Antitumor Effect of the Antiestrogen, Tamoxifen, on a Pregnancy-dependent Mouse Mammary Tumor (TPDMT-4)

Akio Matsuzawa, Yukiko Mizuno, and Tadashi Yamamoto

ABSTRACT

TPDMT-4 mammary tumors, characterized by growth during pregnancy and regression after delivery, show continued growth in female DDD mice carrying pituitary isografts ectopically or a s.c. 17β-estradiol-plus-progesterone pellet. These experimental models were used to investigate the antitumor effect of the nonsteroidal antiestrogen tamoxifen. When tumors grew to significant sizes, tamoxifen was injected s.c. at a daily dose of 800, 400, 200, 100, 50, 5, or 1 μg three times weekly. In pituitary isograft-bearing mice the antitumor effect of tamoxifen was most obvious at the three highest doses was comparable to that of ovariectomy; tumors regressed slowly during the first 2 weeks of treatment and subsequently more rapidly. Tamoxifen at 100 and 50 μg had no influence on tumor growth during the first 2 weeks and subsequently gave rise to rapid regression. The antiestrogen suppressed tumor growth throughout the treatment period at 10 μg, but it had no effect on growth at the two lowest doses. Tamoxifen caused atrophy of the ovaries and mammary glands in a dose-related manner, that of luteal components in the former being especially conspicuous at 200-μg doses and higher. In pellet-carrying mice, tamoxifen suppressed tumor growth completely at 800 μg but partially and insignificantly at 200 μg. Growth of PIMT-16 autonomous mammary tumors included for comparison was not affected either by tamoxifen at 800 μg daily or by ovariectomy. Tamoxifen is suggested to exert an antitumor effect on this particular hormone-dependent mammary tumor model through its direct action on tumor cells and its suppressive action on hormone production by the ovaries and pituitary gland.

INTRODUCTION

Recently, a variety of antihormone agents have been introduced for treatment of breast cancer. TAM is a nonsteroidal compound with potent antiestrogenic activity demonstrated in animal species (3, 4). The mechanism of action of antiestrogens is thought to be through competitive binding of the estrogen receptor in the target organ (11, 14). TAM binds cytoplasmic estrogen receptor and translocates it to nuclear sites like 17β-estradiol (13). It, however, suppresses 17β-estradiol induced uterine weight gain (4) and cytoplasmic progesterone receptor production (5). It has been reported to inhibit or reverse the growth of 7,12-dimethylbenz(a)anthracene-induced mammary tumors in rats (10, 29) and to decrease the frequency of tumor development when administered concurrently with the carcinogen (8). In humans, TAM has induced tumor remission in a significant number of patients with 17β-estradiol receptor-positive breast cancers (16).

A very stable, transplantable pregnancy-dependent mammary tumor line was successfully isolated and designated as TPDMT-4 in DDD mice (18). The TPDMT-4 tumors are characterized by a pregnancy-dependent growth. They grow during pregnancy and regress rapidly following delivery, reaching ascending peaks in subsequent pregnancies in breeders. However, they show practically no growth in virgin mice. They grow without regression in virgin mice with ectopic PI’s or with a s.c. 17β-estradiol plus progesterone pellet (19). 17β-Estradiol and progesterone from the ovaries are essential for the tumor growth in PI-bearing mice. Demonstration of estrogen and progesterone receptor and 17β-estradiol control of progesterone receptor synthesis support the importance of both steroids in tumor growth (17, 23). This experimental system has served as a model for studies on endocrine therapy of hormone-dependent breast cancer (20, 21). A steroid antiestrogen, epilistostanol, which inhibits the binding of 17β-estradiol to estrogen receptor (26), caused the regression of TPDMT-4 tumors in PI-bearing mice and suppressed their growth in 17β-estradiol plus progesterone pellet-carrying mice. In a preliminary study (22), TAM also reversed the tumor growth when injected s.c. at a daily dose of 1 mg 3 times weekly. In the present study, the effect of the antiestrogen on TPDMT-4 tumor growth was investigated at doses varying from 1.0 to 800 μg.

MATERIALS AND METHODS

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 elsewhere (24). Six- to 7-week-old female mice served as recipients, and 2- to 4-month-old male and female mice served as donors of pituitary glands. All mice were fed Laboratory Chow F-2 (Funabashi Nojo Co., Ltd., Funabashi-city, Japan), given water ad libitum, and housed 4 to 8 per cage in a temperature- and light cycle (12 hr light-12 hr dark)-controlled room.

Tumors. PIMT-16 tumors at transplant generation 60 and TPDMT-4 tumors at generations 12 to 15 were used. PIMT-16 is an autonomous line established from a spontaneous mammary tumor of a DDD mouse, and is characterized by insignificant differences in its growth among pregnant, virgin, and ovarioectomized hosts (25). Neither estrogen nor progesterone receptor has been demonstrated in this line. TPDMT-4 is a pregnancy-dependent line established from a spontaneous mammary tumor of a mouse of the same strain. The properties

1 This work is partially supported by a grant-in-aid for cancer research from the Ministry of Education, Culture, and Science, Japan.
2 The abbreviations used are: TAM, tamoxifen; PI, pituitary isograft; PAS, periodic acid-Schiff.
3 Unpublished observations, A. Matsuzawa, Y. Mizuno, and T. Yamamoto.
of this particular tumor line have been described in detail (18, 19).

In Experiment 1, a PIMT-16 tumor was obtained from a virgin mouse and was cut into approximately 2- x 2- x 2-mm pieces. A single piece was implanted into the right inguinal fat pad in virgin mice. Animals were inspected and the 2 diameters, bisecting palpable tumors at a right angle, were measured with vernier calipers twice weekly. The arithmetic mean of these 2 diameters was designated as the tumor diameter, which was used to express tumor growth. When tumors reached 2 to 4 mm in diameter 9 days after implantation, animals were divided into 3 groups, each containing tumors with similar diameters and latent periods (intervals between the tumor implantation and the appearance of palpable tumors). Treatments then followed.

In Experiments 2, 3, and 4 a TPDMT-4 tumor was obtained from a female who was close to the termination of her second pregnancy, and it was cut into approximately 2- x 2- x 2-mm pieces. A tumor piece and 3 Pi's were implanted together into the right inguinal fat pad in 16-day ovariectomized mice. Animals received a s.c. implant of a hormone pentestyl tumor, body, ovarian, and uterine weights and tumor diameters in control and treated groups. The number of animals allocated to each group of the experiments is indicated in Table 1.

**Treatments.** Each test dose of TAM (trans isomer of 1-[4-(2-dimethylaminoethoxy)phenyl]-1,2-diphenylbut-1-ene citrate, ICI 46,474; a gift from ICI Ltd., Pharmaceuticals Division, Alderley Park, Macclesfield, United Kingdom) was suspended in 0.1 ml of an aqueous solution containing 0.9% (w/v) sodium chloride solution, 0.4% (v/v) polysorbate 80, 0.5% (w/v) carboxymethylcellulose, and 0.9% (w/v) benzyl alcohol and was injected s.c. into the neck region 3 times weekly (approximately every other day) in all experiments, starting on the day of animal grouping. Control animals in all experiments and ovariectomized animals in Experiments 1 and 2 received 0.1 ml of the aqueous vehicle alone at the same frequency. The day of the start of treatments was expressed as Day 0. The treatment period was 20, 61, 46, 45, and 22 days in Experiments 1 to 5, respectively.

**Morphology.** At the termination of treatment, all mice were killed, and the uteri, ovaries, and tumors were dissected, cleaned of adhering fat, and weighed wet. Because TAM had no significant influence on the weights of the hypophysis, adrenals, spleen, kidney, liver, and thymus (22), these organs were excluded from the weight determination. The tumors and ovaries were fixed in 10% formalin solution, processed routinely, and stained with hematoxylin and eosin for morphological study. The PAS method was also used for study of the secreted materials. The skins with mammary glands were also fixed in 10% formalin solution to make a whole-mount preparation of the thoracic mammary glands. The degree of mammary gland development was classified according to the criteria described in Ref. 21. Briefly, the extent of lobulolaevalve de-

Table 1

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Treatment</th>
<th>Body wt (g)</th>
<th>Tumor diameter (mm)</th>
<th>Ovarian wt (mg)</th>
<th>Uterine wt (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. PIMT-16, F60 virgin</td>
<td>Vehicle (9)</td>
<td>23.9 ± 0.2</td>
<td>3.2 ± 0.3</td>
<td>19.7 ± 0.26</td>
<td>12.2 ± 0.4</td>
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<tr>
<td></td>
<td>800 Ig TAM (8)</td>
<td>23.0 ± 0.3</td>
<td>3.2 ± 0.4</td>
<td>74.3 ± 0.2</td>
<td>100 ± 4</td>
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<tr>
<td></td>
<td>Ovariectomy (8)</td>
<td>22.8 ± 0.5</td>
<td>3.0 ± 0.3</td>
<td>7 ± 0.37</td>
<td>25.0 ± 0.6</td>
</tr>
<tr>
<td>2. TPDMT-4, F12 PI-bearing</td>
<td>Vehicle (9)</td>
<td>28.2 ± 0.6</td>
<td>7.4 ± 0.6</td>
<td>1.17 ± 0.36</td>
<td>17.0 ± 0.7</td>
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<tr>
<td></td>
<td>800 Ig TAM (9)</td>
<td>27.1 ± 0.6</td>
<td>7.4 ± 0.6</td>
<td>4.7 ± 0.7</td>
<td>0.09 ± 0.02</td>
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<tr>
<td></td>
<td>400 Ig TAM (9)</td>
<td>28.5 ± 0.5</td>
<td>7.4 ± 0.5</td>
<td>4.7 ± 0.8</td>
<td>0.11 ± 0.04</td>
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<tr>
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<td>200 Ig TAM (9)</td>
<td>27.3 ± 0.6</td>
<td>7.4 ± 0.6</td>
<td>5.0 ± 0.5</td>
<td>0.11 ± 0.02</td>
</tr>
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<td></td>
<td>Ovariectomy (9)</td>
<td>27.8 ± 0.4</td>
<td>8.4 ± 0.8</td>
<td>3.5 ± 0.7</td>
<td>0.08 ± 0.04</td>
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<tr>
<td>3. TPDMT-4, F13 PI-bearing</td>
<td>Vehicle (5)</td>
<td>28.4 ± 1.3</td>
<td>9.1 ± 0.4</td>
<td>19.2 ± 2.5</td>
<td>22.9 ± 0.72</td>
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<td>50 Ig TAM (5)</td>
<td>27.9 ± 0.6</td>
<td>9.3 ± 0.6</td>
<td>6.8 ± 0.6</td>
<td>0.17 ± 0.03</td>
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<td>10 Ig TAM (5)</td>
<td>29.2 ± 0.9</td>
<td>9.1 ± 0.3</td>
<td>11.0 ± 1.6</td>
<td>0.56 ± 0.26</td>
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<tr>
<td>4. TPDMT-4, F14 PI-bearing</td>
<td>Vehicle (6)</td>
<td>26.2 ± 0.7</td>
<td>7.7 ± 1.0</td>
<td>15.1 ± 1.2</td>
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<td>5 Ig TAM (6)</td>
<td>26.7 ± 0.7</td>
<td>7.8 ± 1.0</td>
<td>14.9 ± 3.0</td>
<td>1.60 ± 0.85</td>
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<td>1 Ig TAM (6)</td>
<td>26.0 ± 0.9</td>
<td>7.7 ± 0.7</td>
<td>15.6 ± 1.9</td>
<td>1.78 ± 0.62</td>
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<td>5. TPDMT-4, F15 ovariectomy and 17β-estradiol + progesterone pellet</td>
<td>Vehicle (9)</td>
<td>29.4 ± 0.5</td>
<td>4.0 ± 0.6</td>
<td>22.1 ± 2.9</td>
<td>3.97 ± 1.02</td>
</tr>
<tr>
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<td>800 Ig TAM (9)</td>
<td>29.3 ± 0.6</td>
<td>4.2 ± 0.5</td>
<td>8.2 ± 0.8</td>
<td>0.32 ± 0.09</td>
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<tr>
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<td>200 Ig TAM (9)</td>
<td>29.3 ± 0.5</td>
<td>4.3 ± 0.4</td>
<td>18.7 ± 2.1</td>
<td>2.56 ± 0.5</td>
</tr>
</tbody>
</table>
velopment was classified into 5 grades: 0, the presence of end buds and the absence of alveoli; 1, the presence of a few alveoli and few lobules; 2, the presence of many alveoli and a few small lobules; 3, the presence of a considerable number of small and developed lobules; and 4, the presence of highly developed lobules or complete lobuloalveolar development. The glands of Grades 0 to 2 remain at virgin levels. Those of Grades 3 and 4 are comparable to the early to mid- and the mid- to late pregnant states, respectively.

Statistics. The significance of differences in tumor diameter and tumor, body, and organ weights between each treated group and the corresponding control was examined by Student’s t test. The differences were evaluated as significant at p < 0.05.

RESULTS

Experiment 1. Effect of TAM on PIMT-16 Autonomous Tumors in Virgin Hosts. Eight mice with palpable tumors were allocated to each group. The effect of TAM was investigated at a daily dose of 800 µg 3 times weekly. A group of mice was ovariectomized for comparison. Neither TAM nor ovariectomy had a significant inhibitory effect on the growth of PIMT-16 tumors (Chart 1). No significant differences were found in the time course of tumor growth during a treatment period of 20 days and in the final tumor weight between the control and the treated or ovariectomized group (Table 1).

Experiment 2. Effect of TAM at Large Doses on TPDMT-4 Pregnancy-dependent Tumors in Pi-bearing Hosts. The effect of TAM was examined at a daily dose of 800, 400, or 200 µg 3 times weekly. Ovariectomized mice were included for comparison. In the control group tumors showed continued growth throughout a treatment period of 61 days (Chart 2). In contrast, complete arrest of tumor growth occurred immediately, and slow tumor regression continued during the first 2 weeks of treatment, followed by more rapid and progressive regression until the end of treatment in 3 TAM-treated groups. The time course of tumor regression was essentially the same in these groups. The mean tumor weight at the end of treatment was 0.09, 0.11, and 0.11 g in the groups treated with 800, 400, and 200 µg, respectively, with no significant differences among them (Table 1). In ovariectomized mice, tumors continued to regress throughout the treatment period, the regression rate being higher during the first 2 weeks, in agreement with the previous observation (20). Finally, the antitumor effect of TAM at those doses was comparable to that of ovariectomy. TAM elicited ovarian weight loss in a dose-dependent manner (Table 1).

Experiment 3. Effect of TAM at Intermediate Doses on TPDMT-4 Tumors in Pi-bearing Hosts. The effect of TAM was examined at a daily dose of 100, 50, or 10 µg 3 times weekly. Tumors grew without regression in the control group, as in Experiment 2, although more rapidly in this experiment. TAM suppressed tumor growth to a slight degree during the first 2 weeks of treatment and then gave rise to rapid regression at 100 µg (Chart 3). In the group treated with 50 µg TAM, tumors grew at the same rate as that in the control during the first 2 weeks of treatment and then regressed rapidly. Ten µg TAM suppressed tumor growth to a significant extent throughout a treatment period of 46 days. Finally, the antitumor effect of TAM was dose dependent in both tumor diameter and final tumor weight (Table 1). The antiestrogen elicited ovarian weight loss in the ovaries at all 3 doses in a dose-related manner, as in Experiment 2 (Table 1).

Experiment 4. Effect of TAM at Small Doses on TPDMT-4 Tumors in Pi-bearing Hosts. The effect of TAM was investigated at a daily dose of 5 or 1 µg 3 times weekly. The time course of tumor growth was practically the same in the control and the treated groups (data not shown). However, the average weight was rather larger in the treated groups, although the differences did not reach significance (Table 1). In this respect, it was noticeable that the standard errors of the mean tumor diameter and weight were far larger in the treated groups. This indicates that both parameters showed a wider range of distribution in these groups. In fact, the 2 largest tumors were recorded in treated mice, one being 5.70 g in a mouse treated with 5 µg, and the other being 4.62 g in a mouse treated with
1 μg (Chart 5). This suggests that TAM may enhance some hormone-dependent tumors at low doses. This effect may be related to the estrogenic activity of the compound (9). The ovary weight was significantly reduced at 5 μg (p < 0.02), but not at 1 μg (p > 0.05) (Table 1).

Experiment 5. Effect of TAM on TPDMT-4 Tumor Growth Induced by 17β-Estradiol and Progesterone. TPDMT-4 tumors can grow linearly in ovariectomized mice given an implant of a 17β-estradiol plus progesterone pellet (19, 20). The effect of TAM on the tumor growth was examined at daily doses of 800 or 200 μg 3 times weekly in the light of the results obtained in the foregoing experiments. TPDMT-4 tumors grew far more rapidly in 17β-estradiol-plus progesterone-treated mice than in Pi-bearing ones (Charts 2 to 4; Table 1). TAM at 800 μg elicited almost complete arrest of tumor growth but no regression in any mouse (Chart 4). The tumor weight was significantly smaller in the group treated with 800 μg than in the control (p < 0.01) [0.32 ± 0.09 (S.E.) and 3.97 ± 1.02 g, respectively]. In contrast, the antiestrogen had no significant effect on tumor growth, judged by both tumor diameter and weight, at 200 μg (Chart 4; Table 1). Removal of the pellet gave rise to rapid tumor regression under the same conditions in a separate experiment.3

Effects of TAM on Body, Ovarian, and Uterine Weights, Mammary Gland Development, and Ovarian and Tumor Morphology. Body, ovarian, and uterine weights are shown in Table 1. No significant differences in the initial and final body weights were noted between the control and each treated group in Experiments 1 to 4. In Experiment 5, however, the weight gain, probably due to fluid retention caused by 17β-estradiol and progesterone, was inhibited by TAM completely at 800 μg and partially at 200 μg. Interestingly, the former dose of TAM elicited complete arrest of tumor growth and the latter a partial one (Chart 4). In Pi-bearing mice, TAM gave rise to a significant decrease in ovarian weight in a dose-dependent manner at 5-μg doses and higher. The phenomenon was accompanied by conspicuously degenerated luteal elements in the ovaries. The antiestrogen also reduced uterine weight significantly in a dose-related manner at 200-μg and higher doses. On the contrary, the uterine weight increased more with decreasing the dose of TAM from 50 to 1 μg. In addition, 800 μg TAM elicited a significant increase in uterine weight in 17β-estradiol-plus progesterone-treated mice, suggesting the possibility that the compound might antagonize the inhibitory effect of progesterone on 17β-estradiol-induced uterine growth (6).

Mammary gland development was expressed as a function of tumor weight in Experiments 2 to 5. Because the experimental conditions are similar in Experiments 2 to 4, the results in the control mice are indicated collectively (Chart 5). As previously reported (17), Pi's allowed the attainment of the gland to pregnant levels in 19 of 20 mice and to mid- to late pregnant levels or Grade 4 in 15 mice (Fig. 1). Tumor weights were larger in mice with more highly developed mammary glands, supporting the importance of higher hormone levels for growth of TPDMT-4 tumors. Gland development was suppressed to virgin levels in mice treated with 200 to 800 μg TAM or in ovariectomized mice (Fig. 2), all of which produced tumor regression. Control mice of Experiments 2 to 4 are presented together. See "Materials and Methods" for grades of mammary gland development.
enc. In Experiment 5, a 17β-estradiol-plus-progesterone pellet developed mammary glands to pregnant levels in all mice (Chart 6). The treatment was, however, more stimulatory in terms of tumor growth and less so in terms of mammary gland development as compared with PI. TAM at 800 μg maintained the gland at virgin levels and elicited complete arrest of tumor growth (Chart 4). In contrast, 200 μg TAM caused a smaller and insignificant degree of suppression of both mammary gland development and tumor growth.

Ovaries in PI-bearing controls had many apparently active corpora lutea and follicles of various developmental stages (Fig. 3), indicating active hormone production leading to pseudopregnant status. The organ showed practically the same morphology in mice treated with 1 and 5 μg, although lower in weight than in the controls (Table 1). Stronger degeneration of luteal components and a greater decrease in the number of solid, active corpora lutea occurred at higher doses. It is noteworthy that apparently active corpora lutea were lacking in all mice given 50 to 800 μg TAM, and in 2 given 10 μg (Fig. 4). Importantly, all of these mice underwent tumor regression (Chart 5). Three mice treated with 10 μg had a considerable number of active corpora lutea and highly developed mammary glands and produced tumor growth. Folliculogenesis was not so disturbed in any of the treated mice, except for a few treated with 800 μg TAM. Thus, the decreased ovarian weight at all test doses is attributable to the selective effect of the antiestrogen on luteal components (Table 1).

The tumor morphology in PI-bearing mice was practically the same as that reported elsewhere (19, 20). The tumors were composed of small cuboidal, epithelial cells. In the central portion they were arranged either in multiple layers, forming indiscernible acinar and glandular structures, or in single rows, forming acinar and glandular spaces of various sizes which were filled with eosinophilic and PAS-positive milky secretion (Fig. 5). Large cystic lumina filled with a secretion were occasionally found. The periphery was occupied by hyperplastic tubules; tubules filled with the cells either partially, resulting in the formation of an eccentric lumen, or filled completely, resulting in the lack of a lumen (Fig. 6). This morphology is characteristic of mammary plaques (2). A loose stroma divided the tumor into lobules. It varied in amount, being sometimes abundant and sometimes so sparse that adjacent lobules touched each other. The regressing tumors from ovariectomized or TAM-treated mice were characterized by clusters of simple tubules or acini with varying diameters (Fig. 7). They were lined by a single layer of flattened or occasionally cuboidal epithelial cells and contained secreted, inspissated, PAS-positive material, sometimes with concentric markings. The areas occupied by these characteristic structures varied from one tumor to another, with a tendency to be larger in more regressed and small tumors. Similar morphology has been reported in hormone-responsive mammary carcinoma-type P from GR mice (30). In the growing tumors from mice given an implant of 17β-estradiol plus progesterone alone or followed by treatment with 200 μg TAM, small cuboidal cells were more densely scattered and formed lobules in the center (Fig. 8) and hyperplastic tubules at the periphery. The lobules and the tubules were separated from each other by various amounts of loose cellular stroma, and did not show clear acinar and glandular structures because of a complete lack of secretory activity. Static tumors from the mice which received an implant of a pellet and were treated with 800 μg TAM had a few tubules lined by a single layer of cells and were free of secretion in the lumina in some places (Fig. 9).

DISCUSSION

The nonsteroidal antiestrogen TAM elicited a consistent regression of pregnancy-dependent TPDMT-4 tumors at 200 μg doses or higher but had no influence upon the growth of autonomous PIIMT-16 tumors (Charts 1 and 2; Table 1). In agreement with the previous observation (20), ovariectomy caused TPDMT-4 tumors to regress progressively in PI-bearing mice (Chart 2). The time course of tumor regression was similar in mice subjected to ovariectomy and in those treated with 200 to 800 μg TAM, although less rapid regression was recorded during the first 2 weeks after the latter treatment. These TAM-treated mice showed marked atrophy of the ovaries, especially of luteal components which produce progesterone (Fig. 4). Progesterone has been proved to be more important for the growth of TPDMT-4 tumors at later transplant generations (17). This suggests that the antitumor effect of the antiestrogen might be mediated through its inhibitory effect on hormone production by the ovaries. In this respect, it is noteworthy that the organ retains more radioactivity than do the other reproductive organs after a single dose of radiolabeled TAM (15) and that the rise in nidatory plasma 17β-estradiol is eliminated or delayed by TAM treatment (32) in pregnant rats. In addition, TAM inhibits 17β-estradiol-stimulated elevations in plasma prolactin in ovariectomized rats (8). It is therefore possible that the antiestrogen suppresses ovarian function via its inhibitory effect on prolactin secretion by PI's and the pituitary gland in situ.

Moreover, TAM at 800 μg daily completely arrested tumor growth induced by exogenous 17β-estradiol and progesterone in ovariectomized mice (Chart 4; Table 1), suggesting that the antiestrogen can nullify the effect of 17β-estradiol at the tumor site. It is now accepted that an interaction with the 17β-estradiol receptor is fundamental to the mechanism of action of the antiestrogen. In 7,12-dimethylbenz(a)anthracene-induced rat mammary tumors, the percentage of tumor regression caused by TAM is linearly correlated with 17β-estradiol-binding capacity (10), and human estrogen receptor-positive breast carci-
nomas respond to TAM more frequently than do negative ones (27). In cultured breast cancer cells, TAM inhibits estrogen-dependent induction of progesterone receptor at large concentrations (5). In TPDMT-4 tumors, cytoplasmic estrogen receptor is translocated to nuclear acceptor sites after binding with 17β-estradiol and 17β-estradiol stimulates the synthesis of progesterone receptor (23), demonstrating that the mechanism of action of 17β-estradiol through estrogen receptor is intact. In contrast, neither estrogen nor progesterone receptor is detectable in PIMT-16 tumors. Significantly, TAM exerted an antitumor activity in TPDMT-4 but not in PIMT-16 tumors. These data taken together suggest that the antitumor action of TAM in this particular model system is probably at a variety of sites, also, that there are inhibition of ovarian function, suppression of prolactin secretion by Pit and the pituitary gland in situ, and a direct action on the tumor cells via the estrogen receptor system.

TAM has been shown at high doses to produce antiestrogenic effects in terms of response of the vaginal epithelium to 17β-estradiol in the mouse (1, 7). This effect is seen after a short period of estrogenicity. In accord with these observations, more potent antitumor effects of high doses of TAM on TPDMT-4 tumors occurred after 2 weeks of therapy (Charts 2 and 3). On the other hand, at doses lower than that effective against TPDMT-4 tumors, TAM has a potent estrogenic activity in terms of uterine weight gain in ovariectomized mice (9) and stimulation of progesterone receptor synthesis in cultured cells (5) and in ovariectomized rat uteri (12). In this respect, it is noticeable that TAM elicited a dose-related increase in uterine weight by decreasing the dose from 10 to 1 μg in Pit-bearing mice (Table 1) and that the 2 largest tumors were recorded in the groups treated with 5 and 1 μg TAM daily (Chart 5). However, the capability of TAM to elicit the growth of TPDMT-4 tumors in the presence of progesterone in ovariectomized mice is insignificant as compared with that of 17β-estradiol.3

TPDMT-4 tumors have been characterized by different morphology, depending on different hormonal environments (18, 25), endocrine therapies (20), and transplant generations (21). This property was also confirmed in the present study (Figs. 5 to 9). Interestingly, the regressed tumors showed type P structures (30, 31) in many areas, which were composed of epithelial cells and filled with a milky secretion (Fig. 7). Carcinoma type P is pregnancy responsive and arises in mammary plaques. This hormone-responsive preneoplastic lesion has been reported in BR, RIII, GR, DD, and DDD mice, all of which have at least one ancestor of European origin (28). The characteristic morphology suggests that this unique mammary tumor line is related to plaques in its origin. TPDMT-4 tumors show secretory activity in postpartum mice (18) and mice bearing Pit’s (20) (Fig. 5) but not in mice given a s.c. implant of 17β-estradiol plus progesterone (20) (Fig. 8), indicating the importance of prolactin in causing morphological and secretory changes.

A series of studies on TPDMT-4 mammary tumors (17, 20–23), including this one, indicates that this tumor line is a unique model for studies on endocrine therapy of breast cancer and the mechanism of action of hormones in neoplastic growth, especially at early generations.

REFERENCES


A. Matsuzawa et al.


Fig. 1. Thoracic mammary gland (Grade 4) from Pi-bearing mouse given injections of vehicle alone. Note full lobuloalveolar development comparable to late pregnant status. Hematoxylin, X 3.6.

Fig. 2. Thoracic mammary gland (Grade 1) from Pi-bearing mouse given 3 injections/week of 200 µg TAM. Note complete suppression of lobuloalveolar development caused by PI's. Hematoxylin, X 3.6.

Fig. 3. Ovary from Pi-bearing mouse given injections of vehicle alone. Note many large, solid corpora lutea and follicles of various stages. H & E, X 25.

Fig. 4. Ovary from Pi-bearing mouse given 3 injections/week of 400 µg TAM. Note degenerated corpora lutea and size far smaller than that in Fig. 3. H & E, X 25.

Fig. 5. Central portion of growing TPDMT-4 tumor from Pi-bearing mouse given injections of vehicle alone. Note glandular spaces surrounded by small cuboidal epithelial cells in single rows and filled with PAS-positive, milky secretion. H & E, X 440.

Fig. 6. Periphery of the tumor in Fig. 5. Note hyperplastic tubules with lumen filled with milky secretion. H & E, X 440.

Fig. 7. Regressing TPDMT-4 tumor from Pi-bearing mouse given 3 injections/week of 400 µg TAM. Note clusters of simple tubules and acini lined by single layer of flattened and cuboidal epithelial cells and filled with secreted materials. H & E, X 290.

Fig. 8. Growing TPDMT-4 tumor from mouse given implant of 17β-estradiol plus progesterone pellet and given injections of vehicle alone. Note more dense scattered cells and more obscure glandular structures as compared with the tumor in Fig. 5. H & E, X 440.

Fig. 9. Static TPDMT-4 tumor from mouse given implant of 17β-estradiol plus progesterone pellet and given 3 injections/week of 800 µg TAM. Note tubules lined by single layer of cuboidal epithelial cells. Compare with Figs. 6 and 7. H & E, X 440.
Antitumor Effect of Tamoxifen

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Antitumor Effect of the Antiestrogen, Tamoxifen, on a Pregnancy-dependent Mouse Mammary Tumor (TPDMT-4)

Akio Matsuzawa, Yukiko Mizuno and Tadashi Yamamoto

Cancer Res 1981;41:316-324.