Importance of Mammary Gland DNA Synthesis on Carcinogen-induced Mammary Tumorigenesis in Rats

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SUMMARY

DNA synthesis in mammary gland estimated by [3H]thymidine incorporation was significantly higher on the day of proestrus than on the second day of diestrus in 50-day-old female Sprague-Dawley rats. The percentage of progressive mammary tumors, tumor growth rate, and the number and the weight of tumors per tumor-bearing rat were significantly higher in the animals given a single i.v. injection of 5 mg 7,12-dimethylbenz(a)anthracene at proestrus than in the animals given it at diestrus. Inhibition of DNA synthesis at proestrus by 2-bromo-α-ergocryptine also suppressed mammary tumorigenesis by the carcinogen. In 90-day-old rats in which little difference was found in mammary gland DNA synthesis between proestrus and diestrus, there was no difference in mammary tumorigenesis between animals given the carcinogen at proestrus and animals given it at diestrus. On the other hand, the prestimulation of mammary gland DNA synthesis by prolactin increased the growth, the number, and the weight of carcinogen-induced mammary tumors. These results demonstrate the importance of mammary DNA synthesis at the time when a carcinogen acts on the glands in mammary tumorigenesis.

INTRODUCTION

Mammary tumor incidence in rats by polycyclic aromatic hydrocarbons reaches a maximum at 50 to 60 days of age and then declines (1, 2), which is in good correlation with the changes in mammary gland DNA synthesis (3). Moreover, 50-day-old rats given grafts of 3 isologous pituitaries at 30 days of age showed significantly lower mammary gland DNA synthesis than did normal rats of the same age (3), and the incidence of carcinogen-induced mammary tumors was inhibited in these rats (5). These results suggest the dependency of mammary tumorigenesis on mammary DNA synthesis at the time when a carcinogen acts on the glands. Thus, it is plausible that carcinogen-induced mammary tumorigenesis should be controlled by altering the rate of DNA synthesis in the mammary gland, that inhibition should be controlled by reducing high DNA synthesis at about 50 days of age, and that promotion should be controlled by elevating the low synthesis at about 90 days of age.

There may be differences in mammary tumorigenesis between animals given the carcinogen on PE and animals given it on D2, since differences in mammary gland DNA synthesis between PE and D2 have been inferred (H. Nagasawa and R. Yanai, unpublished data).

This paper deals with mammary tumorigenesis in female rats with different mammary gland DNA synthesis at the time of carcinogen administration.

MATERIALS AND METHODS

CB-154 (Sandoz Ltd., Basel, Switzerland), a potent suppressor of pituitary prolactin release, was found to reduce mammary gland DNA synthesis (6). In order to check the time when mammary gland DNA synthesis becomes the lowest after the injection of CB-154 in 50-day-old Sprague-Dawley rats, the rats were given i.p. injections of 1 mg CB-154 suspended in 0.9% NaCl solution once or twice at 24-hr intervals and killed 10, 20, 30, and 50 hr after the 1st injection. The time of the 1st injection was adjusted so that all rats were killed at about 6:00 p.m. on PE. The control rats were killed at about 6:00 p.m. on PE and D2. Two hr before being killed, each rat was given the i.p. injection of 50 μCi [3H]TdR (5 Ci/m mole; The Radiochemical Centre, Amersham, England) per 100 g body weight. [3H]TdR incorporated into mammary gland DNA was determined as described previously (4) as the index of mammary gland DNA synthesis. Since [3H]TdR incorporation was lowest 30 hr after the 1st injection (6 hr after the 2nd injection) of CB-154 (Chart 1A), 50-day-old rats were divided into 3 groups and received a single i.p. injection of 5 mg DMBA (The Upjohn Co., Kalamazoo, Mich.) at about 6:00 p.m. on PE (Group I), on D2 (Group II), or 30 hr after the 1st injection of CB-154 (Group III).

Ovine PRL (NIH-P-S11) was used to increase mammary gland DNA synthesis in 90-day-old rats. In order to determine the changes in DNA synthesis after a single injection of PRL, rats were killed, and [3H]TdR incorporation was assayed 4, 8, 16, 20, 24, and 50 hr after the s.c. injection of 10 mg PRL dissolved in alkaline 0.9% NaCl solution (pH 8). The time of PRL injection was adjusted so that all rats were killed at about 6:00 p.m. on PE. As shown in Chart 1B, [3H]TdR incorporation was highest 24 hr after PRL injection. Thus, 90-day-old rats were divided into 3 groups and received 5 mg DMBA each at about 6:00 p.m. on PE (Group

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2 The abbreviations used are: PE, the day of proestrus; D2, the 2nd day of diestrus; CB-154, 2-bromo-α-ergocryptine; [3H]TdR, [3H]thymidine; DMBA, 7,12-dimethylbenz(a)anthracene; PRL, prolactin.
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IV), on D2 (Group V), or 24 hr after PRL injection (Group VI). Each rat was examined for palpable mammary tumors every 7 days beginning 3 weeks after DMBA injection. The number of tumors and tumor size expressed in terms of the geometric mean of the major 2 diameters were recorded until the 3rd week after the 1st tumor appearance or the 26th week after DMBA injection. When the rats were killed, mammary tumors were removed and weighed.

Throughout the experiments, rats were kept in wire mesh cages (25 x 30 x 20 cm), 3 to 4 animals per cage, maintained in an animal room that was air-conditioned (24 ± 0.5°C; 65 to 70% relative humidity) and artificially illuminated (14 hr light from 5:00 a.m. to 7:00 p.m.), and provided with a commercial diet (CLEA Japan Inc., Tokyo, Japan) and tap water ad libitum.

RESULTS

Mammary Gland DNA Synthesis at Proestrus and Diestrus and Effect of CB-154 and Ovine PRL. In 50-day-old rats, [3H]TdR incorporation into mammary gland DNA on PE was more than 4 times as high as that on D2; this high synthesis was decreased to about 1/10 30 hr after the 1st injection of CB-154, and it returned to the level insignificantly different from that of PE 50 hr after the 1st injection (Chart 1A).

On the other hand, there was no difference in [3H]TdR incorporation between PE and D2 in 90-day-old rats, both being the same levels as that on D2 of 50-day-old rats. [3H]TdR incorporation increased 16 hr after PRL injection, reaching a level similar to that on PE in 50-day-old rats after 24 hr and then decreasing to control levels 50 hr after injection (Chart 1B).

Mammary Tumorigenesis. Mammary tumor incidence, latency period, and the mode of tumor growth in each group are illustrated in Table 1.

There were no significant differences in mammary tumor incidence and latency period either among Groups I to III in 50-day-old rats or among Groups IV to VI in 90-day-old rats. On the other hand, 50-day-old rats given DMBA on PE (Group I) and D2 (Group II) had a higher incidence of mammary tumors than did any group of 90-day-old rats (Groups IV to VI). The latency period in all groups of 50-day-old rats (Groups I to III) was also shorter than that in 90-day-old rats given DMBA on PE (Group IV) or 24 hr after PRL injection (Group VI).

In 50-day-old rats, more than 90% of tumors progressed during the experiment in Group I; the ratio of progressed tumors significantly decreased and the ratios of regressed and/or static tumors increased in Group II as well as in Group III pretreated with CB-154.

In 90-day-old rats, the administration of DMBA 24 hr after PRL injection (Group VI) resulted in a significant increase in the ratio of progressive tumors when compared with the treatment with DMBA on PE (Group IV) or D2 (Group V). There were similar trends in the mode of tumor growth between Groups I and VI and among Groups II to V.

As presented in Chart 2, both the number and weight of mammary tumors per tumor-bearing rat were significantly higher in Group I than in Groups II and III. Similarly, Group VI was higher than Groups IV and V in these traits, although the differences in weight among groups were not statisti-
Groups I and IV were given a single i.v. injection of 5 mg DMBA on the late afternoon of PE. Groups II and V were given DMBA on the late afternoon of D2. Group III received the i.p. injection of 1 mg CB-154 twice at noon on D2 and PE and was given DMBA on the late afternoon of PE (30 hr after the 1st injection of CB-154). Group VI received the s.c. injection of 10 mg ovine PRL (NIH-P-S11) on D2 and received DMBA on the late afternoon of PE (24 hr after PRL injection).

<table>
<thead>
<tr>
<th>Age at DMBA injection (days)</th>
<th>Group</th>
<th>Mammary tumor incidence (%)</th>
<th>Av. latency period (wk)</th>
<th>No. of tumors examined</th>
<th>Regressed</th>
<th>Static</th>
<th>Progressive</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>I (PE)</td>
<td>97.4 (37/38)</td>
<td>11.7 ± 0.8</td>
<td>99</td>
<td>1 (1.0)</td>
<td>5 (5.1)</td>
<td>93 (93.9)</td>
</tr>
<tr>
<td></td>
<td>II (D2)</td>
<td>96.4 (27/28)</td>
<td>10.9 ± 0.8</td>
<td>37</td>
<td>11 (29.7)</td>
<td>5 (13.5)</td>
<td>21 (56.8)</td>
</tr>
<tr>
<td></td>
<td>III(CB-154)</td>
<td>84.0 (21/25)</td>
<td>10.0 ± 1.1</td>
<td>37</td>
<td>3 (8.1)</td>
<td>11 (29.7)</td>
<td>23 (62.2)</td>
</tr>
<tr>
<td></td>
<td>IV (PE)</td>
<td>78.4 (29/37)</td>
<td>16.0 ± 0.8</td>
<td>34</td>
<td>7 (20.6)</td>
<td>6 (17.6)</td>
<td>21 (61.8)</td>
</tr>
<tr>
<td></td>
<td>V (D2)</td>
<td>74.1 (20/27)</td>
<td>13.7 ± 1.1</td>
<td>30</td>
<td>9 (30.0)</td>
<td>6 (20.0)</td>
<td>15 (50.0)</td>
</tr>
<tr>
<td></td>
<td>VI (PRL)</td>
<td>69.7 (23/33)</td>
<td>15.5 ± 0.9</td>
<td>47</td>
<td>1 (2.1)</td>
<td>3 (6.4)</td>
<td>43 (91.5)</td>
</tr>
</tbody>
</table>

Significance: p < 0.05 I, VI/II, IV, V
p < 0.01 I, II/IV, V, VI p < 0.05 I, II, III/IV, VI
p < 0.05 I/III
p < 0.02 I, III/IV, V
p < 0.01 I, VI/II, III, IV, V

* Numbers in parentheses, number of rats with mammary tumors/total number of rats.

† Mean ± S.E.

** DNA Synthesis and Mammary Tumorigenesis**

**DISCUSSION**

In 50-day-old rats in which mammary DNA synthesis was higher on PE than on D2, the administration of DMBA on PE results in higher numbers, weight, and growth rate of mammary tumors than on D2. In 90-day-old rats, no difference in mammary gland DNA synthesis was observed between PE and D2, and there was also little difference in mammary tumorigenesis between animals given DMBA on PE and animals given it on D2. Furthermore, several values as the indices of mammary tumorigenesis were decreased and increased by pretreatment on PD with CB-154 and PRL, respectively, associated with the parallel suppression and stimulation of mammary gland DNA synthesis. DNA synthesis altered by CB-154 and PRL was found to return to control levels by 50 hr after injections of CB-154 and PRL (by 26 and 20 hr after DMBA injection, respectively). Therefore, the differences in mammary gland DNA synthesis among groups were restricted only shortly before and after DMBA injection throughout the experiment. These findings demonstrate that mammary gland DNA synthesis around...
Chart 3. Percentage increase in size of DMBA-induced mammary tumors in each group that appeared first. See Table 1 for description of each group. a, number of tumors examined: I/II, III, IV, V, VI, p < 0.01 at the 3rd week.

the time that the carcinogen acts on the glands is one of the factors controlling the development and growth of mammary tumors. Moreover, they strongly suggest that growth potential of mammary tumors is largely decided at the very initial stages of development.

Even at stages in which average mammary gland DNA synthesis is low, such as on D2 or after CB-154 injection, some or a few mammary foci within individuals would be synthesizing DNA and may be transformed by the carcinogen into tumors. These reflect little difference in mammary tumor incidence (number of rats with tumors/total number of rats) among groups receiving DMBA at stages of different mammary gland DNA synthesis in either 50- or 90-day-old rats.

While there is no difference in rats of different ages in serum PRL level (3), the latency period was shorter in 50-day-old rats than in 90-day-old rats irrespective of estrous stages when DMBA was injected or of pretreatments. This may explain the higher response to PRL of mammary tumors in the younger animals than in the older animals at the initial stages.

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