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Inhibition of Mammary Dysplasia in Estrogen-Treated C3H/HeJ Female Mice by Treatment with 2-Bromo-α-ergocryptine (37836)

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The functional roles of estrogen and prolactin in the complex process of mammary tumorigenesis have been the object of a number of studies. Chronic administration of estrogen (1–3) or prolactin (4–7) to intact female mice or rats results in a marked increase in spontaneous mammary tumor incidence; reduced secretion of these hormones results in a sharp reduction in tumor incidence (1, 3, 8, 9). Mammary tumor incidence is markedly reduced in female mice and rats ovariectomized and adrenalectomized early in life (1, 10) even when chronically treated with prolactin (11), suggesting an essential role for steroids in the developmental phases of murine mammary tumorigenesis. Attempts to induce mammary tumors by estrogen in hypophysectomized mice or rats, on the other hand, have not been fruitful because of a low tolerance in these animals to prolonged estrogen treatment.

Only recently has it become possible to suppress the secretion of prolactin by drugs (12, 13), even though a number of means to increase the secretion of this hormone have been available for years (14, 15). CB-154 (2-bromo-α-ergocryptine),[2] an extract of ergot, has been shown to be a potent suppressor of prolactin secretion in mice and rats, as well as in man (12, 16). These ergots appear to be specific for prolactin, not directly interfering with other hormonal processes (9, 12, 13), and are capable of reducing blood levels of prolactin to those observed in hypophysectomized animals (17). The ergots are also capable of maintaining low blood levels of prolactin, even in animals treated with estrogen (18, 19), a steroid known to enhance prolactin secretion (20). The purpose of this investigation, therefore, is to determine the effects of CB-154 treatment on hyperplastic and neoplastic mammary development in estrogen-treated C3H/HeJ female mice.

Materials and Methods. Nulliparous female C3H/HeJ mice (Jackson Laboratories, Bar Harbor, ME) were used in this investigation. The mice were housed in groups of 10 in a temperature-controlled (75 ± 1°F) and light-controlled (14 hr light/day) environment. Lab-Blox (Allied Mills, Chicago) and water were available *ad lib.*

At 5 months of age each animal was ovariectomized and hysterectomized and placed randomly into two experimental groups. Group I (55 mice) received 17β-estradiol via drinking water. Group II (59 mice) received 17β-estradiol via drinking water plus daily sc injections (0.1 ml) of 100 μg CB-154. Controls (Group I) were injected daily with the diluent alone. All animals of each group were examined monthly for palpable mammary tumors, weighed weekly, and checked for vaginal cornification periodically.

CB-154 was dissolved in a minimum amount of ethanol and diluted to volume with 0.9% saline. Ethanol had a final concentration <1%. 17β-Estradiol was dis-

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solved in a minimum amount of ethanol and diluted with water to a final concentration of 0.5 μg 17β-estradiol/ml drinking water. It was determined, based on water consumption, that each mouse ingested approximately 2.1 μg of the steroid per day.

All mice were treated for 1 year at which time all surviving animals were sacrificed. Adrenal glands were excised and weighed. Inguinal mammary glands were excised and prepared for wholemount evaluation (21). Mammary tumors were excised, fixed in Bouin's fluid, and histologically evaluated.

Mammary glands were rated for development on a 1–6 development scale (22). Number of hyperplastic alveolar nodules (HAN) were counted in the wholemount preparations. Only HAN outgrowths equal to or greater than 0.5 mm diam were recorded for computation. Wholemount preparations were examined under tenfold magnification and coded prior to grading.

Mean differences between body and organ weights, number of HAN, and latency period of tumor development were evaluated statistically by the Student's *t* test. Tumor incidence (total number of tumors per group) was evaluated statistically by chi-square analysis. Mammary gland development was statistically evaluated by the nonparametric Wilcoxon rank procedure test.

Results. Chronic CB-154 administration to estrogen-treated ovariectomized-hysterectomized C3H/HeJ mice significantly (a) inhibited HAN development ($P < 0.001$), (b) reduced the number of mammary tumors ($P < 0.05$), and (c) suppressed mammary gland development ($P < 0.001$) (Table I). 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 absence of alveolar development in the mice treated with estrogen and CB-154. There were 5–6 times the number of HAN in the inguinal mammary glands of mice treated with estrogen alone than in the estrogen- and CB-154-treated mice. Although mammary tumor incidence was significantly decreased in the ergot-treated mice, mean latency period of tumor development was not significantly affected.

There was no significant difference between the two groups of mice in body weight gains, mortality rate, or adrenal gland weights. Histological examination of tumors from both groups showed no apparent differences in morphology. All mice in both groups showed continuous vaginal cornification.

Discussion. Since the pioneering work in 1932 by Lacassagne (23) and subsequently by other laboratories (1–3), it has been known that chronic administration of estrogens to mice or rats causes significant mammary dysplasia characterized most commonly by an increase in hyperplastic and neoplastic mammary development. The results of the present study are in accord with these earlier investigations, as 45% of the ovariectomized

TABLE I. The Effects of CB-154 Treatment in Estrogen-Stimulated C3H/HeJ Mice on Normal, Hyperplastic, and Neoplastic Mammary Gland Development.

	Treatment[a]		
	17β-Estradiol	17β-Estradiol plus CB-154	*P*
Number of mice at beginning of study	55	59	
Number of mice at end of study	20	27	
Mean body weight	28.6 ± 0.9	27.8 ± 0.5	
Number of tumors	25	19	<.05
Number of mice with tumors	24	19	
Mean latency period of tumor appearance (months)	7.7 ± 0.7	8.8 ± 0.8	
Mean number of HAN in inguinal mammary glands	19.3 ± 2.0	3.5 ± 0.6	<.001
Mean (and range) inguinal mammary gland ratings	4.3 (1.5–6.0)	1.6 (1.0–3.0)	<.001

[a] Mice of both groups received daily treatment of 17β-estradiol. In addition, each mouse of one group received 0.1 mg CB-154 daily. These treatments were for a period of 1 year. Means are accompanied by standard error or range.

and hysterectomized C3H/HeJ mice developed mammary tumors after 1 year of estrogen treatment. This is a tumor incidence approximately eight times greater than that observed in intact nontreated nulliparous mice of similar age and strain in our animal colony (9).

Concurrent administration of CB-154 significantly reduced mammary tumor incidence (32%). These results suggest that reduced prolactin secretion can impede murine mammary tumorigenesis, even in animals chronically stimulated with estrogen. The importance of prolactin in the development of mammary tumors in mice and rats has been reported in a number of studies. Treatments which increase prolactin secretion, e.g., pituitary transplants, hypothalamic lesions, or CNS-depressing drugs, markedly increase the incidence of mammary tumors (5–7, 24, 25). More recently, chronic suppression of prolactin secretion in normal nulliparous C3H/HeJ mice has been reported to virtually prevent the appearance of mammary tumors (9). It is also clear from this study that a significant number of mammary tumors (32%) can develop in estrogen-stimulated mice despite the ergot treatment. These results question the validity of the concept, originally proposed by Furth (20), that estrogens are *primarily* mammary oncogenic because of their stimulatory effect on pituitary prolactin secretion. It is probable that estrogens act directly on the mammary tissue, perhaps in synergy with prolactin, in addition to acting via the pituitary as suggested by Furth.

The ergot–estrogen treatment also caused a profound inhibitory effect on the development of mammary hyperplastic alveolar nodules (HAN), an effect much more striking than that observed in mammary tumor inhibition. These hyperplasias have been established by the Berkeley group as antecedents of mammary carcinoma in mice (27) and have been reported to be particularly sensitive to prolactin (9, 28, 29). Thus, an increase or decrease in pituitary prolactin secretion markedly stimulates or suppresses, respectively, HAN development (9, 28). The results of this study provide evidence suggesting that the development of these hyperplasias can be substantially inhibited even during estrogen stimulation by keeping prolactin secretion at a reduced level.

CB-154 is known to be an effective suppressor of prolactin secretion in mice (9, 12, 28), rats (30), cows (31), and man (16). Our laboratory has compared several ergots and ergoline derivatives and found CB-154 to be one of the most effective prolactin suppressors in mice, judged by inhibition of mammogenesis (9, 22). Young nulliparous C3H/HeJ mice chronically treated with CB-154 have normal estrous cycles, yet have mammary glands virtually free of dysplasia (9). Yanai and Nagasawa (12) have reported that multiparous C3H/He mice treated with CB-154 have markedly suppressed mammogenesis and significantly reduced levels of prolactin in their pituitary glands. The ergot appears to act both at the hypothalamic (13) and pituitary (18) levels in reducing prolactin secretion. It is well-known that chronic estrogen treatment to mice or rats significantly increases prolactin secretion (20). Yet, it has been shown that concurrent administration of any of a number of ergots or ergoline derivatives suppresses the estrogen-induced rise in prolactin secretion (18, 19). In accord, mice treated with CB-154 and estrogen in contrast to those treated with the steroid alone, as reported in this study, have considerably fewer normal mammary alveoli, structures known to be markedly sensitive to acute or chronic changes in blood prolactin levels (15).

There have been several reports describing estrogen therapy and consequent mammary dysplasia in women (32). These lesions, however, have been most commonly characterized as ductal hyperplasia or ductal ectasia. Recently, Wellings has provided evidence that a number of lesions, which have been studied in the breasts of patients having a mastectomy, microscopically resembled the hyperplasias (HAN) commonly seen in the mammary glands of mice (33). The hormonal requirements for development and growth of normal and hyperplastic mammary tissue in humans is virtually unknown. Once these requirements are known, and if a prolactin sensitivity can be demonstrated,

prophylaxis of mammary dysplasia may become feasible in women having steroid therapy by drug-induced suppression of prolactin secretion.

Summary. Five-month-old ovariectomized and hysterectomized C3H/HeJ mice were chronically treated for 1 year with 17β-estradiol. Half of the mice concurrently received daily injections of 2-bromo-α-ergocryptine (CB-154), an efficacious inhibitor of prolactin secretion. CB-154 treatment significantly (a) inhibited hyperplastic alveolar nodule (HAN) development, (b) reduced mammary tumor incidence, and (c) suppressed mammary gland development. These results suggest that estrogen-induced mammary dysplasia can be significantly reduced in C3H/HeJ female mice by drug-induced inhibition of pituitary prolactin secretion.

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1. Mühlbock, O., *in* "Biological Activities of Steroids in Relation to Cancer," p. 333. Academic Press, New York (1960).

2. Noble, R. L., and Cutts, J. H., Cancer Res. **19**, 1125 (1959).

3. Dao, T. L., Progr. Exp. Tumor Res. **5**, 157 (1964).

4. Loeb, L., and Kirtz, M. M., Amer. J. Cancer **36**, 56 (1939).

5. Mühlbock, O., and Boot, L. M., Cancer Res. **19**, 402 (1952).

6. Welsch, C. W., Nagasawa, H., and Meites, J., Cancer Res. **30**, 2310 (1970).

7. Welsch, C. W., Jenkins, T. W., and Meites, J., Cancer Res. **30**, 1024 (1970).

8. Moon, H. D., Simpson, M. E., Li, C. H., and Evans, H. M., Cancer Res. **11**, 535 (1951).

9. Welsch, C. W., and Gribler, C., Cancer Res. **33**, 2939 (1973).

10. Durbin, P. W., Williams, M. H., Jeung, N., and Arnold, J. S., Cancer Res. **26**, 400 (1966).

11. Bittner, J. J., and Cole, H. L., J. Nat. Cancer Inst. **27**, 1273 (1961).

12. Yanai, R., and Nagasawa, H., J. Nat. Cancer Inst. **45**, 1105 (1970).

13. Wuttke, W., Cassell, E., and Meites, J., Endocrinology **88**, 737 (1971).

14. Lu, K. H., Amenomori, Y., Chen, C. L., and Meites, J., Endocrinology **87**, 667 (1970).

15. Meites, J., and Nicoll, C. S., Annu. Rev. Physiol. **28**, 57 (1966).

16. del Pozo, E., del Brun, R., Varga, L., and Friesen, H., J. Clin. Endocrinol. Metab. **35**, 768 (1972).

17. Welsch, C. W., Iturri, G., and Meites, J., Int. J. Cancer **12**, 209 (1973).

18. Lu, K. H., Koch, Y., and Meites, J., Endocrinology **89**, 229 (1971).

19. Brooks, C. L., and Welsch, C. W. (unpublished data).

20. Ratner, A., Talwalker, P. K., and Meites, J., Proc. Soc. Exp. Biol. Med. **112**, 12 (1963).

21. Nandi, S., Univ. Calif. Berkeley Publ. Zool. **65**, 4 (1959).

22. Welsch, C. W., and Clemens, J. A., Proc. Soc. Exp. Biol. Med. **142**, 1067 (1973).

23. Lacassagne, A., Compt. Rend. Acad. Sci. **195**, 630 (1932).

24. Bruni, J. E., and Montemurro, D. G., Cancer Res. **31**, 854 (1971).

25. Lacassagne, A., and Duplan, J. F., Compt. Rend. Acad. Sci. **249**, 810 (1959).

26. Furth, J., *in* "Thule Int. Symp. Cancer and Aging," Nordiska Bokhandelns Forlag, Stockholm (1968).

27. Nandi, S., Bern, H. A., and DeOme, K. B., J. Nat. Cancer Inst. **24**, 883 (1960).

28. Yanai, R., and Nagasawa, H., J. Nat. Cancer Inst. **46**, 1251 (1971).

29. Dux, A., and Mühlbock, O., Eur. J. Cancer **5**, 191 (1969).

30. Flückiger, E., and Wagner, H. R., Experientia **24**, 1130 (1968).

31. Schams, D., Reinhardt, V., and Karg, H., Experientia **28**, 697 (1972).

32. Haagensen, C. D., "Diseases of the Breast," 2nd ed., W. B. Saunders Company, Philadelphia (1971).

33. Wellings, S. R., and Jensen, H. M., Proc. Amer. Ass. Cancer Res. **14**, 76 (1973).

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