Enhancement by Human Placental Lactogen of Mammary Hyperplastic Nodules in Ovariectomized Mice

Reiko Yanai and Hiroshi Nagasawa
Pharmacology Division, National Cancer Center Research Institute, Tsukiji 5-1-1, Chuo-ku, Tokyo 104, Japan

SUMMARY

The effect of human placental lactogen on the maintenance and growth of precancerous mammary hyperplastic nodules was studied in ovariectomized, multiparous C3H/He female mice. In mice receiving injections of 0.5 mg of human placental lactogen twice daily for 19 days and on the morning of the 20th day, beginning the morning following bilateral ovariectomy, the number of nodules per mouse was similar to that in the intact controls. The ovariectomized controls had significantly fewer nodules than the intact controls and the ovariectomized, placental lactogen-treated mice. The average size of nodules was significantly larger in the ovariectomized, placental lactogen-treated mice and was smaller in the ovariectomized controls than in the intact controls. The number of very large nodules was extremely high in the ovariectomized, placental lactogen-treated mice. These results suggest that placental lactogen plays an important role in the maintenance and growth of precancerous mammary hyperplastic nodules in mice.

INTRODUCTION

Some mammary tumors in mice grow rapidly during pregnancy but regress after parturition (7). However, up to the present time, no data are available on the precise hormonal mechanism of mammary tumorigenesis during pregnancy, although Foulds (6) described the possible effect of gonadotropic hormones, placental hormones, and the cooperative action of estrogenic and luteal hormones on this process. The development and growth of precancerous HN and their malignant transformation depend primarily upon pituitary prolactin (21-23). Recently, it has been demonstrated that there exists a prolactin-like substance (placental lactogen) in the placentae of mice (13), rats (5, 12, 14-17, 20), goats (3, 4), and monkeys (8-11) as well as humans (19, 20). The similarity of placental lactogen to prolactin in several biological actions has been documented (1, 2) and the predominant participation of placental lactogen in mammary development during pregnancy is suggested (1, 18). In the present paper, we have attempted to study the effect of placental lactogen on the maintenance and growth of HN in mice as 1 step to clarify the hormonal mechanism of the influence of pregnancy on mammary tumorigenesis.

MATERIALS AND METHODS

C3H/He multiparous female mice, without palpable mammary tumors and 10 to 11 months old, were divided into 3 groups. Group 1 remained intact and served as the intact controls. Groups 2 and 3 were bilaterally ovariectomized. Group 2 received no further treatment (ovariectomized controls). Group 3 received injections s.c. of 0.5 mg of HPL (Nutritional Biochemicals, Cleveland, Ohio) dissolved in 0.1 ml of 0.9% NaCl solution, twice daily for 19 days and on the morning of the 20th day. The injections were started on the morning following ovariectomy. They were maintained in an air-conditioned and artificially illuminated room and offered commercial diet and water ad libitum. All the mice were killed 4 to 5 hr after the last injection. The 3rd thoracic mammary glands were excised from both sides and prepared for whole-mount evaluations. The numbers of HN and "ghosts" (remnants of regressed HN) were counted and the size of each HN was expressed as the arithmetic mean of the 2 major diameters. The mammary glands were examined under 10-fold magnification.

RESULTS AND DISCUSSION

The number and size of HN and the number of ghosts in each group are presented in Table 1. The number of HN per mouse in Group 3 was not different from that in Group 1 and both were significantly higher than that in Group 2 (p < 0.05). The average size of HN was significantly smaller in Group 2 and was larger in Group 3 than in Group 1 (p < 0.01). Very large HN were characteristic in the glands of Group 3. The average number of HN greater than 1 mm was 6.2, 2.3, and 14.2 per mouse in Groups 1, 2, and 3, respectively. While the number of ghosts was not significantly different among groups, it was rather large in Group 2. A representative whole-mount preparation from each group is shown in Figs. 1 to 3. The glands of Group 1 consisted of numerous ducts and branches with good lobuloalveolar development and many HN (Fig. 1). On the other hand, the glands of Group 2 were markedly involuted and some small HN were found (Fig. 2). In Group 3, while normal lobuloalveolar system rather regressed, some alveoli...
Table 1
Number and size of mammary HN and number of ghosts in each group

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment*</th>
<th>No. of mice</th>
<th>No. of HN/ mouse</th>
<th>Av. size of HN (mm)*</th>
<th>No. of ghosts/ mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Intact controls</td>
<td>9</td>
<td>33.3 ± 2.5**</td>
<td>0.64 ± 0.03* (299)*</td>
<td>0.7 ± 0.2</td>
</tr>
<tr>
<td>2</td>
<td>Ovx controls</td>
<td>10</td>
<td>20.7 ± 3.7</td>
<td>0.54 ± 0.02 (207)</td>
<td>2.5 ± 1.0</td>
</tr>
<tr>
<td>3</td>
<td>Ovx; HPL, 0.5 mg, 2×</td>
<td>12</td>
<td>33.6 ± 3.5*</td>
<td>0.94 ± 0.02* (403)</td>
<td>0.9 ± 0.3</td>
</tr>
</tbody>
</table>

* Ovx, ovariectomy.
** Sum in bilateral thoracic 3rd mammary glands.
* Arithmetic mean of the major 2 diameters of HN.
** Significant difference, p < 0.05 from Ovx controls.
* Significant difference, p < 0.01 from Ovx controls.
* Significant difference, p < 0.05 from intact controls.
* Total number of HN examined.

showed atypical development and milk secretion was observed in all glands. There existed several huge HN (Fig. 3). The body weight in Group 3 slightly decreased during the experiment, whereas those in Groups 1 and 2 changed little.

These results provide ample evidence that HPL can promote the maintenance and growth of HN in the absence of ovarian steroid hormones, and they imply that placental lactogen is 1 of the important factors in the earlier and higher incidence of spontaneous mammary tumors in multiparous mice than in virgin mice.

The normal mammary system was not always maintained by HPL. We previously observed that involution of the normal mammary gland was not completely prevented by isograft of pituitaries in adrenoovariectomized mice, whereas the maintenance and growth of HN in these mice were good (22). Further experiments on the different responsiveness between normal mammary gland and HN to placental lactogen or pituitary prolactin are now in progress.

REFERENCES
22. Yanai, R., and Nagasawa, H. Enhancement by Pituitary Isografts of
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Fig. 1. Group 1, intact controls. Good lobuloalveolar development and many HN were observed.
Fig. 2. Group 2, ovariectomized controls. Mammary gland regressed and HN were small.
Fig. 3. Group 3, ovariectomized and given injections of 0.5 mg of HPL twice daily for 20 days. Several huge HN were noted.
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