Inhibition of Tamoxifen-stimulated Growth of an MCF-7 Tumor Variant in Athymic Mice by Novel Steroidal Antiestrogens

Marco M. Gottardis, Shun-Yuan Jiang, Meei-Huey Jeng, and V. Craig Jordan

Department of Human Oncology, University of Wisconsin Clinical Cancer Center, Madison, Wisconsin 53792

ABSTRACT

This investigation examines the tamoxifen (TAM)-dependent growth in vivo of an MCF-7 tumor variant, MCF-7TAM, previously reported in this journal (M. M. Gottardis and V. C. Jordan, Cancer Res., 48: 5183-5187, 1988). Ovariectomized athymic mice were implanted with 1-mm³ pieces of MCF-7TAM and were treated with Silastic capsules of varying sizes containing TAM to demonstrate dose-dependent growth over a 10-wk experiment. TAM was necessary to maintain tumor growth. Animals whose capsules were removed at 6 wk showed complete tumor stasis after 20 wk of observation. Removal of TAM after 11 wk caused the rate of tumor growth to decrease compared with TAM-treated animals. Tumor areas were significantly different (P < 0.03) at Wk 20. The growth of TAM-stimulated tumors, MCF-7TAM, was inhibited by the novel steroidal antiestrogens, ICI 164,384 and RU 39,411. TAM-stimulated growth (0.5-cm Silastic capsule) was maintained at control levels by 8 wk of treatment with ICI 164,384 (1 mg s.c. every other day). ICI 164,384 alone had no stimulatory activity. At the same dose, RU 39,411 inhibited TAM-stimulated growth of MCF-7TAM, although not to control levels. RU 39,411 was slightly stimulatory when administered alone. The growth of MCF-7TAM was stimulated by either TAM or 17β-estradiol. The antiestrogens, RU 39,411, effectively inhibited estradiol-stimulated tumor growth. Overall, these studies confirm and extend the previous observation on TAM-stimulated growth of breast cancer cells in vivo and demonstrate the possibility of developing novel antiestrogens to prevent this form of drug resistance should it occur in the clinic.

INTRODUCTION

Tamoxifen, a nonsteroidal antiestrogen, is the adjuvant antihormonal treatment of choice for Stage I and II breast cancer (1-4). Laboratory studies demonstrated that TAM is a tumorstatic agent (5-8) and that continuous adjuvant therapy may be the best strategy to prevent relapse of the disease in patients (1, 3, 4). However, despite continuous TAM therapy, patients do fail this chemosuppressive strategy (1, 3, 4). It is therefore important to investigate TAM resistance to either subvert the process or develop additional therapeutic strategies for the patient.

Tamoxifen is not a pure estrogen antagonist and possesses some estrogen-like properties in animals and patients (9). Clinical evidence of an estrogen-like stimulation of tumors by TAM has been observed in breast cancer patients first starting therapy (10-12). This "tumor flare" seen with TAM appears to be transient, and tumor regression subsequently occurs. Similarly there is an anecdotal report of a patient failing TAM therapy after several years of successful treatment who had a partial regression of the disease following withdrawal of the antiestrogen (13). Furthermore, patients who fail TAM therapy have been known to respond to other endocrine therapies, such as aromatase inhibitors (14-16). Interestingly enough, the reverse is infrequently observed. Overall, these studies point to the possibility that TAM-stimulated growth could occur in patients as one form of drug resistance that results in therapeutic failure.

Our laboratory and others have shown that athymic mice bearing established hormone-dependent MCF-7 tumors eventually fail after long-term TAM therapy (17, 18). We have previously demonstrated that these tumors can be stimulated with either estrogen or TAM (18). A similar phenomenon has been described for human endometrial carcinomas grown in athymic mice (19, 20), and there is a recent report that demonstrates the growth of human endometrial cells in vitro by antiestrogen (21).

The aim of this study was to describe further the characteristics of the MCF-7 breast tumor variant in vivo (designated MCF-7TAM). This paper has also examined the possibility of inhibiting the TAM-stimulated growth of MCF-7TAM with novel, less estrogenic steroidal antiestrogens (Fig. 1).

MATERIALS AND METHODS

The TAM-stimulated breast cancer tumor, MCF-7TAM, was developed in a previous study from an MCF-7 tumor failing TAM therapy (18). This tumor was subsequently maintained by passage in ovariectomized athymic mice implanted s.c. with custom-made 5-mg TAM pellets (Innovative Research of America, Toledo, OH). All tumors used in these experiments were derived from passages 4 and 5, respectively.

Ovariectomized BALB/c 4- to 5-wk-old athymic mice (Harlan-Sprague Dawley, Indianapolis, IN) were implanted s.c. in the axillary mammary fat pads with 1-mm³ pieces of tumor as previously described (18).

Hormone Treatments. Silastic capsules used in experiments were made from Silastic tubing (Dow Corning, Midland, MI) 0.125 inch outer diameter x 0.078 inch inner diameter cut to various sizes and filled with tamoxifen-free base (Stuart Pharmaceuticals, Wilmington, DE) or RU 39,411 (Roussel Uclaf, Romainville, France). Ends were plugged with Silastic cement (Dow Corning). Estradiol capsules were cut to a 1.0-cm size and filled with a matrix consisting by weight of three parts Silastic medical-grade Elastomer (Dow Corning) and one part estradiol (Sigma Chemical Co., St. Louis, MO). All capsules were sterilized by γ-irradiation prior to s.c. implantation on the back of animals with a trochar. Placebo capsules were made of various sizes of empty tubing plugged at both ends. Where indicated, RU 39,411 and ICI 164,384 (provided by Roussel Uclaf) were injected in a 0.1-ml volume s.c. as a fine suspension. This suspension was made by dissolving the compounds in ethanol, mixing in Tween 80 (Sigma Chemical; 1:1 dilution), and adding a 1:10 dilution of isotonic saline to precipitate the compound. This approach was necessary because of the poor bioavailability of ICI 164,384. Silastic capsules of ICI 164,384 do not release the drug.

Tumor Measurements. Tumor measurements were performed weekly using calipers. Tumor area was calculated using the formula

\[ A = \frac{1}{2} \times w \times x \]

Statistical Analysis. Differences in mean tumor area between groups were measured using analysis of variance followed by the unpaired Student t test.
TAMOXIFEN-STIMULATED TUMOR GROWTH

Fig. 1. Tamoxifen and two steroidal antiestrogens, RU 39,411 and ICI 164,384.

Fig. 2. MCF-7TAM tumor growth in athymic mice (n = 6) treated with TAM Silastic capsules of varying sizes (2.0 cm, □; 1.0 cm, ▲; 0.25 cm, ◦; or with an empty 1.0-cm capsule (placebo), ○). Points, mean; bars, SE.

Fig. 3. MCF-7TAM tumor growth in athymic mice (n = 8) treated with 2.0-cm TAM Silastic capsules continuously (▲). Groups of athymic mice bearing MCF-7TAM tumors (n = 8) had TAM capsules removed as indicated by arrows after 6 wk of therapy (○) or 11 wk of therapy (□). Points, mean; bars, SE.

Fig. 4. MCF-7TAM tumor growth in athymic mice (n = 10) treated with 2.0-cm TAM Silastic capsules (▲), 1.0-cm estradiol Silastic capsules (●), or with an empty 1.0-cm placebo capsule (○). Points, mean; bars, SE.

RESULTS

TAM-dependent Growth of MCF-7TAM in Athymic Mice. In these experiments, the dose dependence of MCF-7TAM for TAM was assessed. Animals implanted with MCF-7TAM were also implanted with TAM-containing Silastic capsules of varying sizes (0.25 cm, 1.0 cm, 2.0 cm). Serum levels for these sizes of TAM capsules were established to be in a range of 10 to 15 ng/ml for the 0.25-cm capsules, 18 to 24 ng/ml for 1.0-cm capsules, and 28 to 35 ng/ml for the 2.0-cm capsules.4 No growth is seen by 10 wk in tumor-bearing animals with placebo capsules (Fig. 2). In contrast, mean tumor areas were 1.57 ± 0.16 cm² for the 2.0-cm TAM capsule, 1.03 ± 0.20 cm² for the 1.0-cm TAM capsule, and 0.52 ± 0.06 cm² for the 0.25-cm TAM capsules. Thus, there is a TAM dose-dependent increase in tumor growth for this range of capsules.

To establish the effect of TAM withdrawal on established MCF-7TAM tumors, animals bearing MCF-7TAM were treated continuously with 2.0-cm TAM capsules or had TAM capsules removed at 6 and 11 wk of therapy. Animals maintained continuously on TAM showed a sustained tumor growth that was 1.94 ± 0.33 cm² after 20 wk of therapy. When TAM was removed after 6 wk of therapy, tumor stasis was achieved at 0.43 ± 0.15 cm². TAM removal at 11 wk slowed tumor growth to 1.19 ± 0.25 cm² at 20 wk which was significantly different from continuously TAM-treated animals (P < 0.03). It therefore appears that these MCF-7TAM tumors require TAM administration for sustained growth.

In the next experiment, a comparison was made to determine the ability of either estradiol or tamoxifen to cause growth of MCF-7TAM tumors in athymic mice. Animals implanted with MCF-7TAM tumors were treated with either 2.0-cm TAM capsules or 1.0-cm estradiol capsules (Fig. 4). The estradiol capsules were shown to give a steady-state release of approximately 350 pg/ml of estradiol serum in mice (22). At the end of 12 wk of therapy, the mean ± SE of tumor sizes (n = 12) was 1.26 ± 0.23 cm² for the TAM-treated group and 1.21 ± 0.21 cm² for the estradiol-treated group. Thus, physiological concentrations of estrogen found in premenopausal women could stimulate this MCF-7TAM variant to grow in athymic mice.

Ability of Novel Steroidal Antiestrogens to Inhibit TAM-stimulated MCF-7TAM Growth. In these experiments, we wished to determine whether selected antiestrogens could inhibit TAM-stimulated growth of the MCF-7TAM variant. All animals were implanted with MCF-7TAM tumor and coimplanted with 0.5-cm TAM capsules. A low dose of TAM was used to determine whether the test compounds would have inhibitory activity under optimal conditions. The placebo group and RU 39,411-alone animals were implanted with empty 0.5-cm capsules. Growth of tumors in animals with only TAM capsules was sustained to a mean of 0.78 ± 0.08 cm² after 8 wk of therapy (Fig. 5). RU 39,411 injected every other day dra-

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Novel steroidal antiestrogens have been described in the literature (30, 31). One of these compounds, ICI 164,384, has been shown by Wakeling and Bowler (30) to be less estrogenic than TAM in this same assay.5 This compound and TAM have been shown to inhibit TAM-stimulated growth in athymic mice (19, 20). Indeed, there is a recent report (25) that ICI 164,384 does not stimulate MCF-7TAM tumors to grow and acts as an inhibitory agent. ICI 164,384 also did not increase uterine wet weight of these animals above controls (data not shown).

Finally, we investigated whether one of these novel antiestrogens could also inhibit the estradiol-stimulated growth of this variant of MCF-7 or whether this inhibition was specific to TAM alone (Fig. 7). RU 39,411 inhibited estradiol-stimulated growth by continuous administration of drug with a 2.0-cm Silastic capsule. Animals treated with 1.0-cm estradiol capsules for 12 wk showed a sustained tumor growth of 1.21 ± 0.21 cm² (mean ± SE) compared to a placebo growth of 0.11. The addition of a 2.0-cm RU 39,411 capsule to this dose of estradiol maintained the mean size of tumors at 0.44 ± 0.12 cm² ($P < 0.001$). RU 39,411 alone produced an increase in tumor cross-sectional area (0.40 ± 0.08 cm²) compared with controls ($P < 0.01$). Thus, it appears that RU 39,411 can inhibit either TAM- or estradiol-stimulated tumor growth.

DISCUSSION

The failure of adjuvant TAM therapy for breast cancer is an important pharmacological problem because by the end of this century, more than half a million women will be undergoing long-term treatment in the United States alone. It is therefore necessary to define possible methods of therapeutic failure of TAM in the laboratory to develop potential strategies or novel agents to treat the disease. It is possible that resistance to TAM may be because the tumor perceives TAM as an estrogen. The exact mechanism for this effect is unknown, but it is possible that TAM is metabolized to estrogens in the tumor cells or there are subtle alterations in the form of the ER. In fact, the ER level appears to be elevated in MCF-7TAM tumors (18), and this could be responsible for the hypersensitivity of the tumors to the estrogenic properties of TAM. Possible mechanisms for the development of TAM resistance have recently been reviewed (23). Several examples of the stimulatory properties of TAM have been seen in the normal uterus of humans, rats, and mice (9, 24). Similarly, TAM has been shown to stimulate the growth of human endometrial tumors implanted into athymic mice (19, 20). Indeed, there is a recent report (25) to suggest that long-term (up to 5 yr) adjuvant tamoxifen therapy for breast cancer may facilitate the appearance of invasive endometrial carcinoma.

Human breast cancer cell lines can be stimulated (weakly) to grow in culture in the presence of low concentrations of tamoxifen (26-29), and long-term therapy with TAM encourages the growth of MCF-7TAM tumors in athymic mice (17, 18). Whether this phenomenon will be demonstrated as a reason for TAM failure in the clinics must await the evaluation of the long-term TAM studies.

Novel steroidal antiestrogens have been described in the literature (30, 31). One of these compounds, ICI 164,384, has been shown by Wakeling and Bowler (30) to be less estrogenic in the mouse and rat uterus than TAM (24). We have shown RU 39,411 to be less estrogenic than TAM in this same assay.5 Both of these compounds had the ability to inhibit TAM-stimulated growth to a level achieved by the compound alone and that RU 39,411 showed slight agonist activity in this model.

In a parallel experiment, animals were treated in a similar manner using ICI 164,384 at an identical dosage (Fig. 6). After 8 wk of therapy, ICI 164,384 significantly inhibited TAM-stimulated growth of a 0.5-cm TAM capsule ($P < 0.001$). The mean tumor growth of TAM plus ICI 164,384 was 0.24 ± 0.04 cm² after 8 wk. Interestingly, the group treated with ICI 164,384 alone had a mean growth of 0.15 ± 0.01 cm² which was almost identical to control growth (0.13 cm²). Therefore, it appears that ICI 164,384 does not stimulate MCF-7TAM tumors to grow and acts as an inhibitory agent. ICI 164,384 also did not increase uterine wet weight of these animals above controls (data not shown).

Finally, we investigated whether one of these novel antiestrogens could also inhibit the estradiol-stimulated growth of this variant of MCF-7 or whether this inhibition was specific to TAM alone (Fig. 6). Mice were treated continuously with 0.5-cm Silastic capsules of TAM alone (●), TAM in 0.5-cm capsule and 1 mg of RU 39,411 injected s.c. every other day (Δ), 1 mg of RU 39,411 injected s.c. every other day alone (△), or with an empty 0.5-cm placebo capsule (○). Points, mean; bars, SE.
would have to be used. Indeed this may, in part, be the mechanism for the successful second-line use of aminoglutethimide as an endocrine therapy [14–16].

Since the MCF-7TAM tumor has ER [18], we suggest that these effects are mediated through this mechanism. Nevertheless, these data should be viewed with caution. The results may also represent a modulation of the host/tumor environment. Further studies to define the applicability of this tumor-model system to study TAM-stimulated growth are currently under way in this laboratory.

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