Contrasting Actions of Tamoxifen on Endometrial and Breast Tumor Growth in the Athymic Mouse

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ABSTRACT

The effects of the antiestrogen tamoxifen (TAM) on the growth of two hormone-sensitive human tumors have been examined in athymic mice. The endometrial tumor, EnCa101, was stimulated to grow by TAM either alone or when combined with estradiol. This contrasted with the non-stimulation of the breast tumor, MCF-7, by TAM alone and the antagonistic action of TAM on estradiol-stimulated growth of MCF-7 tumors. The individual tumor responses were observed even when the two tumor types were implanted on opposite sides of the same animal. This suggests that host metabolism of TAM does not dictate tissue response. The conclusion is supported by the finding of very similar patterns of metabolites in the two tumors after administration of [14C]-Tamoxifen. Tissue metabolism therefore is unlikely to be involved. Progestosterone receptor levels were higher in estradiol (376 ± 35 fmol/mg cytosol protein)- or TAM (317 ± 37 fmol/mg cytosol protein)-stimulated EnCa101 tumors than control (42 ± 5 fmol/mg cytosol protein) and increased further with combined treatment (485 ± 75 fmol/mg cytosol protein). Estrogen receptor levels, however, were lower in estradiol (45 ± 11 fmol/mg cytosol protein)-treated tumors than control (92 ± 13 fmol/mg cytosol protein) but higher than control in TAM (200 ± 15 fmol/mg cytosol protein)-treated tumors. Tumors grown with estradiol and TAM had lower estrogen receptor levels (130 ± 7 fmol/mg cytosol protein) than tumors grown with TAM alone. Estrogen receptor levels indicate that TAM may not be acting exactly as estradiol in the EnCa101 tumor. Overall, these findings suggest that the disparate pharmacology of TAM is a tissue-specific phenomenon.

INTRODUCTION

The antiestrogen TAM is as effective in the treatment of hormone-dependent breast cancer as any ablative or additive therapy so far clinically tested, and it has the advantage of fewer side effects than these other treatments (1). However, with the therapeutic regimen of TAM used so far, failure has been a constant occurrence even in cases where the agent has produced a long period of tumor remission (1). The reason for TAM failure is at present unclear, although a number of possible mechanisms are currently being investigated (2).

The growth of hormone-dependent human tumors in athymic mice by estrogen supplementation has provided a useful model to study the antiestrogenic action of TAM (3–6) and perhaps TAM failure. Recently TAM has been shown to stimulate the growth of two human endometrial tumors implanted in athymic mice (7, 8). Endometrial carcinoma has been reported to de-


endometrial tumors during prolonged TAM therapy for breast cancer. A larger cohort of patients under long-term TAM therapy (>5 yr) needs to be monitored for the occurrence of TAM-stimulated endometrial tumors. The endometrial tumors in the athymic mice that grow in response to TAM (7, 8) may ultimately represent this form of TAM failure to act as an antiestrogen.

Previous studies have demonstrated that TAM treatment for 2 to 6 mo does not stimulate the growth of MCF-7 tumors implanted in the athymic mouse, although TAM does stimulate the mouse uterus. TAM metabolites extracted from MCF-7 tumors and athymic mouse uteri indicate the presence of the same nonestrogenic metabolites, suggesting metabolism is not involved in the different tissue responses (6).

The present study now directly compares and contrasts the actions and metabolites of TAM in the EnCa101 tumor and MCF-7 tumor when grown in the athymic mouse.

MATERIALS AND METHODS

Tumors. MCF-7 cells were grown into solid tumors in athymic mice in the manner outlined previously (6), and EnCa101 tumors were grown in athymic mice from a primary endometrial tumor as described (8). Both tumors were transplanted from 17β-estradiol-treated animals by sterile dissection of solid tumors, mincing into 1-mm³ pieces, and s.c. implantation of tumor pieces into the axillary fat pads of 4- to 5- wk-old ovariectomized BALB/c athymic mice (Harlan Sprague Dawley, Indianapolis, IN). Mice were housed in laminar flow hoods with sterile cages and bedding and fed ad libitum autoclaved LM-485 Chow (Teklad, Madison, WI) and sterile water. Tumors were measured with calipers at weekly intervals and the mean cross-sectional area was calculated as length/2 × width/2 × π.

In selected experiments tumors were collected and stored at −70°C until assay of steroid hormone receptors.

Hormone Therapy. TAM (5 mg, 4-wk release) and 17β-estradiol (1.7 mg, 8-wk release) pellets were custom made (Innovative Research of America, Toledo, OH) and implanted s.c. on the back of animals using a trocar following the implantation of tumor pieces. TAM pellets were made using tamoxifen-free base (a gift from Stuart Pharmaceuticals, Wilmington, DE).

Tumor Receptor Measurements. ER levels in tumors were assayed using the commercially available ER-EIA kit (ER-EIA monoclonal; Abbott Laboratories, Chicago, IL) and 17β-estradiol (1.7 mg, 8-wk release) pellets were custom made (Innovative Research of America, Toledo, OH) and implanted s.c. on the back of animals using a trocar following the implantation of tumor pieces. TAM pellets were made using tamoxifen-free base (a gift from Stuart Pharmaceuticals, Wilmington, DE).

PgR levels were determined from tissue homogenized in non-KCl-containing ER-EIA buffer with 10% glycerol added. Ligand-receptor binding was performed using a Rainen progesterone-specific assay kit (New England Nuclear, Boston, MA). This kit used [17α-methyl-3H]-R5020 (86.7 Ci/mmol) and a dextran-coated charcoal separation. PgR was assayed using the recommended kit protocol, and specific binding values were analyzed by Scatchard analysis. Cytosol protein was determined using the commercially available BioRad assay (BioRad Laboratories, Richmond, CA) with a goat IgG protein standard (Sigma Chemical Co., St. Louis, MO).

Tamoxifen Metabolites. Athymic mice implanted with 17β-estradiol pellets and bearing MCF-7 tumors on one side and EnCa101 tumors on the other had the pellets removed more than 3 days prior to study.
Mice were given injections of 50 μCi of \textit{trans-}[ring-3H]TAM (specific activity, 19.9 Ci/mmol; a gift from ICI PLC, Macclesfield, England) in peanut oil into the loose fold of skin on the back of the neck and sacrificed 24 h later. Metabolites of TAM in whole uteri, liver (20 mg), and tumors (30 mg) were analyzed by extraction with acidified methanol (2% acetic acid, v/v), and TLC separation was by the method previously described (6) using the benzene:triethylamine:ethanol (85:10:5) solvent system. The chromatographs shown are representative of results obtained from normal and tumor tissue of athymic mice (n = 3).

RESULTS

Pieces of MCF-7 tumor implanted in athymic mice were stimulated to grow by 17β-estradiol supplementation. In contrast, TAM administration did not produce tumor growth and, when administered with 17β-estradiol, inhibited MCF-7 tumor growth in a dose-dependent manner (Fig. 1). Implants of the endometrial tumor EnCalOl in athymic mice were also stimulated to grow by 17β-estradiol administration; however, this tumor was also stimulated by TAM (Fig. 2). These findings suggest that TAM produces a differential growth response between the two tumor types.

In order to examine these differential tumor responses, pieces of MCF-7 and EnCalOl tumor were implanted on opposite sides of the same animal. In dual transplanted animals receiving no drug treatment (control), no MCF-7 tumor growth was detected, whereas small, but steady, growth of EnCalOl tumors occurred. TAM administration produced a marked increase in EnCalOl tumor growth above control, whereas MCF-7 tumors were not stimulated to grow (Fig. 3). This differential growth response to TAM treatment was also seen in dual transplanted animals when TAM was combined with 17β-estradiol. 17β-Estradiol-stimulated MCF-7 tumor growth was inhibited by TAM, whereas EnCalOl tumors were stimulated to grow even more rapidly than when treated with 17β-estradiol alone (Fig. 4).

TAM and 17β-estradiol significantly increased PgR above control in EnCalOl tumors. The further increase in growth rate of EnCalOl tumors produced by combination of TAM with 17β-estradiol was also reflected in an additional increase in PgR (Table 1). In contrast, the ER content of tumors stimulated...
DISPARATE ACTIONS OF TAMOXIFEN

Table 1. EnCa101 endometrial tumor estrogen receptor (determined by ER-EIA) and progesterone receptor (determined by [3H]RS2020 binding) content

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>ER (fmol/mg cytosol protein)</th>
<th>PgR (fmol/mg cytosol protein)</th>
</tr>
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<tbody>
<tr>
<td>Placebo</td>
<td>92 ± 13° (12)</td>
<td>42 ± 5 (7)</td>
</tr>
<tr>
<td>17β-Estradiol (1.7 mg)</td>
<td>45 ± 11° (8)</td>
<td>376 ± 35 (8)</td>
</tr>
<tr>
<td>TAM (5 mg)</td>
<td>200 ± 15° (12)</td>
<td>317 ± 37 (12)</td>
</tr>
<tr>
<td>17β-Estradiol (1.7 mg) + TAM (5 mg)</td>
<td>130 ± 7° (10)</td>
<td>485 ± 75 (8)</td>
</tr>
</tbody>
</table>

* Mean ± SE.
* Numbers in parentheses, number of tumors.
* Significant difference from other treatment groups by the Mann-Whitney rank test (P < 0.05).

All treatment groups are significantly different from placebo.

to grow by estradiol was significantly lower (P < 0.01) than in control tumors, whereas tumors stimulated by TAM had higher ER receptor levels (P < 0.001) than control. This opposite effect of TAM on EnCa101 tumor ER content compared to 17β-estradiol was further demonstrated by ER levels being significantly lower (P < 0.001) in tumors grown with both TAM and 17β-estradiol than when grown with TAM alone (Table 1).

Radiolabeled metabolites of [ring-3H]TAM were examined in tumors, liver, and uteri of athymic mice dually implanted with MCF-7 and EnCa101 tumors. Chromatograms of liver extracts had three clearly defined peaks of radioactivity: the one eluting furthest along the TLC plate corresponding to the parent compound; the second to a 4-hydroxytamoxifen standard; and the most polar metabolite to a 3,4-dihydroxytamoxifen standard (Fig. 5). Extracts of uteri contained the same metabolites as detected in the liver (Fig. 5). MCF-7 tumors and EnCa101 tumors had qualitatively very similar patterns of metabolites with chromatograms having three peaks of radioactivity again corresponding to tamoxifen, 4-hydroxytamoxifen, and 3,4-dihydroxytamoxifen standards (Fig. 5). These studies detected no differences between metabolites of TAM in the EnCa101 tumors and in the MCF-7 tumors.

DISCUSSION

The stimulation of EnCa101 tumor growth by TAM is consistent with previous reports (8) and demonstrates an agonist action of this agent in the tissue. This contrasts with the nonstimulation of MCF-7 tumors by TAM alone and the antagonist action of TAM on estradiol-stimulated MCF-7 tumor growth. Disparate actions of TAM have previously been noted between species (15). For example, TAM is antiestrogenic in the chick oviduct (16) but estrogenic in the mouse uterus (17). This phenomenon has previously been regarded as a species-specific effect (1).

A species-dependent conversion of TAM to estrogenic metabolites has been examined as a possible mechanism for different responses to TAM. However, no significant difference in metabolism between species and no significant quantities of estrogenic metabolites have been found (15, 18). The present study further supports the conclusion that metabolism does not play a major role in the agonist actions of TAM.

A previous report noted that TAM did not stimulate the growth of MCF-7 tumors implanted in athymic mice, whereas it did produce an increase in uterine weight (4). This suggests that TAM exhibits a tissue-specific pharmacology rather than the species dictating the tissue response. The present study expands this finding with the demonstration that tumors of the same species of origin (human) can have markedly different responses to TAM even when implanted in the same animal.

This implies that the species of tissue origin is not the determining factor in how the tissue will respond to TAM. Whether within a species a particular type of tissue or tumor will respond consistently has not yet been determined. Breast cancer cells
that originally were inhibited by TAM in vitro have been adapted to grow in the presence of TAM. However, this growth is not stimulated by TAM, although a protein induced only by estrogens in the TAM-sensitive cells was induced by TAM in two TAM-resistant cell lines (19).

It is possible that cell adaptation could result in clinical failures observed with this agent. The reports that other endocrine therapies can sometimes be effective in treating breast cancer patients who have had tumor relapses on tamoxifen treatments (20–22) indicate that the hormone sensitivity of these tumors is maintained. The conversion of a cell which recognizes TAM as an antiestrogen to a cell which recognizes TAM as an estrogen would explain this finding.

The lower ER content of 17β-estradiol-treated EnCa101 tumors compared to control may be a consequence of receptor processing in a similar way that the ER content has been observed to decrease in MCF-7 cells cultured with estrogen (23, 24). TAM treatment does not produce the same degree of receptor processing as 17β-estradiol in MCF-7 cells in culture when measured by 17β-[3H]estradiol exchange assay (23) and has been reported to increase the ER content when measured by ER-EIA (25). It is possible that monoclonal antibody detection of ER could be influenced by ligand binding, however, the increase in receptor content in EnCa101 tumors is a similar finding to the increase in the ER content observed in uteri of ovariec-tomized-adrenalec-tomized rats with TAM treatment using a 17β-[3H]estradiol exchange assay (26).

A relationship between ER processing and the induction of PgR has been noted in vitro for estradiol and antiestrogens (24, 27). This relationship does not appear to exist in the EnCa101 tumor as both 17β-estradiol and TAM induced PgR, although PgR concentrations in the present study are lower than previously reported (8). The different influences that 17β-estradiol and TAM had on ER content of EnCa101 cells suggest that TAM may not be acting exactly as 17β-estradiol in this tumor and raise the question whether the effects seen with TAM are selective for this agent or are produced by other antiestrogens.

Until the influence of TAM and other antiestrogens on endometrial cancers has been fully investigated, vigilance by physicians treating patients with these agents is needed to establish the clinical relevance (if any) of these observations.

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REFERENCES

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