Estrogen-like Effects of Tamoxifen on Human Endometrial Carcinoma Transplanted into Nude Mice

P. G. Satyaswaroop, R. J. Zaino, and R. Mortel

Departments of Obstetrics and Gynecology [P. G. S. and R. M.] and Pathology [R. J. Z.] and Cancer Research Center, The Milton S. Hershey Medical Center, Pennsylvania State University, Hershey, Pennsylvania 17033

ABSTRACT

The effect of tamoxifen (TAM) on human endometrial carcinoma was investigated in nude mice bearing an estrogen receptor-positive or estrogen receptor-negative tumor. The tumor-negative tumor grew rapidly, and the rates of tumor growth of 17β-estradiol or TAM-treated animals were identical to the rate of controls. The estradiol receptor and progesterone receptor (PR) concentrations in the tumor cytosol remained undetectable under all experimental conditions. In contrast, the rate of growth of steroid receptor-positive tumor was significantly accelerated in the presence of TAM compared to controls (p < 0.02). The increased tumor growth rate was, however, significantly lower (p < 0.01) than that observed in animals receiving 17β-estradiol. The PR concentration in these tumors was elevated in response to TAM treatment. That the TAM-induced PR was indeed functional was evident from (a) increased activities of the progesterone-sensitive enzyme, 17β-estradiol hydroxysteroid dehydrogenase, and (b) histological appearance of subnuclear vacuolization in these tumors after progesterin administration. These studies indicate that continuous, short-term administration of TAM to nude mice results in an estrogen-like effect on endometrial carcinoma. Based on the finding that TAM induces functional PR, we predict that steroid receptor-positive endometrial carcinoma may show a greater response rate to combined, long-term treatment with TAM and progesterin.

INTRODUCTION

Synthetic compounds of the triphenylethylene series have been widely reported to have both estrogenic and antiestrogenic effects on estrogen target tissues of several experimental systems (5). Particular attention, however, has been devoted to the study of the antiestrogenic actions of TAM, and this, in turn, has resulted in its wide use in treatment of ER-positive breast cancers (16). Encouraged by the results from mammary carcinoma studies, several investigators have suggested the use of TAM in the management of other sex steroid-dependent tumors, such as endometrial carcinoma (2, 21).

We have recently developed a nude mouse model in which the influence of sex steroids on growth and magnitude of steroid receptor concentrations in human endometrial carcinoma could be investigated conveniently (19). In this report, we demonstrate that TAM increases the levels of functional PR, as well as the growth rate of ER-positive endometrial carcinoma and, therefore, it is estrogenic in our experimental system.

MATERIALS AND METHODS

Reagents. The radioactive steroids [1,2,6,7-3H]progesterone (90 Ci/mmol), 17β-[1,2,6,7-3H]estradiol (90 Ci/mmol), 17β-[1,2-3H]estradiol (50 Ci/mmol), and [4-14C]estrone (50 mCi/mmol) were purchased from New England Nuclear and used for receptor measurements and enzyme assays after verification of radiochemical purity. Crystalline steroids, estrone, estradiol, and progesterone were obtained from Steraloids, Wilton, NH, and NAD+ was a gift from Sigma Chemical Co.; MPA was a gift from the Upjohn Company, Kalamazoo, MI. The 17β-estradiol and TAM pellets are products of Innovative Research of America, Rockville, MD.

Animals. Ovariectomized, athymic BALB/c-nu/nu nude mice were purchased from Harlan Sprague Dawley, Inc., Indianapolis, IN and maintained in separate barrier facilities used exclusively for these animals.

Tumors. The 17β-estradiol-sensitive (EnCa-X) and 17β-estradiol-insensitive (EnCa-V) endometrial carcinomas were derived from primary tumors of human endometrium in the nude mouse model. The growth characteristics, as well as ER and PR concentrations, of these tumors have been described (19). The EnCa-X tumor is histologically well differentiated and ER-positive, and its growth rate and PR concentrations are increased significantly by 17β-estradiol. The EnCa-V tumor is histologically poorly differentiated and ER negative, and its growth rate is unaffected by 17β-estradiol.

Tumor Transplantation. The tumor tissue (~100 mg) was transplanted s.c. in the intrascapular region of 4- to 6-week-old, ovariectomized, athymic BALB/c-nu/nu nude mice (Transplant 4). The animals were divided into 2 groups: Group 1: control pellets (cholosteryl: methylcellulose:lactose matrix); and Group 2: TAM pellets (maintain blood levels of 20 to 30 ng/ml for 60 days). Pellets were implanted s.c. in the flank contralateral to the tumor site. The growth rate of tumors was followed by determination of tumor volume with a vernier calipers, at weekly intervals. When the geometric mean diameter of tumors reached 1 to 1.5 cm, the animals were killed by cervical dislocation, and the tumors were excised and weighed. A portion of the tumor was processed for histology. Another portion was used for the determination of cytosolic ER and PR concentrations.

Assay of Cytosol Steroid Receptors. Steroid receptor concentrations (determined by the two-site immunoradiometric assay [22]) were assayed in 105,000 × g supernatants of tumor homogenates in Tris-EDTA-dithiothreitol buffer (20 mM Tris-HCl containing 3 mM EDTA, 1 mM dithiothreitol, and 0.01% sodium azide, pH 7.8), according to procedures described previously (18, 19). The radioactive ligands 17β-[3H]estradiol and 17β-[3H]progesterone were used for ER and PR determinations, respectively. For determination of total bound 17β-[3H]estradiol, portions (200 µl) of cytosol were incubated at 30°C for 3 hr with increasing concentrations of 17β-[3H]estradiol (0.2 to 2 nm) and 100-fold concentrations of dihydrotestosterone. Identical samples containing a 100-fold excess of unlabeled 17β-estradiol was used for determination of nonspecific binding. For determination of total [3H]progesterone binding, 200-µl aliquots of cytosol preparation were incubated at 0–4°C for 3 hr with increasing concentrations of [3H]progesterone (1 to 10 nM) and 100-fold concentrations of cortisol. Identical samples containing a 100-fold excess of unlabeled progesterone served as nonspecific

1 This work was supported by American Cancer Society Grant PDT-239.
2 To whom requests for reprints should be addressed.
3 The abbreviations used are: TAM, tamoxifen; MPA, medroxyprogesterone acetate; ER, estradiol receptor; PR, progesterone receptor; E2DH, 17β-estradiol dehydrogenase; EnCa-X, well differentiated; EnCa-V, poorly differentiated; EnCa-V, poorly differentiated.

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controls. Free, labeled ligands were separated from bound radioactivity by treatment with dextran-coated charcoal (10 min at 0° for ER and 5 min at 0° for PR). Receptor concentrations were estimated by Scatchard plot analysis (20) of specific labeled ligand binding data.

Progestin-stimulated E2DH activity was measured to determine the functional aspect of TAM-induced PR. EnCa-X was transplanted into 2 groups of 6 nude mice each (Transplant 6). Group 1 served as control, while Group 2 had s.c. implants of TAM pellets (20 to 30 ng/ml blood levels for 60 days). Three additional animals were implanted with 17β-estradiol pellets (200 to 300 pg/ml blood levels for 60 days) in order to compare the growth rate of this tumor in the presence of 17β-estradiol. When the geometric mean diameter of tumor reached around 1 cm, 3 animals in each group received a single injection of 0.1 ml of 0.9% NaCl solution (saline) i.m., while the remaining 3 were given a single injection of 1 mg of depo-Provera (MPA) in 0.1 ml of saline i.m. The pellets remained in place during the short-term exposure to MPA. All animals were killed 10 days posttreatment, and E2DH activity in tumor was determined as described previously (18, 19).

E2DH Assay. The 800 x g supernatant of tumor homogenates (1 mg/ml of protein) was incubated with excess substrate, 20 μM [3H]-estradiol and NAD+ (1.4 μM) in 0.05 M Tris, pH 8.0 at 37°, and the rate of estrone formation was monitored at 1, 3, 6, and 9 min. Aliquots (0.1 ml) taken at these intervals were immediately mixed with 2 ml of methanol containing 1000 cpmp of [14C]estrone and 500 ng of estrone and 17β-estradiol. 17β-estradiol and estrone were separated by thin-layer chromatography using the solvent system chloroform:ethyl acetate (4:1). The isolated radioactive products were counted in a Beckman 7500 liquid scintillation spectrometer, with counting efficiencies of 48% for tritium and 71% for 14C. The enzyme activity was expressed as nmol of estrone formed per mg of protein per hr and was calculated as reported previously (17, 18).

The protein concentration was determined by the Coomassie dye method (3).

Histological examination of hematoxylin-and-eosin-stained sections from each tumor was carried out for monitoring the secretory response to progestin treatment.

Statistical significance was determined using Student's t test.

RESULTS

The 17β-estradiol-insensitive EnCa-X tumor grew rapidly and reached a geometric mean diameter of more than 1 cm within 3 weeks of tumor transplantation (Chart 1). Administration of TAM or 17β-estradiol did not alter the growth rate. We have reported previously on the lack of 17β-estradiol sensitivity of this poorly differentiated carcinoma (19). The inability of TAM or 17β-estradiol to influence its growth was further corroborated by the absence of any measurable ER in this tumor. In addition, administration of TAM or 17β-estradiol did not alter the PR levels in this tumor, which were undetectable in all 3 groups (results not presented).

The growth rate of 17β-estradiol-sensitive EnCa-X tumor was significantly (p < 0.02) increased in the presence of TAM (Chart 2). The EnCa-X tumor continued to grow rapidly in nude mice implanted with 17β-estradiol pellets as reported previously (19). It is interesting to note that the tumor growth rate was significantly (p < 0.01) lower in TAM-treated animals than that seen with 17β-estradiol, although TAM pellets were designed to maintain 100-fold excess concentration compared to 17β-estradiol pellets [TAM (20 to 30 ng/ml) versus 17β-estradiol (200 to 300 pg/ml)]. EnCa-X tumors exposed to TAM weighed more than twice as much as control tumors (Table 1).

In addition to its 17β-estradiol-like effects on EnCa-X tumor growth, TAM also increased the tumor PR concentration (Table 1). While the cytosol progesterone-specific binding sites were undetectable in control EnCa-X tumor, those grown in the presence of TAM showed a dramatic increase to 707 ± 255 (S.D.) fmol/mg of protein. The cytosolic ER concentration in control tumors was 260 ± 180 fmol/mg of protein, whereas TAM treatment resulted in a reduction of 17β-estradiol-specific sites to 36 ± 25 fmol/mg of protein, presumably due to their nuclear translocation by TAM.

Increased E2DH activity and the histological appearance of subnuclear vacuolization are 2 characteristic progestin effects in human endometrium. Both these responses were assessed in EnCa-X tumors after administration of MPA, in order to determine...
if increased cytosolic progesterone-binding sites that result from TAM treatment are functional. While administration of progesterin to control tumors (PR negative) had no effect on E2DH activity, progesterin treatment to TAM-exposed animals resulted in more than 2-fold increase in E2DH activity in 2 of 3 tumors (Table 2). There was considerable variation in E2DH activity in TAM + progesterin-treated animals and, therefore, individual values are presented. However, histological examination of all 3 tumors in the TAM + progesterin group showed the characteristic secretory response to progesterin treatment, namely, the subnuclear vacuolization (Fig. 1). The subnuclear vacuolization was absent in control or TAM treatment groups.

There was no detectable difference in the rate of EnCa-X tumor growth in TAM-treated animals during the 10-day exposure to MPA.

**DISCUSSION**

Increased PR levels and tissue growth are 2 responses of endometrium to 17β-estradiol. In the ovariectomized nude mouse system we demonstrated previously that 17β-estradiol elicits both these responses in the estrogen receptor-positive endometrial carcinoma (19). The present studies show that TAM also increases growth, as well as PR concentrations, in EnCa-X tumor and is therefore estrogenic. The receptor-negative tumor, on the other hand, fails to respond to either TAM or 17β-estradiol. The correlation between receptor presence and responses to TAM provides support for the idea that TAM effects are mediated through ER.

The functional nature of the progesterone-binding sites which are elevated in EnCa-X tumor cytosol, as a consequence of TAM exposure, is evident upon subsequent treatment of these animals with progesterin. The 2 characteristic progestational responses, namely, increased E2DH activity and appearance of subnuclear vacuolization, are observed only in TAM + progesterin-treated tumors. The reason for the considerable variation in E2DH activity in these tumors is unclear. This may be related to the differential sensitivity of different areas of the tumor to progesterin, which, in turn, might be reflected by the presence of PR at these sites at different times after TAM treatment. Differential enzyme induction at different sites within the same tumor needs to be examined. The presence of necrotic areas within the tumor sampled for enzyme assay may also contribute to the large differences in E2DH activity. However, after 5 to 6 weeks of TAM exposure, the EnCa-X tumor is relatively small (~1 cm geometric mean diameter), and there is generally little necrosis. In contrast to the marked variation in E2DH activity, the histological examination of hematoxylin-eosin-stained tumor sections unequivocally reveals characteristic subnuclear vacuolization in all 3 TAM + progesterin-treated tumors. Tumor sections from animals exposed to either TAM or progesterin alone failed to show subnuclear vacuolization. These findings are consistent with the concept that E2DH activity and subnuclear vacuolization are progesterone-specific events which are mediated through PR. Similar effects of progesterin on E2DH activity and subnuclear vacuolization in EnCa-X tumors grown in the presence of 17β-estradiol was reported previously (19).

It is generally recognized that the physiological responses commonly observed in normal endometrium are preserved to a considerable degree in the well-differentiated endometrial carcinoma. Results of the present studies further demonstrate that 17β-estradiol and progesterone regulated sequence of events can be elicited in a predictable fashion during repeated transplantations of the endometrial tumor in the nude mouse model.

As indicated earlier, wide-ranging studies on the antiestrogenic activities of TAM (and other triphenylethylene derivatives) led to its use in the treatment of ER-positive breast cancer. However, several reports on the estrogenic actions of these compounds in humans and other experimental systems have also appeared. These include TAM-mediated increases in transcoritin, sex hormone-binding globulin, testosterone-estradiol-binding globulin, pregnancy zone protein, and other plasma proteins (4), suppression of follicle-stimulating hormone and prolactin levels in postmenopausal women (7), increased PR concentration in human breast carcinoma (8), endometrial carcinoma (14), hamster uterus (11), dimethylbenz(a)anthracene-induced estrogen-sensitive tumors in rats (22), growth of dimethylbenz(a)anthracene-induced rat mammary tumor (6), mouse vaginal and uterine cell proliferation (13), increased growth and PR concentrations in rat uterus (1), and mammary tumor MXT-3590 (23). The data presented here are further demonstrative of the estrogenic actions of TAM on endometrial carcinoma transplanted into nude mice. In light of these findings, it is necessary to routinely monitor growth effects of TAM in breast and other target tissues in patients receiving this hormone for the treatment of breast carcinoma. Evidence for a 3-fold increase in growth rate of lung metastasis in a postmenopausal breast cancer patient has been presented (12). A recent study reported on the detection of low-grade endometrial carcinoma in 3 women undergoing TAM treatment for breast cancer (10).

Progestins have been shown to be effective in the treatment of endometrial carcinoma (9). However, progestins also lower PR concentrations and thereby reduce their own effectiveness. Therefore, any agent that augments PR concentrations within the tumor may be expected to potentiate the effectiveness of progestin therapy. The results presented here show that, while TAM is estrogenic in regard to growth as well as PR induction, its growth-promoting effect is significantly lower than that observed in the presence of 100-fold lower concentration of 17β-estradiol. Based on these data, we postulate that combination of TAM + progestin, administered either simultaneously or sequentially, may prove to be more effective in the treatment of estrogen receptor-positive endometrial carcinoma. Indeed, our preliminary results in the nude mouse system (15) show that sequential administration of TAM + progestin may be superior to either TAM or progestin alone in the control of this disease.

**ACKNOWLEDGMENTS**

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**Table 2**

<table>
<thead>
<tr>
<th>E2DH activity (nmol of estrone formed/mg of protein/hr)</th>
<th>Control</th>
<th>depo-Provera</th>
<th>TAM</th>
<th>TAM + depo-Provera</th>
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<td></td>
<td>2.8; 2.2; 1.9</td>
<td>2.4; 1.3; 1.8</td>
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<td>4.6; 11.9; 1.1</td>
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**Results were obtained from EnCa-X Tumor Transplant 6.**
REFERENCES

Fig. 1. Sections of EnCa-X tumor grown in nude mice. A, control; B, MPA; C, TAM; D, TAM + MPA. H & E, × 200.
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