Inhibition by early treatment with bromocriptine of spontaneous mammary tumour development in rats with no side-effects

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Abstract. Temporary inhibition by CB-154 (bromocriptine) of pituitary prolactin secretion, which induced a decline in mammary gland DNA synthesis for 4–11 weeks of age, resulted in a marked suppression of spontaneous mammary tumour development in rats after one year of age. The treatment had no effect on reproduction and subsequent lactation immediately after the CB-154 injection or growth and function of the mammary glands and pituitary prolactin secretion in later life.

Mammary gland DNA synthesis is a primary factor for mammary tumour development (Nagasawa et al. 1976; Nagasawa 1977). The synthesis in virgin rats is generally high in early life showing a peak around 50 days and declines with age (Nagasawa & Yanai 1974a; Nagasawa & Vorherr 1977), and is largely dependent upon prolactin (Nagasawa et al. 1976). Following the previous paper (Nagasawa & Morii 1981), we report the inhibition of spontaneous mammary tumourigenesis in aged rats in which pituitary prolactin secretion and consequent mammary gland DNA synthesis were temporarily inhibited by CB-154 during youth. It is plausible that any short- and long-term after-effects of CB-154 on pituitary prolactin secretion and mammary gland growth and function may contribute to the prevention of mammary tumourigenesis at advanced ages. This paper deals also with this problem.

Materials and Methods

Experiment I

Sprague-Dawley female rats were divided into 4 groups at 4 weeks of age. The 1st and the 2nd groups were given daily sc injections of 0.5 mg CB-154 suspended in 0.05 ml olive oil for 7 weeks beginning at 4 and 11 weeks of ages, respectively. The 3rd and the 4th groups received vehicle only for the respective periods and served as controls. Throughout the experiments, rats were kept in cages (30 × 40 × 17 cm) with wood shavings 6 each, placed on an iso-rack (SANKI Scientific Co. Ltd., Tokyo, Japan). From the back wall of the rack, fresh air filtered by double microfilters entered gently and horizontally. All feeding conditions were the same as detailed previously (Nagasawa & Morii 1981). Each rat was checked for palpable mammary tumours every 7 days until 3 weeks after the first tumour appearance or 26 months of age. Vaginal smears were taken every morning for more than a week before sacrifice. All rats were killed by decapitation. Blood was collected from the trunk and serum was immediately frozen and kept at −20°C. Mammary tumours were fixed in Bouin’s solution, sectioned and stained with haematoxylin-eosin. A portion of thoracic mammary glands was prepared for wholen mount evaluation. Mammary lobulo-alveolar development was rated from 1 to 7 in increments of 1 (Nagasawa et al. 1980). The in vitro incorporation of [3H]thymidine into DNA as an index of mammary gland DNA synthesis was deter-
Changes in cumulative incidence of spontaneous mammary tumours in each group. Daily dose of 0.5 mg CB-154 suspended in 0.05 ml olive oil was injected sc for 7 weeks beginning at 4 and 11 weeks of ages to groups I and II, respectively. Group III (controls) received vehicle only. Number of rats examined is indicated in the parentheses. The difference in the incidence is statistically significant between group I and groups II or III at all months ($P < 0.05, 0.01$ or $0.001$) and between groups II and III at 18, 20 and 26 months ($P < 0.05$).

Determined using bilateral inguinal glands by the same procedures as reported previously (Nagasawa & Yanai 1974b). Serum prolactin was assayed by the double-antibody homologous radioimmunoassay using the kit supplied by NIH, USA. Data of prolactin levels at different oestrous stages were pooled under Results section in each group, since the levels differed little between stages, which might largely be due to the irregular cycles at advanced ages. Moreover, other data in the controls were also pooled, since all conditions in both controls were the same except for the time of vehicle injection and no difference was seen in any experimental value.

**Experiment II**

Sprague-Dawley female rats were divided into 2 groups at 4 weeks of age. The 1st and the 2nd groups were given daily sc injection of 0.5 mg CB-154 and vehicle only.
Table 2.
Mammary gland activity, serum prolactin level and organ weight at advanced ages in each group (mean ± SEM).

<table>
<thead>
<tr>
<th>Group and treatment¹</th>
<th>No. of rats</th>
<th>Body weight (g)</th>
<th>Mammary gland</th>
<th>Serum prolactin level (ng/ml)</th>
<th>Organ weight (mg)</th>
<th>No. and age of rats which died without mammary tumours</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>DNA synthesis (²H]thymidine incorporated: DPM/µg DNA)</td>
<td>Rating</td>
<td>Anterior pituitary</td>
<td>Adrenal</td>
<td>Ovary</td>
</tr>
<tr>
<td>I. CB-154 (4–11 weeks)</td>
<td>20</td>
<td>381 ± 10</td>
<td>467 ± 103</td>
<td>4.4 ± 0.2</td>
<td>447 ± 152</td>
<td>23.6 ± 1.6</td>
</tr>
<tr>
<td>II. CB-154 (11–18 weeks)</td>
<td>18</td>
<td>410 ± 21</td>
<td>603 ± 102</td>
<td>4.6 ± 0.2</td>
<td>595 ± 285</td>
<td>28.1 ± 3.7</td>
</tr>
<tr>
<td>III. Control</td>
<td>25</td>
<td>419 ± 16</td>
<td>531 ± 77</td>
<td>5.0 ± 0.3</td>
<td>486 ± 95</td>
<td>23.6 ± 2.7</td>
</tr>
</tbody>
</table>

¹ See Fig. 1 for details of treatment.

Table 3.
Reproductivity immediately after CB-154 injection in each group.

<table>
<thead>
<tr>
<th>Group and treatment¹</th>
<th>No. of rats</th>
<th>No. and % of rats which became pregnant</th>
<th>Body weight at parturition (g)</th>
<th>Litter size</th>
<th>Av. pup's weight (g)</th>
<th>% increase in pup's weight²</th>
<th>Rearing rate (%)³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental (CB-154)</td>
<td>15</td>
<td>13 (86.7)</td>
<td>242 ± 8</td>
<td>9.8 ± 0.5</td>
<td>6.3 ± 0.1</td>
<td>25.6 ± 0.5</td>
<td>42.9 ± 0.9</td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
<td>9 (90.0)</td>
<td>249 ± 6</td>
<td>8.1 ± 1.7</td>
<td>6.2 ± 0.4</td>
<td>23.3 ± 2.0</td>
<td>44.3 ± 2.6</td>
</tr>
</tbody>
</table>

¹ Experimental or control rats received daily sc injection of 0.5 mg CB-154 or vehicle only for 7 weeks beginning at 4 weeks of age and mated with males for 30 days from one day after the last injection. Number of pups was adjusted to 8 on the day of parturition (day 0 of lactation).

² % increase in pup's weight = \( \frac{\text{Av. pup's weight on day 20 or 12} - \text{Av. pup's weight on day 0}}{\text{Av. pup's weight on day 0}} \times 100 \).

It was calculated in rats with 100% rearing rate on each day.

³ Rearing rate (%) = \( \frac{\text{No. of pups on day 20 or 12}}{\text{No. of pups on day 0} [8]} \times 100 \).

⁴ Pup's age or day of lactation.

⁵ Means ± SEM.
during 4—11 weeks of age, respectively. Rats were kept in a wire-mesh cage (24 × 30 × 15 cm) 4 each. Beginning one day after the last injection, all rats of both groups were mated with males for a period of 30 days. Care was taken to rotate the males through the cages every day so that each male was exposed to each female of both experimental and control groups. Pregnant rats were placed individually in the same size of cages with wood shavings. At parturition, the number of pups was adjusted to 8 (4 females and 4 males) and nursed normally. Parameters shown in Table 3 were used as the indices for reproductivity.

Statistics
The difference in mammary tumour incidence at each age and other parameters were evaluated by χ²-test and Duncan's multiple range test, respectively.

Results

Experiment I
Mammary tumourigenesis. The results are illustrated in Fig. 1. Mammary tumour incidence in rats receiving CB-154 for 4—11 weeks (group I) was significantly lower than in rats given CB-154 for 11—18 weeks (group II) and in the control (group III) at all months of age (P < 0.05, 0.01 or 0.001). On the other hand, the difference in the incidence between groups II and III was statistically significant at 18, 20 and 26 months of age (P < 0.05).

While mammary tumours appeared after 13 and 11 months of age in groups II and III, respectively, group I developed the first tumours at 19 months of age and the onset of mammary tumours in group I (22.7 ± 0.6 months) was significantly greater than that in group II or III (19.8 ± 0.8 or 19.6 ± 0.7 months) (P < 0.02), between which no difference was observed.

There was no apparent difference between groups in the incidence of different types of mammary tumours (Table 1).

As shown in Table 2, little difference was seen between groups in DNA synthesis, or rating of mammary glands, serum prolactin levels, body weight and organ weights. Furthermore, these parameters differed little between tumorous and not-tumorous rats in all groups. The ages of rats which died without tumours are more than 21 months in each group.

Experiment II
Table 3 shows the similarity between groups in any parameter for reproductivity, including average pup's weight and its per cent increase, both being often used as the indices for lactational performance.

Discussion
Mammary tumour results in Experiment I demonstrated that spontaneous mammary tumour development up to 26 months of age is prevented by the temporary suppression by CB-154 of pituitary prolactin secretion, inducing a marked decline in mammary gland DNA synthesis during youth (4—11 weeks). Meanwhile, treatment for 11—18 weeks showed only slight effects. No difference between groups was seen in mammary gland activity and serum prolactin levels as well as body and organ weights at advanced ages. Moreover, in Experiment II, reproduction and lactation were quite normal even immediately after the termination of CB-154 injection. All results indicate that chronic treatment with CB-154 had no side-effects and that inhibition by CB-154 of spontaneous mammary tumourigenesis in rats may primarily be ascribed to the temporary suppression of mammary gland DNA synthesis during treatment, but to the short- and long-term after-effects of CB-154 on the mammary glands and pituitary.

Acknowledgments
We wish to thank Dr. R. Yanai, Miss Y. Nakajima and H. Taniguchi, National Cancer Center Research Institute, Tokyo, Japan, for their help. Our thanks are also due to Prof. E. Flückiger, Sandoz Ltd., Basel, Switzerland, for CB-154 and Pituitary Hormone Program, NIAMDD, NIH, Bethesda, Maryland, USA, for the kit for radioimmunoassay of rat prolactin.

References


Received on November 1st, 1981.