Prophylaxis of Spontaneous Mammary Tumorigenesis by Temporal Inhibition of Prolactin Secretion in Rats at Young Ages

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ABSTRACT
The effects of temporal prolactin suppression during the limited periods of early age on spontaneous mammary tumorigenesis at advanced ages were examined in rats. Daily s.c. injections of 0.5 mg 2-bromo-α-ergocryptine mesylate, a potent suppressor of pituitary prolactin secretion, into virgin rats for 7 weeks beginning at 4 weeks of age resulted in almost complete prevention of mammary tumor appearance by 20 months of age. The incidence of tumors in this group (Group 1) was 3.3% (1 of 30), significantly smaller than that of the control [Group 2, 47.6% (10 of 21)]. Treatment given between 11 and 18 weeks of age inhibited mammary tumor incidence to a lesser extent [Group 3, 20% (6 of 30)], although tumor incidence was still significantly smaller than that in the corresponding control [Group 4, 41.2% (7 of 17)]. Serum prolactin level was decreased significantly by 2-bromo-α-ergocryptine mesylate; the levels on the evening of proestrus in the last week of injection were 18 ± 3 (S.E.), 326 ± 37, 15 ± 2, and 460 ± 55 ng/ml in Groups 1, 2, 3, and 4, respectively. Body weight change and pattern of estrous cycles were not affected by the treatment.

INTRODUCTION
Mammary gland DNA synthesis as estimated by [3H]thymidine incorporation into mammary gland DNA has been shown to be a limiting factor for mammary tumorigenesis (5). In cyclic rats, mammary gland DNA synthesis is generally high during youth and declines with the advance of age; in rats approximately 50 days old, the synthesis is significantly higher at proestrus, estrus, and metestrus than at diestrus, although only at estrus in rats aged 90 days and older (7, 9). It has also been found that the synthesis is largely controlled by prolactin (9, 16). Both animals and humans are constantly exposed to several kinds of carcinogenic agents throughout their lifetimes, and therefore it appears that, the longer the total periods of low mammary gland DNA synthesis, the smaller is the risk of mammary gland tumors. From this viewpoint, it is of much interest to study whether the temporal inhibition of mammary gland DNA synthesis through suppression of pituitary prolactin secretion in rats at young ages would result in the prevention of spontaneous mammary tumorigenesis in those rats at advanced ages. This paper deals with this problem as a possible step to determine an efficient way for prophylaxis of human breast cancer.

RESULTS
Mammary Tumorigenesis. The incidence and age of onset of mammary tumors in each group are presented in Table 1. While there was little difference between groups in the age of onset, mammary tumor appearance was almost completely prevented in rats treated with CB-154 for 7 weeks beginning at 4 weeks of age (Group 1). The incidence in this group was significantly smaller than that in the other 3 groups. Administration of CB-154 from 11 weeks of age (Group 3) also significantly decreased mammary tumor incidence when compared to the controls.

All rats developed only one tumor each during 3 weeks after the first tumor appearance except for 2 rats in Group 3 which...
tumor appearance was checked until 20 months of age. More than 87% of mammary tumors were diagnosed as benign tumors, predominantly fibroadenomas, and no difference between groups was observed in the frequency of occurrence of each type of tumor (Table 2).

**Serum Prolactin Levels.** As shown in Table 3, serum prolactin level was significantly decreased by chronic administration of CB-154 (Groups 1 and 3), showing that prolactin surge on the evening of proestrus was completely inhibited by CB-154.

**Body Weight and Estrous Cycle.** No difference was seen between groups in body weight changes during CB-154 injections or in the weight at the time of tumor appearance. The treatment also showed no effects on the estrous cycle; mostly regular 4-day cycles were observed in all rats.

No rat in any group had died by 20 months of age.

**DISCUSSION**

This study shows that spontaneous mammary tumor appearance is prevented by temporal treatment with CB-154 in rats at young ages. This is primarily due to the suppression of mammary gland DNA synthesis through inhibition of pituitary prolactin secretion by CB-154. Mammary gland DNA synthesis is very high on the evening of proestrus in rats consonant with the occurrence of each type of tumor (Table 2).

Serum prolactin levels in each group

Table 3

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>No. of rats</th>
<th>No. of rats with tumors</th>
<th>Onset age (mos.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CB-154 (4–11 wk)</td>
<td>30</td>
<td>1 (3.3)</td>
<td>18.3</td>
</tr>
<tr>
<td>2</td>
<td>Vehicle (4–11 wk)</td>
<td>21</td>
<td>10 (47.5)</td>
<td>17.0 ± 0.6</td>
</tr>
<tr>
<td>3</td>
<td>CB-154 (11–18 wk)</td>
<td>30</td>
<td>6 (20.0)</td>
<td>17.2 ± 0.6</td>
</tr>
<tr>
<td>4</td>
<td>Vehicle (11–18 wk)</td>
<td>17</td>
<td>7 (41.2)</td>
<td>17.0 ± 0.4</td>
</tr>
</tbody>
</table>

- Numbers in parentheses, percentage of incidence.
- Differs from the other 3 groups at p < 0.001.
- Mean ± S.E.
- Differs from Groups 2 and 4 at p < 0.05.

The pattern of changes in mammary gland DNA synthesis in humans is shown to be similar to that in rats. The synthesis is high during youth and is decreased by aging (4), pregnancy, and lactation (7). Thus, the present results would provide a possibility that human breast tumors may also be prevented by a temporal application of CB-154 during a critical period of early age. It has also been reported that prolactin is mitogenic to human breast tissue in vitro (2) and that CB-154 plays an ameliorative role in benign human breast diseases (3, 10). In the present experiments, a body weight changes and estrous cycles were not affected by CB-154. Furthermore, that there are few deleterious effects of chronic treatment with CB-154 on reproduction has already been confirmed in both mice (15) and humans (1, 11).

**ACKNOWLEDGMENTS**

We thank Professor E. Flückiger, Sandoz Ltd., Basel, Switzerland, for CB-154 and Pituitary Hormone Program, National Institute of Arthritis, Metabolism and Digestive Diseases, NIH, Bethesda, Md., for the kit for radioimmunoassay of rat prolactin. Collaboration by Dr. Reiko Yanai and technical help by Yuko Nakajima and H. Taniguchi in our laboratory are also acknowledged.

Table 2

Histological type of mammary tumors in each group

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Adenocarcinoma</th>
<th>Carcinoma</th>
<th>Intraductal papilloma</th>
<th>Medullary tubular carcinoma</th>
<th>Tubular carcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CB-154 (4–11 wk)</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Vehicle (4–11 wk)</td>
<td>2</td>
<td>5</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>CB-154 (11–18 wk)</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Vehicle (11–18 wk)</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- Numbers in parentheses, percentage of incidence.
- Differs from the other 3 groups at p < 0.001.

Markedly in the latter (20%). This is ascribed to the difference in mammary gland DNA synthesis between the 2 ages. Synthesis is much higher at 4 to 11 weeks than at 11 to 18 weeks (7, 9), and therefore, suppression of synthesis by CB-154 in rats at younger ages would bring on a more marked efficiency in those rats at advanced ages.

The pattern of changes in mammary gland DNA synthesis in humans is shown to be similar to that in rats. The synthesis is high during youth and is decreased by aging (4), pregnancy, and lactation (7). Thus, the present results would provide a possibility that human breast tumors may also be prevented by a temporal application of CB-154 during a critical period of early age. It has also been reported that prolactin is mitogenic to human breast tissue in vitro (2) and that CB-154 plays an ameliorative role in benign human breast diseases (3, 10). In the present experiments, a body weight changes and estrous cycles were not affected by CB-154. Furthermore, that there are few deleterious effects of chronic treatment with CB-154 on reproduction has already been confirmed in both mice (15) and humans (1, 11).

Welsch et al. (12–14) reported the prophylaxis by synthetic alkaloids of preneoplastic and neoplastic mammary development in mice. However, in these studies, the alkaloid treatments were given throughout the experiments for more than 9 months. These long periods of treatment may be essential, since mammary gland DNA synthesis in mice bearing mammary tumor virus is little related to age, which is quite different from synthesis in rats, and the synthesis in mice is generally 2 to 4 times as high as the maximum value in rats at proestrus (6).
REFERENCES


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