Production of Ovarian Tumors by Hormone Administration

SUMIO ARAKI

Department of Obstetrics and Gynecology, Kurume University School of Medicine, Kurume 830 Japan

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Summary: The influence of various extrinsic hormones was observed by using the clipping method to imbed and fix chemical carcinogens in the ovarian parenchyma.

Among the hormones used, ACH and estrogen appeared to accelerate tumor growth. Although the incidence of solid tumors showed little difference among the test groups by the 20th week, the incidence increased to 90% by the 40th week in the ACH group. In hormone-given groups, incidence of tumors was low until the 20th week, particularly in the androgen and progesteron groups. After that point the incidence of tumors increased more in the hormone-given groups than in the control group. The estrogen group had the highest total incidence of cystic tumor, when compared with the control.

The process of generating adenocarcinoma and sarcoma was fundamentally similar in the treated groups and the control. However, the time of appearance of tumors is the hormone-treated groups, except in the ACH group, differed from the control, particularly in the estrogen group.

Keywords: experimental ovarian tumor — clipping method — ACH — estrogen, progesteron theca cell tumor

Introduction

There appears to be a relationship between hormones and ovarian tumors as the ovaries themselves participate in hormone production. Such a relationship has been suggested from clinical and experimental data about granulosa cell tumors (Butterworth, 1937; Traut et al, 1937; Mühlböck, 1953). However, as other tumors are difficult to produce experimentally, few observations have been made.

In our department chemical carcinogens are imbedded in the ovarian parenchyma using the clipping method; this results in a high incidence of adenocarcinoma (Kato, 1973, 1974, 1975).

In the present study, the authors produced experimental ovarian tumors with various chemical carcinogens using the clipping method. The influence of administration of hormones on this tumor production is discussed here.

Materials and Methods

1. Subjects

Wistar female rats weighing 150 to 180 grams were allowed to adapt to the environmental conditions in the laboratory for about 2 weeks before the experiment. Food and water were given freely.

2. Tumor production

Knots were made in size-3 black silk surgical thread of soft type at an interval of 0.5 to 1 mm. A chemical carcinogen, which had been melted in a small test tube
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dipped in a silicon oil bath, was applied to each knot. Two such knots were imbedded in the ovarian parenchyma of each rat.

3. Chemical carcinogens
There are several hundred carcinogens. The author used 9, 10-dimethyl-1, 2-benzanthracene (DMBA), 20-methylcholanthrene (20-MC), 3, 4-benzpyrene (Benz-p) an aromatic hydrocarbon, and 4-nitroquinolin-N-oxide (4 NQO) a heterocyclic compound.

4. Hormones
Five hormones were used: Estriol-depot (Est.) of estriol preparation, New progest-depot (Prog.) of progesteron preparation, Enarmon-depot (And.) of testesterone preparation, Dexascheroson (ACH) of dexamethasone preparation, and HCG. The dosage schedule was shown in Table 1.

Results
1. Tumor production by chemical carcinogens
Animals were sacrificed weekly for 40 weeks after performing the clipping method to observe the incidence of tumors

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Drugs of hormones</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hormone</td>
<td>Component part</td>
</tr>
<tr>
<td>Estrogen</td>
<td>• Estriol tripropionate</td>
</tr>
<tr>
<td>Progesterone</td>
<td>• Progesterone • 17-hydroxyprogren-4ene-3, 20-dione hexaneate</td>
</tr>
<tr>
<td>Androgen</td>
<td>• Testosterone enanthate • Testosterone heptoate • 17 β-hydroxy-4-androsten-3-one 17-heptoate</td>
</tr>
<tr>
<td>Adrenal Cortical hormone</td>
<td>• Dexamethason sulfat-Natrium</td>
</tr>
<tr>
<td>HCG</td>
<td>• Human chorionic gonadotropin</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>TABLE 2</th>
<th>Production of tumors by various chemical carcinogens</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20-MC (120 cases)</td>
</tr>
<tr>
<td></td>
<td>Cystic tumor</td>
</tr>
<tr>
<td>20w</td>
<td>0</td>
</tr>
<tr>
<td>30w</td>
<td>8 (20%)</td>
</tr>
<tr>
<td>40w</td>
<td>8 (20%)</td>
</tr>
</tbody>
</table>
by the chemical carcinogens. The term "solid tumor" refers to all tumors which are entirely solid and cystic tumors which are partially fluid filled.

Tumor incidence is shown in Table 2. Among the four carcinogens, the incidence of solid tumors was the highest with DMBA; 15% up to the 20th week, 30% up to 30th week, and 60% up to the 49th week. A similar pattern did not exist for cystic tumors. The incidence of cystic tumor was rather low in the DMBA group. Among the animals sacrificed in the 40th week, the incidence of solid tumor was high in the 20-MC group, similar to the DMBA group, but was very low in the Benz-P and 4 NQO groups (4% and 6.6%, respectively).

2. Incidence of tumors with various hormones

A) Dosage and administration method of hormones

To determine the dosage of hormones, a pre-test was performed by selecting several doses calculated from human doses. The maximal dose that did not cause depilation, reduction of body weight, or death was thus determined. The change in the vaginal smears of the rats was used as an index for the estrogen preparation in the pre-test. It was observed that an intravascular injection of 10 mg of Estriol depot kept the rats in a state of estrus for at least 1 week longer than the control (Fig. 1, Photo 1). Because a solid tumor was observed in the 10th week in the pre-test, administration of a drug was started in the 8th week (table 3).

B) Assay

The above-described clipping method was employed for producing tumors. DMBA, which yielded the highest incidence of tumors, was used as the chemical carcinogen. For unifying the influence on the incidence of tumors, the experiment was performed under uniform conditions. A total of 671 rats were used: 135 for the control of DMBA, 96 for Est, 96 for ACH, and 93 for HCG.

Rats were sacrificed 10, 20, 30 and 40 weeks after treatment with chemical carcinogens and the incidence and histological type of tumors produced were examined. The results obtained under various conditions were compared and studied.

C) Results

(1) Incidence of ovarian solid tumors

Fig. 2 shows the incidence of solid ovarian tumors. In the DMBA control group solid tumors were first noted in the 10th week (5%), with the incidence rising to 62% by the 40th week. This was not significantly different from the results of the pre-test.

In the ACH group, tumor incidence
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TABLE 3
Dosage and administration method of hormones

<table>
<thead>
<tr>
<th>Drags</th>
<th>No. of rats</th>
<th>administration and dose</th>
<th>total dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estrogen</td>
<td>104</td>
<td>10 mg → 1 × 4/week (im)</td>
<td>40 mg</td>
</tr>
<tr>
<td>Progesterone</td>
<td>147</td>
<td>75 mg → 1 × 4/week (im)</td>
<td>300 mg</td>
</tr>
<tr>
<td>Androgen</td>
<td>96</td>
<td>25 mg → 1 × 4/week (im)</td>
<td>100 mg</td>
</tr>
<tr>
<td>Adrenal Cortical hormone</td>
<td>96</td>
<td>2.5 mg → 1 × 4/week (im)</td>
<td>10 mg</td>
</tr>
<tr>
<td>HCG</td>
<td>93</td>
<td>500 I. u. → 1 × 6/week (im)</td>
<td>3000 I. u.</td>
</tr>
</tbody>
</table>

Fig. 2. Incidence of solid tumor

increased rapidly after the 21st week compared with the control. The incidence was 79% in this group by the 30th week and 90% by the 40th week. In the groups of Est., Prog., HCG, and And, the incidence of solid tumors was low in an early stage, particularly Prog. and And. groups.

(ii) Incidence of cystic tumor

Cystic tumors are sometimes difficult to classify pathomorphologically into non-neoplasma and neoplasma. The term “cystic tumor” here refers to one in which a cystic change is caused in a DMBA-treated ovary almost without the normal ovarian parenchyma (Photo 2). Fig. 3 shows the change in incidence compared with the control. In the ACH, Prog., HCG, and And. groups, the incidence of cystic tumor was high by the 10th week and then subsequently declined. In the Est. group, however, the incidence of cystic tumor was very high even by the 30th week.

(iii) Incidence of histological types of solid tumors

On the basis of histological examination, the solid tumors produced were divided into 3 types; fibroma (or adenofibroma), sarcoma, and adenocarcinoma. Fig. 4 shows the incidence of tumors caused by hormone administration by week. In the 10th week, fibroadenoma (Photo 3) and adenocarcinoma (Photo 4) were each seen in 50% of the controls. Among the hormone-treated groups the ACH group was similar in outcome to the control group. In that group the same two types of tumors were found in almost equal ratios. In the other hormone groups, solid tumors did not appear until the 10th week, as described above.

The tumors produced by Est. were all of the adenofibroma type. In the 20th week, solid tumors were seen in all of the
EXPERIMENTAL OVARIAN TUMOR

Photo 2

Fig. 3 Incidence of cystic tumor

Fig. 4 Incidence by week of the histological types of solid tumors
test groups. In the And. and Prog. groups, all the tumors were either of the fibroadenoma or fibroma type, similar to the Est. group. In the HCG group both types of tumors were found, but the fibroadenoma type predominated. In the 30th and the 40th weeks, sarcoma-type tumors (Photo 5) were observed. They were found in the control, in the 30th week and in the Est. and Prog. groups, in the 40th week. Sarcoma-type tumors were not confirmed in the And., ACH. and HCG groups by the 40th week.

| TABLE 4 |
| Weight of the solid tumor produced |

<table>
<thead>
<tr>
<th>experimental orgp</th>
<th>weeks</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMBA only (control)</td>
<td></td>
<td>M±S.D.</td>
<td>M±S.D.</td>
<td>M±S.D.</td>
<td>M±S.D.</td>
</tr>
<tr>
<td>DMDA+ACH</td>
<td></td>
<td>2.0±1.24</td>
<td>4.3±1.90</td>
<td>9.45±2.13</td>
<td>13.9±3.45</td>
</tr>
<tr>
<td>DMBA+Est.</td>
<td></td>
<td>2.8±1.06</td>
<td>8.4±3.11</td>
<td>11.6±2.95</td>
<td>18.7±3.76</td>
</tr>
<tr>
<td>DMBA+Prog.</td>
<td></td>
<td>3.0±1.13</td>
<td>8.3±2.05</td>
<td>13.1±2.18</td>
<td>19.3±3.72</td>
</tr>
<tr>
<td>DMBA+And.</td>
<td></td>
<td>5.1±1.92</td>
<td>9.7±2.72</td>
<td>14.3±2.81</td>
<td></td>
</tr>
<tr>
<td>DMBA+HCG</td>
<td></td>
<td>4.2±2.43</td>
<td>8.5±2.86</td>
<td>15.8±3.31</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.9±3.56</td>
<td>10.2±2.81</td>
<td>15.1±3.03</td>
<td></td>
</tr>
</tbody>
</table>
(iv) Weight of solid tumors

Solid tumors were measured and weighed (Table 4). The average weight of tumors produced by ACH and Est. was generally greater than the control, but the other groups were not significantly different from the control. The largest tumors in each group were: 42.5 g in the control, 146 g in the Est. group, 120 g in the ACH group, 38 g in the Prog. group, 50 g in the And. group, and 36 g in the HCG group. The largest tumor was in the Est. group (Photo 6).

Discussion

Retrospective and prospective approaches are usually employed to examine human tumor tissue and tumor production.

Observations of experimental ovarian tumors have shown that some tumors are produced by X-ray irradiation (Furth et al., 1936; Traut et al., 1937; Butterworth, 1937; Mandel et al., 1956), some by intrasplenic ovarian transplantation (Biskind et al., 1944, 1948; Iglesias et al., 1953; Gardner, 1957), and others by chemical carcinogens or parabiosis. Tumor produced by X-ray or intrasplenic ovarian transplantation are primarily granulosa-theca cell tumors. There have been many reports on the genetic mechanism of this tumor and its relation to hormones based on experimental results.

Kato and Yakushiji in our department established the clipping method for imbedding and fixing chemical carcinogens in the ovarian parenchyma to produce adenocarcinoma in 1973. Their report on production of these tumors was the first on this subject. Since that time there have been continuous studies on the histological genesis of the tumor and its properties with emphasis on inheritability (Yakushiji et al., 1979).

The present study was one of these experiments. The initial assumption is that tumors can be produced by external hormones. In the 10th or the 20th week, changes in the remaining, contralateral ovaries were morphologically observed. When the rats were sacrificed, however, no special morphological changes were found except in a few cases. However, as cystic tumors appeared more in the treated group than in the control, and in several rats in the groups of Est. and HCG some changes were observed in the stroma of the regenerative gland mainly of ovarian follicles. There were also changes in the vaginal smears. These results seem closely related to the imbalance of the body endocrine adjusting mechanism caused by extrinsic hormones. These data also raise the question of transformation of tumor type. The solid tumors produced were of three types; adenofibroma, adenocarcinoma, and sarcoma. The controls exhibited similar types. The type of tumor produced depends on the chemical carcinogen used, as the tumors were produced by local action of the chemical carcinogens. The activity of DMBA appears to be rather strong, however, and it is possible that DMBA overrides the effect of extrinsic hormones.

All solid tumors in the Est.-given rats found in the 10th week were of the adenofibroma type. It is also interesting to note that most of the solid tumors which were produced by hormones, except ACH, and which appeared early, were of the adenofibroma type. It can be said that in the process of producing adenocarcinoma as reported previously, fibroadenoma is a fundamental type which develops into adenocarcinoma or sarcoma, though the influence of extrinsic hormones cannot be denied as the time of appearance of the tumor differs greatly from that of the control.

Generally, the type of tumor and the immune mechanism contribute to the proliferation or development of a tumor, and in the present experiment considerably larger tumors were produced in the groups...
of ACH and Est. This may be related to the growing rate of the tumor. ACH and Est. are known to accelerate proliferation of the tumor.

Recently hormone administration in chemotherapy for ovarian malignancies has been suggested. Based on our experimental results, patients treated with ACH and Est. must be carefully followed.

Hormones have been linked to tumor production for some time. Clinical observations supported this link, including the increased incidence of uterine cancer in women after menopause and the relation between lactation and breast cancer. Experimental support was first reported early in this century by Lacassagne, Loeb, and other researchers. They showed the increase in the incidence of breast cancer and uterine cancer in mice when ovarian follicle hormone was used. They also showed that ovarian excision prevented the appearance of breast cancer. Various authors later showed that tumors were produced in various organs and tissues when hormones were used (Nelson, 1937, 1939; Lipschutz, 1938; Iglesias, 1938; Moricard, 1938; Cauchox, 1939).

The effect of estrogen on mice, however, was not clarified in these early studies. Synthetic estrogen has been considered a cyclic type of carcinogenic hydrocarbon. It has been proposed that uterine cancer results from a direct action, similar to carcinogenic chemicals (Gardner, 1938, 1939; Allen, 1941; Pan, 1948; Williams, 1953). However, there has been no evidence to prove this until now. In an experiment by Taki et al. (1963) in which a tumor was produced by applying chemical carcinogens directly to the uterus, estrogen showed a significant accelerating effect, while progesterone and testosterone had inhibiting effects. Although the present work involved ovarian rather than uterine tumors, the finding of high total incidence of cystic and solid tumors in the estrogen group, combined with the lower early incidence in progesterone and androgen groups, suggests the same links between hormones and tumor production.

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