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Stimulation of Mammary Tumorigenesis and Suppression of Uterine Adenomyosis by Temporary Inhibition of Pituitary Prolactin Secretion during Youth in Mice[1] (41492)

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Abstract. The effects of daily subcutaneous injections of 0.2 mg CB-154 (bromocriptine-mesilate), a potent suppressor of pituitary prolactin secretion, between 4 and 11 weeks of age on the occurrence of spontaneous mammary tumors and adenomyosis, a hyperplasia of endometrial tissue, were studied in the SHN strain of virgin mice. While there was little difference in mammary tumor incidence between experimental and control mice until 9 months of age, mammary tumor incidence in the experimental mice given CB-154 was significantly enhanced and surpassed that in the control after 10 months; 25 (53.2%), 31 (66.0%), and 36 (75.5%) of 47 experimental mice and 20 (27.5%), 23 (33.3%), and 34 (49.3%) of 69 control mice developed mammary tumors at 10, 11, and 12 months of age, respectively. In contrast, no adenomyosis appeared in 39 experimental mice at necropsy at 12 months of age, while 15 (46.9%) of 32 control mice developed it. Furthermore, five mice (15.6%) of the control had numerous subserosal nodules, an advanced state of adenomyosis. No significant correlation was observed in the control mice between the occurrence of mammary tumors and that of adenomyosis.

Mammary gland DNA synthesis, which is primarily controlled, in part, by prolactin (1, 2), is a limiting factor for mammary tumorigenesis (3, 4). DMBA (7,12-dimethylbenz[*a*]anthracene)-induced mammary tumorigenesis was much more marked in rats given DMBA at proestrus, when both circulating prolactin and mammary gland DNA synthesis were high, than in rats given DMBA at diestrus, when prolactin and DNA synthesis were low (5). Suppression by CB-154 of the high prolactin at proestrus resulted in the decline of mammary gland DNA synthesis and the inhibition of DMBA-induced mammary tumorigenesis. By contrast, the single prolactin injection elevated mammary gland DNA synthesis and stimulated DMBA-induced mammary tumorigenesis (5). Moreover, temporary suppression of pituitary prolactin secretion during youth protected markedly spontaneous mammary tumor development in rats (6, 7). Mammary gland DNA synthesis in this species is high only during youth with a peak around 7 weeks of age decreasing thereafter with increasing age (5, 8). Russo and Russo (9) also found that mammary gland of 50-day-old virgin rats contained much higher number and labeling index of terminal ducts and end buds, from which DMBA-induced mammary tumors arise, than the glands of 180-day-old virgin and multiparous rats. Thus, prolactin suppression after the peak of mammary gland DNA synthesis (11–18 weeks of age) had little effect on prophylaxis of mammary tumors at advanced ages (6, 7).

On the other hand, mammary gland DNA synthesis in SHN mice, a high mammary tumor strain, changes little with age and much higher than that in rats (10). Although chronic prolactin suppression was found to inhibit markedly spontaneous mammary tumor development in C3H mice (11–13), no data are available in this species on the effects of temporary suppression of prolactin secretion during youth on mammary tumorigenesis. The effects of short-term

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and long-term suppression of prolactin secretion on mammary tumors are two different problems.

It has recently been observed that SHN virgin mice develop spontaneously adenomyosis, hyperplasia of endometrial tissue, both glandular and stromal components in the myometrium (14, 15). The development of adenomyosis is strongly prolactin dependent in the presence of ovarian hormones; ectopic pituitary grafts have been reported to enhance the appearance of the condition in intact animals (14, 15), however, the mechanism of its development is little understood.

The primary objective of this experiment was to study the effects of temporary suppression of prolactin secretion during youth on the development of spontaneous mammary tumors and adenomyosis at advanced ages in SHN mice.

Materials and Methods. *Mice.* SHN strain of mice maintained by brother × sister mating were used at the 45th generation. One of the characteristics of this strain is the high and early development of mammary tumors; the final incidence and onset age of tumors in virgin mice are 100% and 8.9 months, respectively (16). They also develop adenomyosis after 7 months of age with the incidence of about 50% at 12 months (14, 15). Throughout the experiment, five or six mice each were kept in Teflon cages (15 × 18 × 13 cm) with wood shavings, maintained in an air-conditioned (24 ± 0.5°C and 65–70% relative humidity) and artificially illuminated (14 hr of light from 5:00 AM to 7:00 PM) animal room and provided with a commercial diet and tap water *ad libitum*.

CB-154 treatment. A daily dose of 0.2 mg CB-154 (bromocriptine-mesilate: Sandoz Ltd., Basel, Switzerland), a potent suppressor of pituitary prolactin release (17–19), suspended in 0.05 ml olive oil with the Teflon glass homogenizer was injected subcutaneously each into 47 experimental mice between 4 and 11 weeks of age. Sixty-nine control mice received vehicle only.

Mammary tumorigenesis. Each mouse was checked for palpable mammary tumors every 7 days between 3 and 12 months of age.

Adenomyosis. All mice were killed by cervical dislocation at 12 months of age. Uteri were fixed in Bouin's solution, embedded in paraffin, sectioned at 7 μm, and stained with hematoxylin–eosin for histological determination of adenomyosis (14, 15).

Statistics. Statistical evaluation of mammary tumorigenesis was performed using the multiple classification method (two-way analysis of variance) (20). By this method, the statistical sequence of the incidence and onset age of mammary tumors could be determined simultaneously. The difference in the incidence of adenomyosis or subserosal nodules was evaluated by χ^2 test.

Results. The results of mammary tumor development are presented in Fig. 1. In both experimental and control groups, the cumulative incidence of mammary tumors increased with age. However, the increasing rate was more marked in the experimental mice given CB-154 than in the control, especially after 10 months. The number (and percentage) of mice with tumors at 10, 11, and 12 months were 25 (53.2%), 31 (66.0%), and 36 (75.5%) of 47 experimental mice and 20 (27.5%), 23 (33.3%), and 34 (49.3%) of 69 control mice, respectively. Therefore, mammary tumorigenesis in the experimental group was significantly higher than that in the control

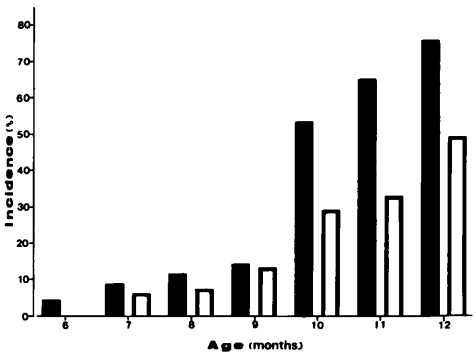

FIG. 1. Cumulative incidence of mammary tumors in experimental mice receiving CB-154 between 4 and 11 weeks of age (■) and the controls given vehicle only (□).

when evaluated by analysis of variance ($P < 0.05$).

In contrast, none of 39 experimental mice developed adenomyosis by 12 months of age, whereas in the controls, 15 or 46.9% of 32 mice developed it and five mice (15.6%) further had several subserosal nodules. The differences between experimental and control groups in the incidences of these pathological endometrial states were statistically highly significant ($P < 0.01$) (Table I).

No significant relationship was seen in the control mice between the occurrence of mammary tumors and that of adenomyosis; 6 out of 10 mice bearing mammary tumors (60%) and 9 out of 22 mice with no tumors (41%) developed adenomyosis.

The numbers of mice which died without tumors during the experiment were two and three in the experimental and control groups, respectively.

The body weight at the beginning of CB-154 injection (4 weeks of age) was 17.6 ± 0.2 (SE) g (pooled data of experimental and control groups) and the weights at the end of injection (11 weeks of age) were 26.9 ± 0.5 and 27.7 ± 0.4 g in the experimental and control groups, respectively, showing no difference between groups.

Discussion. This study shows that mice treated with CB-154 at an early age enhanced mammary tumorigenesis when compared with the control. Two major factors could be responsible for the enhanced mammary tumorigenesis seen in this study—pituitary and ovarian secretion of mammotropic hormones and mammary gland susceptibility to these hormones. Pituitary prolactin and ovarian estrogen and progesterone are prerequisite for the development of mammary tumors in mice, while established mammary tumors in many strains do not require hormonal support and, therefore, are autonomous (21). The enhancement of mammotropic hormone secretion as a result of terminating CB-154 treatment is unlikely, since normal reproductivity was observed in studies involving chronic administration of CB-154 (7, 22) and prolactin secretion at an advanced age was not altered by the treatment (7).

Mammary gland susceptibility to mammotropic hormones may be more important for normal and neoplastic mammary gland development than the secretion of mammotropic hormones (2). It has been reported that mammary glands exposed to abnormal hormonal conditions during early development show an increased susceptibility to mammotropic hormones (2). The enhanced mammary tumorigenesis in this study may be ascribed to the long-term effects of stimulated mammary gland susceptibility.

In any case, the present results suggest that the temporary inhibition of mammary gland DNA synthesis through prolactin suppression (6, 7) is not effective in preventing mammary tumorigenesis in species where mammary gland DNA synthesis continues at a high rate throughout the lifetimes.

The occurrence of adenomyosis at an advanced age (12 months) was completely eliminated by the temporary CB-154 treatment. While no information is available on the mechanism of adenomyosis development in mice except for its high prolactin dependency (14, 15), this study has demonstrated that there is a critical period for its

TABLE I. INCIDENCE OF ADENOMYOSIS AT 12 MONTHS OF AGE IN EXPERIMENTAL AND CONTROL MICE

Group[a]	No. of mice	No. (and %) of mice with	
		Adenomyosis	Subserosal nodules
Experimental	39	0 (0)[b]	0 (0)[d]
Control	32	15 (46.9)[c]	5 (15.6)[e]

[a] Experimental and control mice received daily subcutaneous injections of CB-154 (0.2 mg) and vehicle only for 7 weeks between 4 and 11 weeks of age, respectively.

b/c; d/e: $P < 0.01$.

expression similar to that observed in rats for spontaneous mammary tumors (6, 7). Normal uterine growth is dependent upon estrogen and progesterone from the ovary and the susceptibility of uterine cells to these hormones changes little with age (23). However, exposure of uterus to abnormal hormonal conditions during the early developmental stages often induces a decrease in the susceptibility to estrogen (24). Thus, the present findings may indicate a continuous decline in uterine susceptibility to prolactin and ovarian steroid hormones as a result of CB-154 injection during youth and this could contribute to the complete elimination of adenomyosis at advanced ages.

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