Differential Response of an Ovarian-responsive Mouse Mammary Tumor to Androgenic and Estrogenic Agents

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ABSTRACT

An ovarian-responsive mammary tumor subline, T4-OR26, was isolated from an outgrowth of a progressed TPDMT-4 pregnancy-dependent mammary tumor in a virgin DDD mouse. T4-OR26 tumors were characterized by significantly faster growth in virgin mice than in ovariectomized mice. Both estrogen and progesterone were important for growth of the subline, as they were for that of the parent. Tamoxifen (TAM), with estrogenic activity, and epithiostanol (EPI) and testosterone propionate, with androgenic activity, which all caused TPDMT-4 tumors to regress, were compared for antitumor potency against the new subline by 3 s.c. injections weekly in virgins. EPI at 300 µg and testosterone propionate at 1000 µg elicited immediate tumor growth suppression with subsequent slight regression as did ovariectomy. TAM at 1000 µg caused tumor growth suppression after 2 weeks without subsequent regression. At 600 µg, EPI but not TAM significantly inhibited 17β-estradiol plus progesterone-induced tumor growth; at 400 µg, neither had any significant effect on the tumor growth induced by 17β-estradiol alone. With regard to their effect on hormone receptors, it was noted that EPI and testosterone propionate treatments with tumor regression, caused significant reduction in cytoplasmic progesterone receptor, but TAM treatment, which does not influence tumor growth, did not cause such reduction. The results provide evidence that hormone-dependent mammary tumors may acquire greater resistance to estrogenic than to androgenic therapeutics with progression.

INTRODUCTION

A transplantable pregnancy-dependent mammary tumor line was successfully isolated from a spontaneous, virus-related mammary tumor and designated as TPDMT-4 in DDD mice (16). TPDMT-4 tumors have been characterized by continuous growth during pregnancy and rapid regression after parturition in breeders as well as by ductal-lobular-alveolar outgrowth in the gland-free fat pads and insignificant outgrowth in the intact fat pads of virgin mice (13, 16). Tumors can grow without regression in pseudopregnant mice carrying ectopic pituitary isografts (17). This system has provided a unique model for studies on endocrine therapy of breast cancer (14, 18, 20, 21). An androgenic antitumor, EPI, and an estrogenic antitumor, TAM, have been proven to exert similar antitumor potency in the model system. Both agents are suggested to give rise to tumor regression by the same action mechanism. On the other hand, TPDMT-4 tumors have progressed toward autonomy, although slowly, with serial passage as observed in other hormone-dependent tumors (9, 28). As a result, they have acquired greater tumor potential in virgins and have become less sensitive to EPI in pituitary isograft-bearing mice. Late-generation TPDMT-4 tissue produced significant tumorous growth after a latency period of over 4 months in virgins (12). The resulting tumors were still dependent or responsive to the ovarian hormones. From one of them, a transplantable ovarian-responsive mammary tumor subline was successfully established (12). The subline of tumors can grow in both virgin and ovariectomized mice, but significantly more rapidly in the former. This property is also fairly stable. The present study was conducted to investigate whether EPI and TAM have the same antitumor effect on the subline as on the parent line. TES, which also has an antitumor effect on the parent tumor (18), was included for comparison.

MATERIALS AND METHODS

Chemicals and Reagents. [3H]Estradiol (specific activity, 150 Ci/mmol), [6,7-3H]R5020 (specific activity, 56.5 Ci/mmol), and unlabeled R5020 were purchased from New England Nuclear (Boston, Mass.). Radiopurity of the compounds was checked by thin-layer chromatography on silica gel and was found to be over 95%. Unlabeled estradiol of analytical grade was obtained from Tokyo Kasei Company (Tokyo, Japan) and used for determination of unspecifically bound [3H]estradiol. Estradiol and PG for treatment of mice were obtained from Sigma Chemical Company (St. Louis, Mo.). Norit A was from American Norit Company (Jacksonville, Fla.), and Dextran T70 was from Pharmacia (Uppsala, Sweden). TES was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). EPI and TAM were gifts from Shionogi Co., Ltd. (Osaka, Japan) and ICI, Ltd., Pharmaceuticals Division (Alderley Park, Macclesfield, United Kingdom), respectively. All other compounds were commercial preparations of analytical grade.

Mice. DDD mice were bred and maintained in the mouse colony operated by the Laboratory Animal Research Center, Institute of Medical Science, University of Tokyo. The origin and the characteristics of the strain of mice have been described in detail elsewhere (23). Seven- to 10-week-old female mice were used throughout the study. All mice were fed Laboratory Chow F-2 (Funabashi Nojo Co., Ltd., Funabashi-city, Japan), given water ad libitum, and housed 5 to 7 per cage in a temperature- and light cycle (12 hr light-12 hr dark)-controlled room.

Tumor Growth. In all experiments, tumor appearance was checked by palpation twice weekly, and palpable tumors were measured for 2 perpendicular diameters with vernier calipers. The arithmetic mean of both diameters was designated as tumor diameter and was used for expression of tumor growth.

Received January 12, 1983; accepted May 11, 1983.
Antiestrogen and Ovarian-responsive Mammary Tumor

The intact inguinal fat pad of a 3-week-old female mouse, which was maintained as a virgin. A palpable tumor appeared on Day 150 and grew to 13 x 14 mm in diameter on Day 247 of implantation. This tumor was implanted in the same manner into virgin mice, which immediately received bilateral ovariectomy and s.c. insertion of the back of a pellet containing 0.16 mg estradiol, 39.90 mg PG, and 9.94 mg cholesterol. When tumors became barely palpable on Day 10 of implantation, mice were divided into groups as mentioned above. Treatments then followed. In Experiment 6, the effects of TAM and EPI on the tumor growth induced by estradiol alone were observed. For this purpose, the largest tumor (10 x 10 mm in diameter; 0.29 g) obtained from the estradiol-treated group of Experiment 1 was implanted in the same manner into ovariectomized mice carrying a pellet containing 0.16 mg estradiol and 49.84 mg cholesterol. When tumors grew to 3 to 9 mm in diameter on Day 35 of implantation, mice were divided into groups as mentioned above. Treatments then followed.

**Treatments.** The test doses indicated in Table 2 for TAM, EPI, and TES were each suspended in 0.1 ml of an aqueous solution containing 0.9% (v/v) sodium chloride, 0.4% (v/v) polysorbate 80, 0.5% (w/v) carboxymethylcellulose, and 0.9% (v/v) benzyl alcohol and was injected s.c. into the back region 3 times weekly (approximately every other day) in all experiments, starting on Day 0 when mice were grouped. The intact and ovariectomized controls in each experiment received 0.1 ml of the aqueous vehicle alone at the same frequency. The treatment period was 60 days (21). Tumor growth was followed for 48 days.

**Hormone Responsiveness.** In Experiment 1, the effect of estradiol and PG, alone or in combination, on the growth of T4-OR26 tumors was investigated, since both ovarian steroids were important for the growth of the parent tumors (17). Ovariectomized mice received implantation of a 2 x 2 x 2-mm tumor piece into the right inguinal fat pad and s.c. insertion into the back of a 50-ng pellet containing none, either, or both of 0.16 mg estradiol and 39.90 mg PG in addition to cholesterol used as a base. The pellet provided continuous hormone stimulation for at least 60 days (21). Tumor growth was followed for 48 days.

**Antitumor Effect.** In Experiments 2 to 4, the effects of TAM, EPI, and TES on the tumor growth were observed at various doses in virgin mice. A T4-OR26 tumor was cut into approximately 2 x 2 x 2-mm pieces. Single pieces were implanted into the right inguinal fat pads of mice. When tumors grew to 5 to 14 mm in diameter on Day 35 of implantation, mice were divided into groups as mentioned above. Treatments then followed. In Experiment 5, the effects of TAM and EPI on the estradiol plus PG-induced tumor growth were observed. Tumor pieces were implanted in the same manner into virgin mice, which immediately received bilateral ovariectomy and s.c. insertion into the back of a pellet containing 0.16 mg estradiol, 39.90 mg PG, and 9.94 mg cholesterol. When tumors became barely palpable on Day 10 of implantation, mice were divided into groups as mentioned above. Treatments then followed. In Experiment 6, the effects of TAM and EPI on the tumor growth induced by estradiol alone were observed. For this purpose, the largest tumor (10 x 10 mm in diameter; 0.29 g) obtained from the estradiol-treated group of Experiment 1 was implanted in the same manner into ovariectomized mice carrying a pellet containing 0.16 mg estradiol and 49.84 mg cholesterol. When tumors grew to 3 to 9 mm in diameter on Day 35 of implantation, mice were divided into groups as mentioned above. Treatments then followed.

**Morphology.** At the termination of treatment, all mice were killed, and the uteri, ovaries, and tumors were dissected, cleaned of the adhering fat and tissue, and weighed wet. The tumors and ovaries were fixed in 10% formalin solution, processed routinely, and stained with hematoxylin and eosin for histological study. The skins with mammary glands were also fixed in the same fixative to make the whole-mount preparations of thoracic mammary glands. Mammary gland development was graded into 5 classes: Class 0, lateral buds absent or scarce; Class 1, many lateral buds along the main ducts but acini scarce or absent; Class 2, small clusters of acini; Class 3, large clusters of acini; and Class 4, profuse acinar development (24) in Experiments 2 and 3.

**E2R and PGR Assays.** T4-OR26 tumors were implanted in virgin mice as described above. When tumors grew to 15 to 20 mm in diameter, mice were divided into 4 groups of 5 each. A group of mice were given 3 s.c. injections per week of the vehicle, 600 µg TAM, 600 µg EPI, or 1000 µg TES for 2 weeks. Tumors were obtained 24 hr after the last injection, trimmed of the surrounding fat and tissue, weighed, washed twice with chilled phosphate-buffered saline solution (0.05 M NaH₂PO₄, Na₂HPO₄, and 0.12 M NaCl, pH 7.4), dried on filter paper, and stored frozen at −80° until assay. Tumors from Experiment 2 were processed in the same way and included for reference. Cytoplasmic and nuclear E2R were determined by the methods described elsewhere (1, 15) with some modification. Briefly, single or pooled tumors were minced with scissors and homogenized in 6 volumes of 10 mM Tris-HCl buffer (pH 7.4) containing 1.5 mM EDTA and 1.5 mM 2-mercaptoethanol using a glass-Teflon homogenizer. The homogenate was centrifuged at 2000 rpm for 10 min at 0-4°. The supernatant and the pellet were used for cytoplasmic and nuclear E2R determination, respectively. The cytosol was prepared by centrifuging the supernatant at 100,000 x g for 30 min at 0-4°. To determine the total binding, at least 5 different concentrations of [³H]estradiol ranging between 0.5 and 10 nM were used. The nonspecific binding for estradiol was determined in the presence of a 100-fold or more excess of nonradioactive estradiol. Incubation was carried out in duplicate at 0° overnight. After equilibrium was reached, the unbound steroid was removed by adding dextran-coated charcoal suspension (0.5% charcoal and 0.05% dextran in the buffer for homogenization). After additional incubation with agitation at 4° for 20 min, the tubes were centrifuged at 1600 x g for 5 min at 4°. The 500-µl portions of the supernatant were counted. On the other hand, the nuclear pellet was washed with 5 to 10 volumes of 10 mM buffer (pH 7.4) containing 1.5 mM EDTA, 10 mM 2-mercaptoethanol, 0.25 mM sucrose, and 3 mM MgCl₂.
3 times by centrifuging at 2000 rpm for 5 min at 4°C. The pellet was suspended in the same buffer at a concentration of 0.1 g, wet weight tissue, per ml. The 500-µl portions of the suspension were incubated with at least 5 different concentrations of [3H]estradiol ranging between 0.5 and 10 nM with frequent agitation at 30°C for 3 hr in the presence and absence of nonradioactive estradiol to obtain the nonspecific and the total binding, respectively. In preliminary studies, exchange assays with nuclear suspensions from tumors removed 1 hr after estradiol injection were run at 4°C, 15°C, 25°C, 30°C, and 37°C for between 10 min and 10 hr. Based on the results obtained, a 3-hr incubation at 30°C was adopted for T4-OR26 tumors in consideration of inactivation of the nuclear E2R. The reaction mixture was cooled on ice for 15 min and centrifuged at 1800 rpm for 5 min. The pellet was washed with 1 ml of the buffer 3 times and extracted with 1 ml of ethanol at room temperature for 30 min for counting.

Cytoplasmic PGR was assayed as described elsewhere (22). The procedure was practically the same as that for E2R. In this case, however, 10 mM Tris-HCl buffer (pH 7.4) containing 10 mM thyroglobulin, 1.5 mM EDTA, and 10% glycerol was used in place of the buffer containing 1.5 mM EDTA and 1.5 mM 2-mercaptoethanol, and R5020 in place of estradiol. The specific binding values were calculated by subtracting the nonspecific binding from the total binding and plotted according to the method of Scatchard (25) to determine the receptor concentration and the apparent dissociation constant (Kd).

The protein concentration of the cytosol and the DNA content of the nuclear suspension were estimated by the methods of Lowry et al. (11) and Burton (3) using bovine serum albumin and thymus DNA as standards, respectively.

Statistics. The data were analyzed for statistical difference by Student's t test. The difference was evaluated as significant at p < 0.05.

RESULTS

Experiment 1. Effect of Estradiol and PG on Growth of Ovarian-responsive T4-OR26 Tumors. The new subline tumor T4-OR26, isolated from a TPDMT-4 tumor, was characterized by significantly more rapid growth in virgins than in ovariectomized mice (Chart 2). Parent tumors need pituitary hormones and estradiol and PG from the ovary for their growth (17, 19). Thus, this experiment was conducted to investigate the role of both steroids in the growth of T4-OR26 tumors. As shown in Table 1, the tumor growth in the presence of both estradiol and PG was significantly faster than that in the presence of either hormone or in their absence. The subline is similar to the parent line in hormone requirement, although the former can grow at lower hormone levels. Moreover, it was noted that significant growth occurred in a few estradiol-treated mice. In fact, an estradiol-responsive tumor was successfully isolated from the largest tumor in this group and used in Experiment 6.

Experiment 2. Effect of TAM and EPI on Growth of T4-OR26 Tumors in Virgin Mice. Seven mice with palpable tumors were allocated to each group. The effect of TAM and EPI was investigated at daily doses of 400 and 600 µg, respectively, 3 times weekly. These doses were selected, since they gave rise to immediate regression of the parent tumor in pituitary isograft-bearing mice (14, 18). A group of mice were ovariectomized at the start of treatment and included for comparison. In the control group, tumors grew continuously without regression throughout a treatment period of 40 days (Chart 3). In the TAM-treated group, they grew in the same course as in the control for the first 20 days, followed by progressive delay of their growth. In the EPI-treated and ovariectomized groups, however, the tumor growth was immediately suppressed almost completely, and a slight degree of tumor regression commenced around Day 20 of treatment. EPI was comparable to ovariectomy in antitumor activity at the dose used. Consequently, the tumor weight at the end of treatment was significantly lower in the EPI-treated and ovariectomized groups, but it was not significantly lower in the TAM-treated group than it was in the control group (Table 2). However, TAM and EPI had the same potency in causing atrophy
Table 2

Tumor, body, ovarian, and uterine weights and tumor diameters in control and treated groups in Experiments 2 to 6

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Treatment</th>
<th>Body wt (g)</th>
<th>Tumor diameter (mm)</th>
<th>Tumor wt (g)</th>
<th>Ovarian wt (mg)</th>
<th>Uterine wt (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 (virgin)</td>
<td>Vehicle (7)</td>
<td>27.2 ± 0.6g</td>
<td>8.4 ± 1.0</td>
<td>19.0 ± 1.8</td>
<td>2.53 ± 0.69</td>
<td>19.1 ± 1.2</td>
</tr>
<tr>
<td></td>
<td>400 µg TAM (7)</td>
<td>27.3 ± 1.6</td>
<td>8.3 ± 1.0</td>
<td>16.5 ± 1.0</td>
<td>1.66 ± 0.25</td>
<td>9.2 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>600 µg EPI (7)</td>
<td>26.2 ± 0.3</td>
<td>8.3 ± 0.9</td>
<td>9.1 ± 1.1</td>
<td>0.40 ± 0.1f</td>
<td>9.7 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>Ovariectomy (7)</td>
<td>28.4 ± 1.4</td>
<td>8.4 ± 1.1</td>
<td>9.4 ± 0.8</td>
<td>0.28 ± 0.03</td>
<td>28 ± 1</td>
</tr>
<tr>
<td>3 (virgin)</td>
<td>Vehicle (7)</td>
<td>28.9 ± 0.5</td>
<td>8.9 ± 1.1</td>
<td>17.5 ± 0.3</td>
<td>1.75 ± 0.3</td>
<td>16.0 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>1000 µg TAM (7)</td>
<td>26.5 ± 0.7</td>
<td>8.8 ± 0.7</td>
<td>13.9 ± 1.4</td>
<td>1.12 ± 0.3</td>
<td>7.4 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>1000 µg TES (7)</td>
<td>27.4 ± 1.2</td>
<td>9.0 ± 0.7</td>
<td>9.2 ± 1.0</td>
<td>0.35 ± 0.03</td>
<td>7.6 ± 0.4</td>
</tr>
<tr>
<td>4 (virgin)</td>
<td>Vehicle (7)</td>
<td>25.7 ± 1.2</td>
<td>8.5 ± 0.8</td>
<td>15.8 ± 1.9</td>
<td>1.57 ± 0.53</td>
<td>18.2 ± 1.5</td>
</tr>
<tr>
<td></td>
<td>300 µg EPI (7)</td>
<td>26.1 ± 0.6</td>
<td>8.5 ± 1.0</td>
<td>8.6 ± 1.3</td>
<td>0.35 ± 0.13</td>
<td>11.1 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>100 µg TAM (7)</td>
<td>26.8 ± 0.9</td>
<td>8.4 ± 1.2</td>
<td>11.4 ± 2.5</td>
<td>0.70 ± 0.22</td>
<td>11.4 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>50 µg TAM (7)</td>
<td>26.6 ± 0.9</td>
<td>8.4 ± 0.9</td>
<td>12.6 ± 1.4</td>
<td>0.84 ± 0.34</td>
<td>13.3 ± 0.9</td>
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<tr>
<td></td>
<td>300 µg TES (7)</td>
<td>25.6 ± 0.7</td>
<td>8.4 ± 0.8</td>
<td>11.2 ± 1.3</td>
<td>0.53 ± 0.18</td>
<td>10.0 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>100 µg TES (7)</td>
<td>25.7 ± 0.3</td>
<td>8.4 ± 1.1</td>
<td>13.6 ± 1.7</td>
<td>0.94 ± 0.28</td>
<td>12.8 ± 0.6</td>
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<tr>
<td></td>
<td>50 µg TES (7)</td>
<td>26.5 ± 0.9</td>
<td>8.3 ± 1.0</td>
<td>14.5 ± 1.7</td>
<td>1.20 ± 0.40</td>
<td>17.9 ± 0.8</td>
</tr>
<tr>
<td>5 (estradiol + PG treated)</td>
<td>Vehicle (6)</td>
<td>31.7 ± 0.4</td>
<td>2.7 ± 0.8</td>
<td>16.7 ± 1.5</td>
<td>1.64 ± 0.44</td>
<td></td>
</tr>
<tr>
<td></td>
<td>600 µg TAM (8)</td>
<td>32.4 ± 0.6</td>
<td>2.9 ± 0.8</td>
<td>15.1 ± 1.4</td>
<td>1.31 ± 0.31</td>
<td></td>
</tr>
<tr>
<td></td>
<td>600 µg EPI (8)</td>
<td>32.2 ± 0.7</td>
<td>2.6 ± 0.9</td>
<td>10.9 ± 1.3</td>
<td>0.49 ± 0.18</td>
<td></td>
</tr>
<tr>
<td>6 (estradiol treated)</td>
<td>Vehicle (6)</td>
<td>29.4 ± 0.4</td>
<td>4.8 ± 0.8</td>
<td>11.6 ± 0.6</td>
<td>0.44 ± 0.08</td>
<td>213 ± 32</td>
</tr>
<tr>
<td></td>
<td>400 µg TAM (6)</td>
<td>29.0 ± 0.4</td>
<td>5.0 ± 0.8</td>
<td>10.5 ± 0.9</td>
<td>0.40 ± 0.13</td>
<td>226 ± 25</td>
</tr>
<tr>
<td></td>
<td>400 µg EPI (6)</td>
<td>29.7 ± 0.7</td>
<td>4.9 ± 0.8</td>
<td>9.6 ± 1.0</td>
<td>0.35 ± 0.10</td>
<td>190 ± 14</td>
</tr>
</tbody>
</table>

*The indicated dose was injected s.c. 3 times weekly.

b Information in parentheses, status of the host.

c Numbers in the parentheses, number of mice included.
d Mean ± S.E.

e Significantly different from the control value at p < 0.05.
f Significantly different from the control value at p < 0.01.
g Significantly different from the control value at p < 0.02.

of the ovary, and the ovarian weight loss reached significant levels in the groups treated with these drugs. Morphologically, atrophy of the luteal components was conspicuous in these mice as observed in the pituitary isograft-bearing mice under similar conditions (14, 18). TAM and EPI showed the same suppressive effect on the host mammary gland development. The degree of gland development was expressed as grade scores of 2.14 ± 0.26 (S.E.), 1.29 ± 0.29, 1.29 ± 0.29, and 0.29 ± 0.18 in the control, TAM-treated, EPI-treated, and ovariectomized groups, respectively. It was therefore noted that TAM and EPI had similar effects on the ovary and mammary gland but different ones on the tumor growth. TAM decreased but EPI increased the uterine weight. TAM and EPI showed the same suppressive effect on the host mammary gland development. The degree of antitumor potency comparable to those of ovariectomy and 600 µg EPI (Charts 3 and 4). In the TES-treated group, the tumor growth was suppressed almost completely immediately after starting treatment, with slight regression commencing 15 days later. Tumors obtained at the end of treatment were significantly smaller in both tumor diameter and weight in this group than in those from the control group (Table 2). TAM and TES produced significant ovarian weight loss which was morphologically ascribable to selective degeneration of the luteal components. TAM also significantly decreased the uterine weight. TES appeared to suppress development of the host mammary gland to a slightly higher degree than did TAM. The degree of the gland development was expressed as 2.29 ± 0.34, 1.43 ± 0.30, and 1.00 ± 0.22 in the control, TAM-treated, and TES-treated groups, respectively. Thus, the effect of this dose of TES was comparable to that of 600 µg EPI in this respect.

Experiment 4. Comparison of Antitumor Effect on T4-OR26 Tumors between EPI and TES. It became evident from Experiments 2 and 3 and the previous study (18) that TAM and EPI exerted a slight insignificant antitumor effect on T4-OR26 tumors at 400 µg. Therefore, the effect of the antitumor effect was investigated again at a higher dose, 1000 µg daily. TES also gave rise to regression of the parent tumors, although its antitumor potency was 3 times weaker or more than that of EPI on a weight basis (18). Accordingly, TES was also tested at 1000 µg daily. Seven mice with palpable tumors were allocated to each group. TAM manifested a stronger antitumor activity and almost completely suppressed the tumor growth 5 days earlier at this dose than at 400 µg (compare Charts 3 and 4). As a result, the final tumor diameter was significantly smaller in the TAM-treated group than in the control group (Table 2), but the tumor weight was not. In contrast, a 1000-µg dose of TES had an effect on antitumor potency comparable to those of ovariectomy and 600 µg EPI (Charts 3 and 4). In the TES-treated group, the tumor growth was suppressed almost completely immediately after starting treatment, with slight regression commencing 15 days later. Tumors obtained at the end of treatment were significantly smaller in both tumor diameter and weight in this group than in those from the control group (Table 2). TAM and TES produced significant ovarian weight loss which was morphologically ascribable to selective degeneration of the luteal components. TAM also significantly decreased the uterine weight. TES appeared to suppress development of the host mammary gland to a slightly higher degree than did TAM. The degree of the gland development was expressed as 2.29 ± 0.34, 1.43 ± 0.30, and 1.00 ± 0.22 in the control, TAM-treated, and TES-treated groups, respectively. Thus, the effect of this dose of TES was comparable to that of 600 µg EPI in this respect.
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suppressed. Consequently, the antitumor effect of EPI was concluded to be approximately 3 times stronger than that of TES on the weight basis. They also gave rise to ovarian weight loss in a dose-related manner (Table 2).

Experiment 5. Effect of TAM and EPI on Estradiol plus PG-induced Tumor Growth. It was evident from Experiment 1 that both estradiol and PG are important for growth of T4-OR26 tumors as well as for the parent tumor (17). Tumor growth suppression and regression were associated with atrophy of the ovary throughout Experiments 2 to 4. This experiment was conducted using estradiol plus PG-treated, ovariectomized mice with palpable tumors in order to elucidate the possible direct action of TAM and EPI on tumor cells. They were used 3 times weekly at a daily dose of 600 µg. Eight mice were allocated to each agent. TAM suppressed the tumor growth to a slight degree throughout a treatment period of 30 days, and EPI also suppressed it to the same degree for the first 20 days but thereafter suppressed it completely (data not shown). Consequently, tumors were significantly smaller in both tumor diameter and weight in the EPI-treated group but not significantly smaller in the TAM-treated group than in the control group (Table 2).

Experiment 6. Effect of TAM and EPI on Estradiol-induced Tumor Growth. The estradiol-responsive tumor obtained from a mouse with an estradiol pellet in Experiment 1 was investigated for response to TAM and EPI at 400 µg daily. Six estradiol pellet-carrying, ovariectomized mice with palpable tumors were allocated to each group. Both TAM and EPI appeared to suppress the tumor growth to the same degree, although their antitumor effects did not reach significant levels in any parameter (Table 2).

Effects of TAM, EPI, and TES on E2R and PGR of T4-OR26 Tumors. When tumors grew to sizes large enough for receptor assays in virgin mice, 600 µg TAM or EPI or 1000 µg TES were administered 3 times weekly for 2 weeks. Tumors were obtained 24 hr after the last injection. They showed the same effects as those seen in Experiments 2 and 3 even on such large tumors. Tumors followed the same course of growth in the control and the TAM-treated groups, whereas they regressed immediately in a similar course in the EPI-treated and the TES-treated groups (data not shown). Both cytoplasmic and nuclear E2Rs were detected at significant levels in all groups except for the TAM-treated in which neither receptor was undetectable (Table 3). It was noticed that the cytoplasmic E2R level was higher whereas the nuclear E2R was lower in the EPI-treated and the TES-treated groups than in the control. Cytoplasmic PGR is under the control of estradiol in hormone-dependent mouse (22, 31) and rat (2) mammary tumors. The receptor was detectable in all groups. Noticeably, the level was significantly lower in the EPI-treated and the TES-treated groups in which tumor regression occurred (Table 3). Similar results were obtained in the tumors from Experiment 2, although E2R was detected at a low level in both cytosol and nuclei of TAM-treated tumors (data not shown).

DISCUSSION

It is generally accepted that hormone-dependent mammary tumors progress toward greater autonomy with the lapse of time (30) or during serial passage (5, 8, 9, 26). Along with the progression, tumors altered their biological properties (8) and their response to therapeutic agents (26, 27). This rule also holds true of the TPDMT-4 tumor, a transplantable mouse mammary tumor which is exceptionally stable in pregnancy dependence. TPDMT-4 tumors can grow continuously in the presence but not in the absence of ectopic pituitary isografts in virgin mice (17). In these pseudopregnant hosts, EPI, an antiestrogen with androgenic activity, gave rise to immediate tumor regression at early transplant generations but suppressed the tumor growth only partially at later generations (18, 20). This indicates that TPDMT-4 tumors have become more resistant to the antiestrogen with advancing passages. In addition, a TPDMT-4 transplant at generation 29 produced slow but significant growth in a virgin mouse and the ovarian-dependent subline from the sporadic outgrowth has served as a new model for study on endocrine therapy of
breast cancer (21). In the current study, another new subline, T4-OR26, was isolated from a TPDMT-4 transplant at generation 38 after a long latency period in a virgin mouse (see "Materials and Methods"); Chart 1). The subline has been characterized by significantly faster growth in virgin than in ovariectomized mice, i.e., by “ovarian-responsive” growth (Chart 2). As shown in Table 1, the new subline is similar to the parent line as regards the importance of both estradiol and PG for their growth. However, their significant growth in virgins suggests that T4-OR26 tumors can grow at lower hormone levels than do TPDMT-4 tumors. On the other hand, the slow but significant growth of T4-OR26 tumors in the absence of the ovary (Table 1; Chart 2) may be explained by the appearance of autonomous cells as observed during serial passage of hormone-dependent mammary tumors in GR mice (28). However, these cells may be partially autonomous, since the tumors from ovariectomized mice contained significant levels of cytoplasmic E2R and grew more rapidly when retransplanted into fresh virgin mice and still more rapidly during pregnancy when the mice were mated.4 Similar autonomous cells in which hormone responsiveness was maintained were isolated from TPDMT-4 tumors which had passed through continuous hormonal stimulation (12); it has been suggested that these cells ultimately progress toward full autonomy.4 TAM and EPI exerted the same degree of antitumor activity against TPDMT-4 tumors at early generations and gave rise to immediate tumor regression at 100 μg and higher doses (14, 18). Their effects were equivalent to that of ovarioectomy. In these experiments, both antiestrogens also suppressed ovarian and mammary gland development to the same degree, and the tumor regression and the atrophy of these organs were in parallel with each other in degree. In the current study conducted using ovarian-responsive T4-OR26 tumors isolated from a progressed TPDMT-4 tumor, however, similar doses of the androgen gave rise to growth suppression immediately and regression subsequently (Chart 4) without reducing cytoplasmic E2R. In fact, the receptor level was somewhat higher in the treated than it was in control tumors (Table 3). On the other hand, TAM elicited a marked reduction in cytoplasmic E2R in both rat (6, 7, 10) and mouse (Table 3) mammary tumors, whereas it caused rat mammary tumors to regress immediately but had no influence on mouse mammary tumors during the first 2 weeks (Charts 3 and 4). Consequently, it was noted that the rat and mouse mammary tumors responded similarly to TES in their growth habit and to TAM in their cytoplasmic E2R levels but differently to TAM in the former parameter and to TES in the latter. Higher cytoplasmic E2R levels and lower nuclear E2R and cytoplasmic PG levels in EPI- and TES-treated than in control tumors (Table 3) were of great significance, since PG production is under the control of estradiol (22, 31). However, more extensive work should be conducted to clarify the mechanism by which these agents act directly on tumor cells through the machinery of hormone receptors.

REFERENCES

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Differential Response of an Ovarian-responsive Mouse Mammary Tumor to Androgenic and Estrogenic Agents

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