolactin secretion, however, reduced the number of HN and incidence of mammary tumors in these animals. These results suggest that reduced prolactin secretion can impede murine mammary tumorigenesis even in animals chronically stimulated with estrogen. Although the prolactin-suppressed, estrogen-treated mice did not have nearly as many HN as those treated with the steroid alone, the difference in incidence of mammary tumors in these groups was not striking. It is feasible that the secretion of prolactin in the estrogen-ergot-treated mice, although lower than in animals treated with the steroid alone (2), was sufficient to contribute to this neoplastic process. It is well known that the administration of estrogens to rodents markedly increases pituitary prolactin secretion (13). A number of laboratories have reported the effectiveness of CB-154 or MEA in inhibiting the secretion of pituitary prolactin in normal as well as estrogen-treated rodents (2, 3). It appears that these drugs are relatively specific for prolactin, at least in rodents. Mice treated with these drugs have normal estrous cycles (except for an occasional shortening of the diestrus period) and normal endocrine weights (10-12). In addition, growth hormone secretion does not appear to be affected by the ergot alkaloids (14). In our studies, the nulliparous mice treated with CB-154 had normal estrous cycles, yet were virtually free of mammary tumors, a particularly interesting observation, as it suggests that mammary tumorigenesis may be blocked, even in animals with normal ovarian activity, as long as prolactin secretion is kept minimal. The only apparent disparity between controls and the mice with chronically suppressed prolactin levels was in the mammas, i.e., a striking inhibition of normal, hyperplastic, and neoplastic mammary epithelial development. Of the hormones believed to be most important in mammary tumorigenesis (e.g., estrogen, progesterone, growth hormone, and prolactin), prolactin would appear to be a top candidate for chronic suppression in the prophylaxis of this disease. It is relatively specific for the mammary gland, aside from its luteotrophic and luteolytic effects in rodents, although it has been implicated in a number of other physiological processes (5). Whether or not this hormone is critically involved in normal, hyperplastic, or neoplastic breast development in humans is at present unknown and represents an area of investigation of potentially great significance.

Acknowledgments

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References


Figs. 1 and 2. Representative whole mounts of inguinal mammary glands from CB-154-treated 14-month-old nulliparous mice.
Fig. 3. Whole mount of an inguinal mammary gland from a CB-154-treated 14-month-old nulliparous mouse showing a hyperplastic growth.
Fig. 4. Representative whole mount of an inguinal mammary gland from a control 14-month-old nulliparous mouse.
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