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Influence of Chronic Prolactin Suppression during Puberty on the Development of Dimethylbenz(a)anthracene-Induced Mammary Tumors (41163)

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Abstract. In order to assess the effect of early prolactin suppression on the subsequent development of dimethylbenz(*a*)anthracene (DMBA)-induced mammary cancers, the dopamine agonist, CB-154, was chronically administered to female Sprague–Dawley rats from Day 35 to Day 50 of age. DMBA was then administered and tumor development assessed over a 25-week period. It was found that animals treated with CB-154 exhibited decreased tumor incidence, a longer latent period, and fewer tumors/animal, when compared to vehicle controls. However, statistical analysis showed that inhibition of tumor development was significant only with regard to the total number of tumors/tumor-bearing animal; differences in tumor incidence and latency failed to attain statistical significance. These results are consistent with the hypothesis that the sensitivity of the mammary gland to polycyclic aromatic hydrocarbon (PAH) carcinogenesis is determined by the level of differentiation of the gland at the time of carcinogen administration. Accordingly, perturbations in prolactin secretion patterns, early in life, may accelerate or retard the differentiation of the mammary gland thereby rendering it less susceptible to the carcinogenic effects of PAH.

A variety of experimental studies have shown that increased prolactin secretion can have diametrically opposed effects on the development of the dimethylbenz(*a*)anthracene-induced rat mammary adenocarcinoma depending on when prolactin secretion is enhanced (1). For example, selective elevation of serum prolactin titers by either pharmacological (2) or surgical (3) means *prior to* carcinogen administration results in an inhibition of tumor development, while elevation of prolactin *after* carcinogen administration results, paradoxically, in a pronounced stimulation of tumor growth (1). As these facts indicate, prolactin functions in an entirely different manner depending on whether its target is a normal or a transformed mammary parenchymal cell.

The inhibitory effect of early hyperprolactinemia on DMBA-carcinogenesis has been ascribed to the ability of prolactin to accelerate the rate of maturation of the mammary gland during puberty (4). Once in a differentiated state, the gland apparently becomes less susceptible to the carcinogenic effects of polycyclic aromatic hydrocarbons (PAH). The nature of this decreased susceptibility is uncertain but may involve a reduced capacity of mammary parenchymal cells to metabolize DMBA to its putative reactive intermediates (5–8), or a diminution in the number of mitotically active target (end-bud) cells in the gland (9). The stimulatory influence of hyperprolactinemia, on the other hand, has been attributed to the ability of prolactin to function as a classical promoter substance in the DMBA-tumor system (10, 11). In this capacity prolactin acts as a modulator of the development of incipient or latent mammary neoplasms.

Since accelerated maturation of the mammary gland, induced by hyperprolactinemia, apparently renders the gland less susceptible to DMBA, one might infer that the converse may also be true; namely, that delaying the maturation of the gland by suppression of prolactin secretion during puberty, may also render the gland less susceptible to DMBA action. In this case, however, the less susceptible state would be a reflection of a retarded rather than an accelerated rate of maturation.

The drug of choice for selectivity suppressing prolactin secretion is 2-bromo-α-ergocryptine (CB-154). A dopaminergic agonist, CB-154, inhibits prolactin secretion in both rodents and man by an action at the level of the hypothalamus and the pituitary gland (12). Moreover, suppression of prolactin secretion by CB-154 is obtained at dose levels which are nontoxic and which

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do not interfere with other endocrine processes (13). In light of the above considerations, and in order to further explore the role of prolactin in mammary gland carcinogenesis, this study examines the influence of chronic prolactin suppression during puberty on the subsequent development of DMBA-induced mammary tumors.

Methods. *1. Experimental.* Forty-eight weanling female Sprague–Dawley rats (Sprague–Dawley, Madison, Wisc.) were randomized on Day 35 of age into two groups of 24 animals each. The experimental groups received sc injections (0.1 ml) of 2-bromo-α-ergocryptine-methanesulfonate (CB-154, Sandoz Ltd., Basel, Switzerland) dissolved in corn oil. Injections were five to six times per week at a dose level of 3 mg/kg body wt (14). The control group received similar injections with the corn oil vehicle alone. On Day 50 of age, after each animal had received a total of 11 injections of CB-154, dimethylbenz(*a*)anthracene (DMBA) (Eastman Kodak Co., Rochester, N.Y.; 5 mg/rat, dissolved in corn oil), was administered to all animals by intragastric instillation (15).

Animals were housed three to a cage in polycarbonate cages equipped with stainless-steel tops. The animal room was temperature (74 ± 2°), light (12-hr cycle) and humidity (50%) controlled. Purina Lab Chow (Ralston Purina, St. Louis, Mo.) and tap water were provided *ad libitum*. At weekly intervals each rat was weighed and palpable mammary tumors were recorded. The experiment was terminated 26 weeks after DMBA administration. All gross lesions were excised and processed for histological examination.

2. Statistical procedures. Differences in median latent period for treatment and control groups were analyzed using the Mann–Whitney rank sum test. Differences between mean numbers of tumors/tumor-bearing animal were analyzed by a two-sample, two-tailed t test. The difference in cumulative tumor incidence between treatment and control groups was assessed by use of a BMDP program (16) which calculates Kaplan–Meier estimates. The cumulative incidence of one or more, two or more, three or more, etc., tumors/animal in each test group was determined by the method of Gail *et al.* (17). Weight gain data were analyzed using an SAS program (18) written to carry out a multivariate analysis of variance on repeated measures.

Results. The effect of CB-154 treatment on tumor incidence and total number of tumors can be seen in Fig. 1. Statistical analysis demonstrated that differences in tumor incidence at termination of the experiment in treated and control groups were not significant. However, treatment with the prolactin-suppressing drug CB-154, prior to DMBA administration, did significantly reduce the number of mammary tumors/tumor-bearing animals compared to untreated controls. The mean tumor of tumors/tumor-bearing animal in treated and control animals, at termination of the experiment, was $\bar{x} = 1.7$ ($N = 11$) and $\bar{x} = 2.8$ ($N = 13$), respectively. The difference between means was statistically significant ($t = 2.24$, $P < 0.036$).

Analysis of tumor-free survival times using the Kaplan–Meier statistic indicated that although the tumor-free survival probability was greater in the experimental group ($P = 0.547$) than the control group ($P = 0.4193$), the difference was not statistically significant (Fig. 2). Using the method

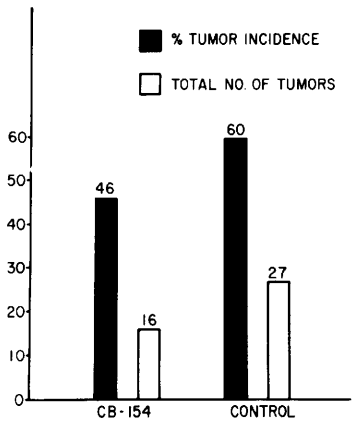

FIG. 1. Effect of chronic prolactin suppression (CB-154) beginning on Day 35 of age and ending on Day 50 of age in Sprague–Dawley rats on DMBA-induced tumor incidence and total number of mammary tumors at termination of study (26 weeks). For results of statistical evaluation, see Results. $N = 24$ and 22 for experimental and control groups, respectively.

of Gail *et al.* (17) which takes the number of tumors/animal as well as cumulative tumor incidence into account, it was found that the differences in combined cumulative tumor yield between treatment and control groups fell just short of significance (Z = 2.49 vs a critical value of 2.51 for a 5% significance level).

The differences in time to appearance of first tumor or median latent period between treated and control groups were not significant. Time to first tumor in each group was 98 days, while the median latent period was 131 and 124 days, respectively, for treated and control groups.

Multivariate analysis of variance on repeated measures of weight during the course of the experiment indicated that treatment with CB-154 had no measurable effect on food consumption or animal weight gain (data not shown). Histological examination revealed that at least 95% of tumors were adenocarcinomas. Two animals in the control group died during the course of the experiment.

Discussion. It is shown here that chronic suppression of prolactin secretion by CB-154, prior to initiation by DMBA, causes a significant decrease in tumor multiplicity. However, with regard to tumor incidence and latent period, differences between treated and control groups did not attain significance.

Two related studies on the effect of early prolactin suppression on mammary carcinogenesis have been reported (19, 20). In the first, Nagasawa *et al.* (19) found that administration of an acute dose of CB-154, 50 hr before DMBA administration, resulted in a significant decrease in mammary gland DNA synthesis and tumor multiplicity compared to untreated controls. No significant changes were reported with regard to tumor incidence or latent period. In the second study, by Welsch *et al.* (20), it was reported that CB-154-induced suppression of prolactin secretion for 60 days, beginning on Day 21 of age, lowered the number of mammary carcinomas/rat in methylcholanthrene-treated Lewis rats, although this reduction just fell short of the 5% level of statistical probability. Despite some methodological differences, in general, all three studies suggest that early treatment with CB-154 tends to inhibit the subsequent development of PAH-induced mammary tumors.

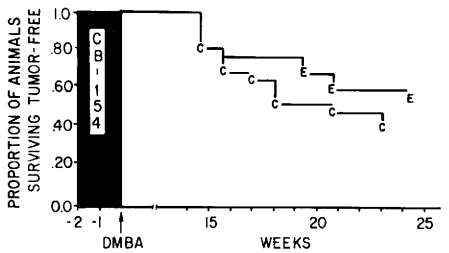

FIG. 2. Effect of chronic prolactin suppression (CB-154 treatment) beginning on Day 35 of age and terminating on Day 50 of age on cumulative incidence of DMBA-induced mammary tumors in Sprague–Dawley rats. The two curves were found to be not significantly different by both the Breslow ($P < 0.39$) and the Mantel–Cox statistic ($P < 0.37$). C = vehicle control; E = CB-154.

Advis *et al.* (21) have demonstrated that at Day 35 of age, onset of puberty, as determined by vaginal opening and a proestrus-like prolactin surge, can be observed in approximately 40% of female Sprague–Dawley rats. Between Days 35 and 42 all rats begin cycling and exhibit cyclic prolactin surges. Simultaneously, the mammary gland begins to differentiate. Hence, in this study CB-154 was administered at the inception of and during puberty and mammary gland maturation.

The importance of this period to PAH-induced mammary carcinogenesis was first noted by Huggins *et al.* (15), who demonstrated that susceptibility of the mammary gland to a single dose of PAH was highest at ages 50–55 and decreased markedly in younger and older animals. More recently Russo *et al.* (9) analyzed this phenomenon by histological techniques and demonstrated that during maturation of the rat mammary gland mitotically active terminal end-buds gradually appeared and reached their maximum number at ages 50–55. Moreover, it was shown that DMBA-induced tumors originated from such end-buds. Hence, it was concluded that the sensitivity of the mammary gland to DMBA carcinogenesis was a function of the number of sites in the mammary gland

available for interaction with DMBA or its metabolites.

These considerations provide a possible clue to the differential effect of CB-154 treatment on the three key parameters of tumor development, namely, incidence, multiplicity, and latency reported in the present study. Since CB-154, at the dose given, only partially suppresses prolactin secretion (21), it may be inferred that prolactin-dependent maturation of some, but not all, ductal elements to the critical end-bud stage, will be blocked at Day 50 of age. In this manner while the total *number* of tumors per animal may be significantly inhibited, the *frequency* of animals with at least one tumor may be reduced only slightly. Direct proof of this, however, will have to await a longitudinal analysis of mammary gland maturation in CB-154-treated animals.

Although the relationship between prolactin and human breast cancer is uncertain (22), the results of the present study may provide important insights into the role prolactin plays during the early stages of breast cancer in humans. For example, both rat and human breast cancers apparently arise from morphologically similar elements in the ductal system of the mammary gland (9, 23). Moreover, the risk of breast cancer is decreased in women with late onset of menarche or a full-term pregnancy before the age of 18 (24). In light of the fact that both puberty and pregnancy are accompanied by marked alterations in prolactin secretion patterns (25), the results of the present study suggest that altered prolactin release patterns during puberty and mammary gland development may influence the subsequent development of human breast cancer.

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1. Welsch, C. W., and Nagasawa, H., Cancer Res. **37**, 951 (1977).
2. Welsch, C. W., and Meites, J., Experientia **26**, 1133 (1970).
3. Welsch, C. W., Clemens, J. A., and Meites, J., Cancer Res. **29**, 1541 (1969).
4. Nagasawa, H., Med. Hypoth. **5**, 499 (1979).
5. Sinha, D. K., and Dao, T. L., J. Nat. Cancer Inst. **64**, 519 (1980).
6. Shepherd, R. E., and Bryan, A. H., Proc. Amer. Assoc. Cancer Res. **18**, Abstr. No. 837 (1977).
7. Greiner, J. W., Malan-Shibley, L. B., and Janss, D. H., Life Sci. **26**, 313 (1980).
8. Yang, S. K., Easton, G. D., and Chou, M. W., *in* "Microsomes, Drug Oxidations, and Chemical Carcinogenesis" (M. J. Coon, A. H. Conney, R. W. Estabrook *et al.*, eds.), Vol. II, p. 1185, Academic Press, New York (1980).
9. Russo, J., and Russo, I. H., J. Nat. Cancer Inst. **61**, 1451 (1978).
10. Kim, U., and Furth, J., Vitamin Horm. **34**, 107 (1976).
11. Pearson, O. H., Arafah, B., and Manni, A., *in* "Central and Peripheral Regulation of Prolactin Function" (R. M. Macleod and U. Scapagnini, eds.), pp. 237–242, Raven Press, New York (1980).
12. Fluckiger, E., Acta Endocrinol. (Suppl. 216), **88**, 111 (1978).
13. Heuson, J.-C., Waelbroeck, C., Legros, N., Gallez, G., Robyn, C., and L'Hermite, M., Gynecol. Invest. **2**, 130 (1971/1972).
14. Chan, P. C., and Cohen, L. A., J. Nat. Cancer Inst. **52**, 25 (1974).
15. Huggins, C., Grand, L. C., and Brillantes, F. P., Nature (London) **189**, 204 (1961).
16. BMDP-79, BioMedical Computer Program P-Series, (W. G. Dixon and M. B. Brown, eds.), Univ. Calif. Press, Berkeley, Calif. (1979).
17. Gail, M. H., Santner, T. J., and Brown, C. C., Biometrics **36**, 255 (1980).
18. SAS Users Guide, 1979 edition (J. T. Helwig, and K. A. Council, eds.), Published by SAS Inst. Inc., Raleigh, N.C., pp. 245–265 (1979).
19. Nagasawa, H., Yanai, R., and Taniguchi, H., Cancer Res. **36**, 2223 (1976).
20. Welsch, C. W., Brown, C. K., Goodrich-Smith, M., Van, J., Denenberg, B., Anderson, T. M., and Brooks, C. L., J. Nat. Cancer Inst. **63**, 1211 (1979).
21. Advis, J. P., Simpkins, J. W., Chen, H. T., and Meites, J., Endocrinology **103**, 11 (1978).
22. Nagasawa, H., Europ. J. Cancer **15**, 267 (1979).
23. Wellings, S., Adv. Cancer Res. **31**, 287 (1980).
24. Kelsey, J. L., Epidemiologic Rev. **1**, 74 (1979).
25. Vorherr, H., *in* "Breast Cancer: Epidemiology, Endocrinology, Biochemistry and Pathobiology" Urban & Schwartzenberg Inc., Baltimore, Md, (1980).

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