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Antitumor Actions of Keoxifene and Tamoxifen in the *N*-Nitrosomethylurea-induced Rat Mammary Carcinoma Model[1]

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ABSTRACT

We have compared the antitumor activities of the antiestrogens, keoxifene (LY 156758) and tamoxifen (TAM), using the *N*-nitrosomethylurea (NMU) rat mammary carcinoma model.

To establish an effective antitumor dose of TAM in this model, rats were treated 2 wk after initiation with NMU for 8 wk with s.c. daily injections of 6.25 μg, 25 μg, or 100 μg of TAM in peanut oil. At the 25-μg and 100-μg daily doses, TAM completely inhibited tumor appearance during the therapy period. An effective antitumor dose of TAM (100 μg daily) was compared to 20-, 100-, or 500-μg daily doses of keoxifene 7 wk after NMU initiation. None of the keoxifene-treated groups prevented the appearance of tumors as effectively as TAM during 13 wk of therapy. When keoxifene was compared to TAM at equivalent daily doses of 100 and 500 μg daily starting 2 wk after NMU injection, the keoxifene groups again failed to prevent the appearance of all tumors during 10 wk of therapy. TAM, however, completely suppressed any tumor formation.

In the same experiment, animals treated with 500 μg of TAM had therapy stopped after 10 wk, and tumors started to appear 6 wk later. No tumors appeared when animals (n = 25) were treated continuously for 23 wk with 100 μg of TAM.

In separate experiments, keoxifene (500 μg daily) and TAM (500 μg and 100 μg daily) administered for 1 wk blocked the binding of [^{3}H]-estradiol in NMU tumors and in uteri. The effect lasted for up to 5 wk after antiestrogen therapy was stopped.

These experiments demonstrate that keoxifene is not as effective in its antitumor action as TAM in the NMU model.

INTRODUCTION

Earlier experiments using the DMBA[3]-induced rat mammary carcinoma model demonstrated that 1 mo of TAM therapy (started 1 mo after carcinogen administration) can inhibit the induction of tumors in a dose-dependent manner (1). Furthermore, these studies showed that an almost complete inhibition of tumor appearance occurred with a 4-mo continuous TAM treatment schedule compared with 1 mo of therapy (1). Unfortunately, DMBA-induced rat mammary tumors are predominantly dependent upon prolactin for growth, which contrasts with the estrogen dependency of human breast cancer cells (2, 3). Therefore, the potential relevance for the treatment of breast cancer may be questionable, although the DMBA model shows that TAM is a tumoristatic agent. Interestingly though, studies *in vitro* with the human breast cancer cell line, MCF-7, also provide supportive evidence that TAM is a tumoristatic rather than a tumoricidal agent (4, 5). Similarly, pilot clinical data on long-term (>5 yr) adjuvant therapy with TAM demonstrate an advantage for these patients when compared with short-term adjuvant therapy (6). However, as yet only the impact of short-term TAM administration (2 yr) has been studied in randomized trials (7).

In the present study, we had two principal aims. (*a*) We wished to provide further laboratory support for clinical studies in the use of long-term TAM therapy. We selected the NMU-induced rat mammary tumor model as our experimental system because it has proved to be more estrogen dependent than the DMBA model (8, 9). Furthermore, earlier reports demonstrated that tumor growth can be inhibited by antiestrogens (10). (*b*) We wished to evaluate the antitumor activity of a new antiestrogen, keoxifene (LY 156578) (Fig. 1), which has a high binding affinity for the estrogen receptor and a low reported level of estrogenic activity (11). These properties of keoxifene may avoid some of the potential estrogen-related clinical side effects (thromboembolic disorders) that may arise with the use of long-term TAM adjuvant therapy (6, 12, 13). It should, however, be pointed out that an earlier study that used the DMBA tumor model demonstrated an activity for keoxifene and structurally related high affinity antiestrogens to be equal to or less than that of TAM (14–16). However, in the present study, we sought to delay tumor appearance rather than to cause the regression of established mammary tumors.

MATERIALS AND METHODS

Treatment of Rats

NMU was purchased from ICN Pharmaceuticals, Inc., Plainview, NY. An aqueous solution of 10 mg/ml was made by wetting NMU powder with 3% acetic acid and dissolving in distilled water (17). Fresh solutions of NMU were injected within 45 min.

Virgin female Sprague-Dawley rats (King Labs, Oregon, WI) were given injections i.v. at 50 and 57 days of age with 5 mg of NMU/100 g of body weight (18). Rats were randomized 2 wk later for all experiments. Tamoxifen, a gift from Stuart Pharmaceuticals, Wilmington, DE, was dissolved in ethanol, and the required volume was added to peanut oil. The ethanol was evaporated with gentle mixing under a stream of nitrogen. Doses in 0.1 ml of peanut oil were administered daily by s.c. injection. Keoxifene (LY 156578), a gift from the Eli Lilly Company, Indianapolis, IN, was injected s.c. daily as a suspension in 0.1 ml of peanut oil.

Rats were housed during the experimental period in AALAS-accredited facilities and fed Purina rat chow and water *ad libitum*. All animals were palpated weekly, and in some groups tumor sizes were measured using calipers. Tumor area was calculated using the formula

$$\pi \times \frac{L}{2} \times \frac{w}{2}$$

Three separate experiments were undertaken to determine the efficacy of the antiestrogens as antitumor agents. The protocols are summarized in Table 1.

Experiment 1: Tamoxifen Dose Response. Two wk after the second injection of NMU, rats were divided into groups of 15 animals and treated for 8 wk with daily s.c. injections of 6.25, 25, or 100 μg of tamoxifen. The control group received peanut oil.

Experiment 2: Tamoxifen *versus* Keoxifene Dose Response. Seven wk after the second injection of NMU, rats were divided into groups of 15 animals and treated for 13 wk with 100 μg of TAM and 20, 100, and 500 μg of keoxifene. The control group received peanut oil alone.

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[3] The abbreviations used are: DMBA, dimethylbenzanthracene; TAM, tamoxifen; NMU, *N*-nitrosomethylurea; AALAS, American Association for Laboratory Animal Science; ER, estrogen receptor; MHT, monohydroxytamoxifen; AEBS, antiestrogen binding site.

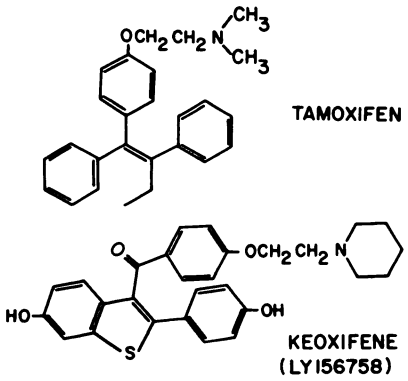

Fig. 1. Chemical structures of tamoxifen and keoxifene (LY 156758).

Table 1 *Experimental protocols to study the antitumor effects of keoxifene and TAM in the NMU-induced rat mammary carcinoma model*

Experiment	Compounds (daily doses/μg)	Treatment periods after NMU (wk)	
		Range	Duration
1	TAM (6.25) TAM (25) TAM (100)	2–10	8
2	TAM (100) Keoxifene (20) Keoxifene (100) Keoxifene (500)	7–20	13
3	TAM (100)	2–25	23
	TAM (500)	2–12	10
	Keoxifene (100)	2–12	10
	Keoxifene (500)	2–12	10

Experiment 3: Keoxifene *versus* Tamoxifen Long Term. Two wk after the second injection of NMU, rats were divided into 4 groups of 25 animals and given injections daily for 10 wk with 100 and 500 μg of keoxifene or 100 and 500 μg of TAM. The control group received peanut oil alone. The group treated daily with 100 μg of TAM was continued for 23 wk.

Duration of Action of Tamoxifen and Keoxifene in Rat Mammary Tumors and Uteri

NMU tumor-bearing rats (14 wk after final NMU injection) with one tumor ≥2 cm^2 were randomized into groups of 20 animals. Rats were given injections daily for 1 wk with either 100 μg of TAM or 500 μg of keoxifene. Controls received peanut oil alone. Five animals from each group were sacrificed at the end of treatment (0 wk) and 1, 3, and 5 wk later. Tumors were excised and stored at −70°C for estrogen receptor determination. Uterine wet weights were also noted for each animal.

Six-mo-old virgin female untreated rats were ovariectomized under ketamine anesthesia. Animals were randomized 2 wk later into groups of 20 animals. Groups received daily injections for 1 wk of 100 and 500 μg of TAM and 100 and 500 μg of keoxifene. Controls received peanut oil alone. Five animals were sacrificed at the end of therapy (0 wk) and 1, 3, or 5 wk later. Uteri were excised and frozen at −70°C for estrogen receptor determinations.

Estrogen Receptor Assays

NMU tumors were minced and homogenized in glass tubes with Teflon-coated pestles in a 9:1 (w/v) dilution with 10 mM Tris buffer (pH 7.4) (Sigma) containing 1 mM EDTA (Sigma) and 0.5 mM dithiothreitol (Sigma). Uteri were homogenized using a Polytron tissue homogenizer with three 10-s bursts with the same Tris buffer. All tissue was kept on ice during these procedures. Tissue homogenates were spun at 100,000 × *g* for 1 h to produce cytosols. An amount of 0.2 ml of cytosols which gave a 1- to 2-mg/ml protein concentration was used in each tube of the single-point binding assays. For the ER assay (done in triplicate), a 5 nM final concentration of [2,4,6,7-^{3}H]estradiol (New England Nuclear) was used with and without a 100-fold excess of cold estradiol (Sigma).

Unoccupied sites were determined by incubation of the mixtures for 16 h at 4°C. In order to adsorb free ligands, 1 ml of dextran-coated charcoal (0.25%) (Wien Laboratories) was added to the incubate.

Statistical Analysis of Data

Differences in the significance of tumor incidence between groups were determined using Fisher's exact test. Differences in average numbers of tumors per group were determined by analysis of variance and the unpaired Student *t* test. Analysis of receptor data and uterine weights was done using the Mann-Whitney rank test.

RESULTS

Experiment 1:Tamoxifen Dose Response. In the first experiment, varying doses of TAM were injected daily to test for antitumor activity. Cumulative tumor incidence (Fig. 2) was 92% at wk 20 in the control group compared to 67% in the 6.25-μg TAM treatment group ($P < 0.01$ from control). No tumors appeared in the 25- and 100-μg groups during therapy, and these groups had a final tumor incidence of 30% and 26% ($P < 0.001$ from control), respectively. Although the 6.25-μg TAM dose did not prevent tumor breakthrough during the treatment schedule, there was a 47% reduction ($P < 0.02$ from control) in the average number of tumors per animal (Fig. 3) compared to controls.

Experiment 2: Tamoxifen *versus* Keoxifene Dose Response. Unlike the first experiment where tamoxifen therapy was started 2 wk after NMU, all drug treatments were started 7 wk after NMU. The aim was to determine the activity of keoxifene under severe experimental conditions. The effect on tumor incidence with daily doses of 20, 100, or 500 μg of keoxifene was compared to a 100-μg daily dose of TAM (Fig. 4). TAM treatment almost completely suppressed the appearance of mammary tumors; 2 tumors appeared on 15 animals during therapy. In contrast, keoxifene was less effective in its antitumor activity. The cumulative tumor incidence for animals in the keoxifene treatment groups was 46% for the 100-μg group and 53% for the 500-μg group ($P < 0.01$ from controls). The group

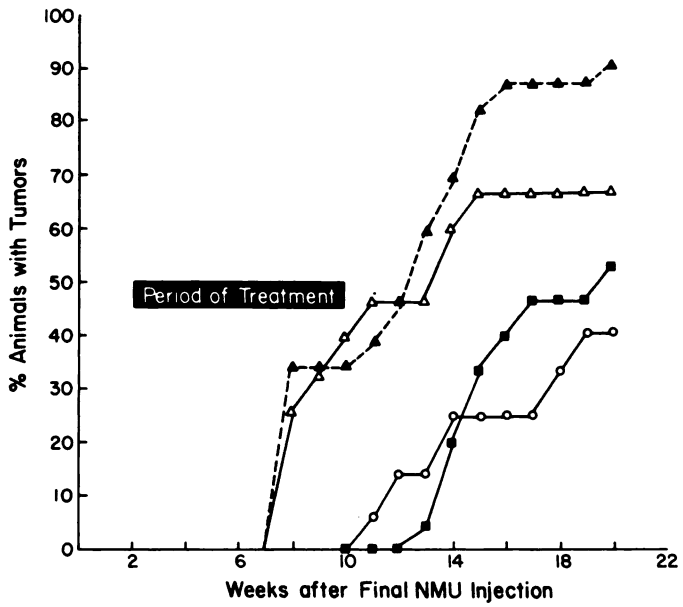

Fig. 2. Rat mammary tumor incidence in groups of rats (n = 15 per group) treated with tamoxifen for 8 wk, from wk 2 to wk 10, after final NMU injection. Vehicle (▲); TAM at 6.25 μg (△), 25 μg (■), and 100 μg (○).

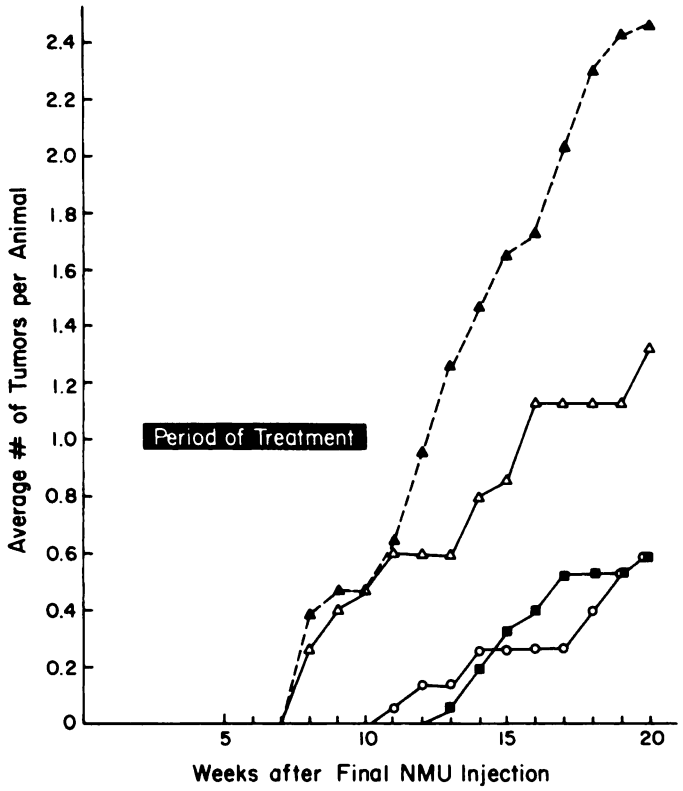

Fig. 3. Average number of tumors/rat after 8 wk of TAM treatment. Vehicle (▲); TAM at 6.25 μg (△), 25 μg (■), and 100 μg (○).

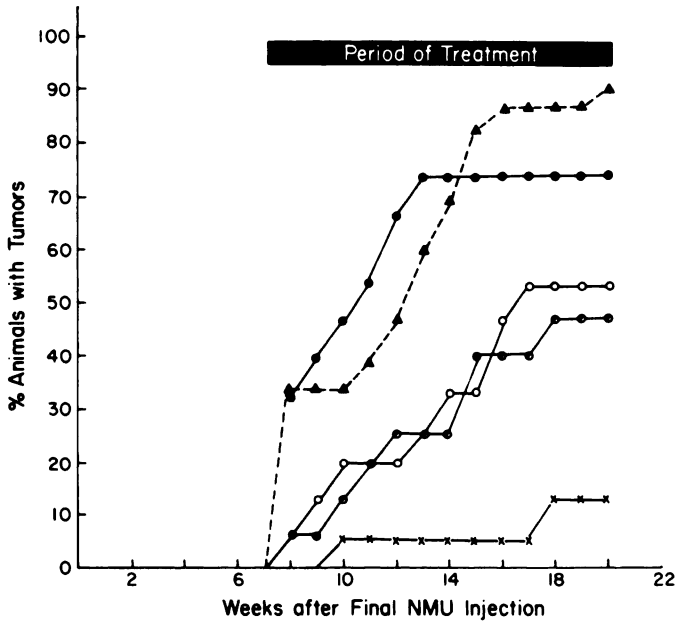

Fig. 4. Rat mammary tumor incidence in groups of rats (n = 15 per group) treated with tamoxifen or keoxifene for 13 wk from wk 7 to wk 20 after final NMU injection. Vehicle (▲); TAM at 100 μg (×); keoxifene at 20 μg (●), 100 μg (◐), and 500 μg (○).

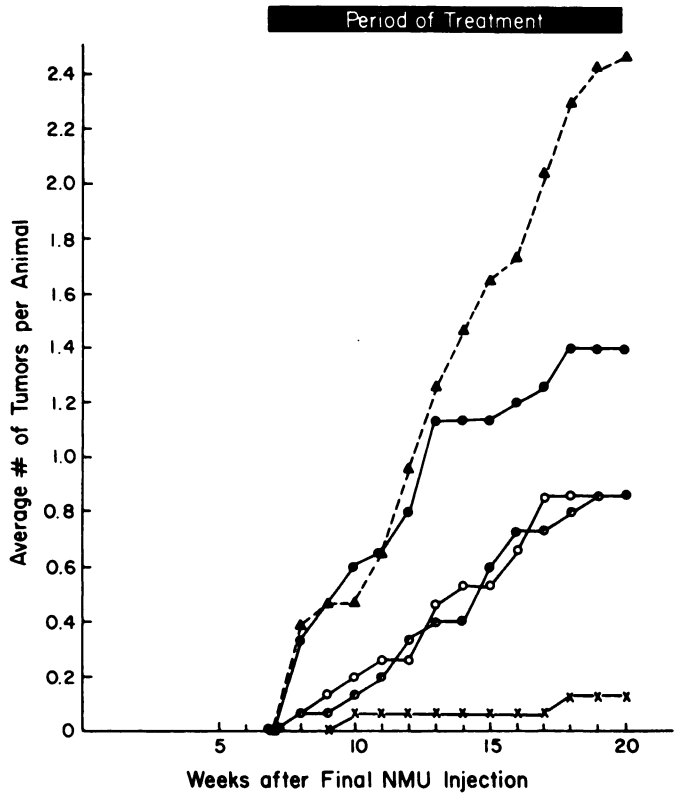

Fig. 5. Average number of tumors/rat after 13 wk of TAM or keoxifene. Vehicle (▲); TAM at 100 μg (×); keoxifene at 20 μg (●), 100 μg (◐), and 500 μg (○).

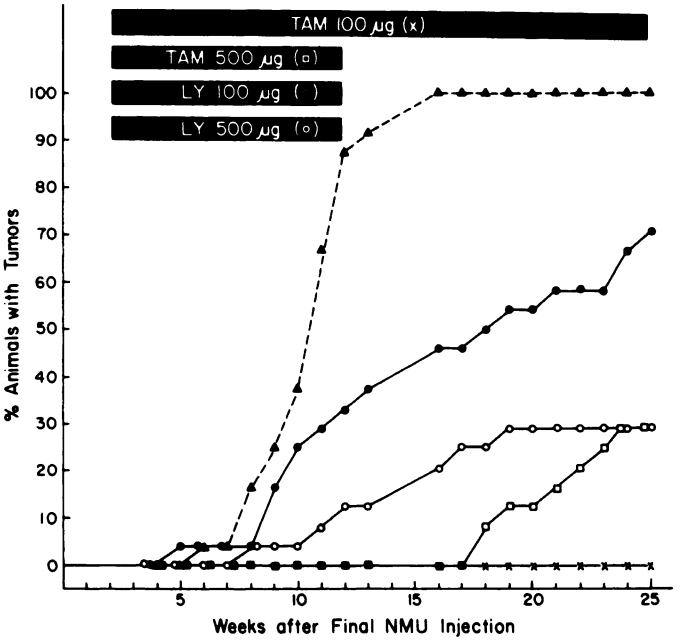

Fig. 6. Rat mammary tumor incidence in groups of rats (n = 24 per group) treated as shown. Vehicle (▲); TAM at 100 μg (×), 500 μg (□); keoxifene at 100 μg (●), 500 μg (○).

of rats treated with the lowest dose of keoxifene (20 μg) had a tumor appearance rate similar to controls; however, there was a 43% drop in the average number of tumors per animal compared to controls ($P < 0.03$) (Fig. 5).

Experiment 3: Keoxifene *versus* Long-Term Tamoxifen Treatment. There were two aims in this experiment: (*a*) to determine the effect of either high-dose (500 μg) TAM therapy for 10 wk *versus* continuous (23 wk) low-dose (100 μg) TAM therapy and (*b*) to determine the effects of 10 wk of keoxifene therapy starting 2 wk after NMU in light of the failure of the therapy starting at 7 wk.

Continuous TAM treatment with either dose completely inhibited the appearance of mammary tumors. However, when therapy with TAM (500 μg daily) was stopped, these animals began to develop tumors 6 wk later. Eventually this group had a cumulative tumor incidence of 28% (Fig. 6). In contrast, no tumors appeared in animals treated with TAM (100 μg daily)

for 23 wk. Keoxifene was not as effective as TAM at stopping the appearance of mammary tumors. Tumors appeared during therapy, and the final tumor incidence was 29% and 71% for the 500- and 100-μg doses of keoxifene, respectively. Animals in the control group reached 100% tumor incidence at wk 15 of the experiment. Keoxifene at the 100-μg dose, however, was effective in reducing average number of tumors per animal by 4-fold compared to controls ($P < 0.001$) (Fig. 7).

Ovariectomies were performed on control animals and in tumor-bearing animals from the keoxifene groups where tumor had achieved >3.0 cm^2. This was to assess tumor hormone dependency. Fourteen of 16 tumors regressed in control animals, while 3 of 4 tumors regressed in the 500-μg TAM-treated group. In animals where tumors appeared during treatment with keoxifene, 4 of 5 tumors regressed (data not shown). This indicated an incomplete antihormonal action of the drug.

Duration of Action of Tamoxifen and Keoxifene in Rat Mammary Tumors and Uteri. The next set of experiments studied duration of the blockade of TAM or keoxifene on the estrogen receptor in target tissue. NMU tumor-bearing rats were given 100 μg of TAM or 500 μg of keoxifene for 1 wk, as described in "Materials and Methods." Keoxifene completely blocked the measurement of NMU tumor ER at the level of sensitivity of the assay (10 fmol/mg of protein) for up to 5 wk after cessation of therapy (Table 2). TAM similarly blocked ER levels in this competitive ligand binding assay; however, 3 wk after therapy was stopped, the tumor ER levels were detectable. These values were significantly lower than controls ($P < 0.02$) for up to 5 wk after cessation of therapy.

Both antiestrogens lowered the uterine wet weight compared to control; however, only in the uterine weights of the keoxifene-treated animals was this difference significant ($P < 0.04$) (Table 3). This is in accordance with keoxifene's lower estrogenic effects in the uterus compared with TAM. Five-day monitoring of vaginal smears indicated no real difference in estrous cycles between control and antiestrogen-treated groups. Over 50% of all animals had abnormal estrous cycles exhibited by constant presence of squamous cells in vaginal smears.

Ovariectomized nontumor-bearing animals were also treated with the same schedule of therapy to analyze the effect on hormone receptor status of the uteri of these animals (Table 4). Available ER was significantly lower ($P < 0.02$) in both the TAM-treated groups compared to controls and lower compared to keoxifene-treated groups 5 wk after cessation of therapy. ER values in the 100-μg keoxifene group increased significantly above both TAM treatment groups ($P < 0.02$) 1 wk after therapy. In contrast, the 500-μg dose of keoxifene produced similar ER values to TAM treatment groups up to 3 wk after therapy.

Overall, these experiments demonstrated that the antiestrogens could block ER in tumors and uteri during therapy and that the effects of the compounds took several weeks to wear off. There was no evidence that the compounds had too short a duration of action as antiestrogens and were unable to gain access to target tissues (*e.g.*, uterus and tumor).

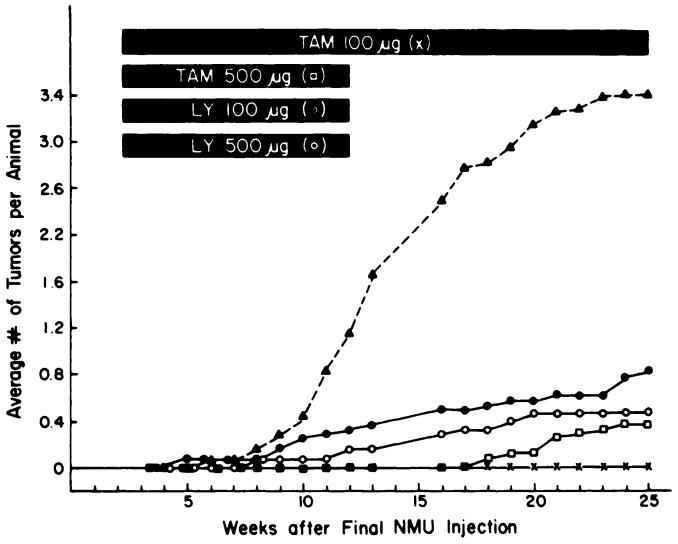

Fig. 7. Average number of tumors per rat. Vehicle (▲); TAM at 100 μg (×), 500 μg (□); keoxifene at 100 μg (●), 500 μg (○).

Table 2 *Estrogen receptor values in NMU-induced rat mammary tumors after 1 wk of treatment with antiestrogens*

Time 0 is the last day of treatment.

Group	Time after treatment (wk) 0	1	3	5
Control	221 ± 16[a,b]	96 ± 10	43 ± 11	68 ± 15
TAM (100 μg/day)	<10	<11	19 ± 7	26 ± 7[c]
Keoxifene (500 μg/day)	<10	<10	<10	10 ± 1[d]

[a] Mean ± SE (fmol/mg cytosol protein).
[b] $n = 10$.
[c] Significantly different from control ($P < 0.02$) by the Mann-Whitney rank test.
[d] Significantly different from control ($P < 0.0001$) by the Mann-Whitney rank test.

DISCUSSION

These studies demonstrate the efficacy of long-term TAM therapy in the NMU-induced rat mammary carcinoma model. We have shown that NMU-initiated rats treated continuously for 6 mo with TAM (100 μg daily) had no mammary tumors. Conversely, if animals were treated with a much larger dose of TAM (500 μg daily) for only 10 wk and therapy was stopped, tumors began to appear 6 wk later. This result concurs with preliminary clinical studies on the use of long-term TAM and provides more evidence for its advantage over short-term TAM regimens (6, 7). Although the DMBA model is less directly responsive to estrogen than the NMU model, both models

Table 3 *Uterine wet weight in mg of NMU tumor-bearing animals after 1 wk of antiestrogen treatment*

Time 0 is the last day of treatment.

Group	Time after treatment (wk) 0	1	3	5
Control	743 ± 121[a,b]	603 ± 80	536 ± 48	651 ± 147
TAM (100 μg/day)	551 ± 88	417 ± 78	477 ± 101	535 ± 90
Keoxifene (500 μg/day)	345 ± 19[c]	366 ± 35[d]	397 ± 29	362 ± 21

[a] Mean ± SE.
[b] $n = 5$ per value.
[c] Significant from control ($P < 0.02$) by the Mann-Whitney rank test.
[d] Significant from control ($P < 0.04$) by the Mann-Whitney rank test.

Table 4 *Estrogen receptor values in ovariectomized rat uteri after 1 wk of antiestrogen treatment*

Time 0 is the last day of treatment.

Group	Time after treatment (wk) 0	1	3	5
Control	431 ± 48[a,b]	417 ± 32	574 ± 23	422 ± 23
TAM (500 μg/day)	33 ± 4[c]	36 ± 2	67 ± 2	135 ± 16
TAM (100 μg/day)	45 ± 5[c]	79 ± 6	51 ± 6	123 ± 18
Keoxifene (500 ng/day)	18 ± 3[c]	22 ± 5	91 ± 17	264 ± 25
Keoxifene (100 ng/day)	14 ± 6[c]	230 ± 21	233 ± 20	265 ± 36

[a] Mean ± SE (fmol/mg of cytosol protein).
[b] $n = 5$ per value.
[c] All antiestrogen-treated groups significant from control for all time points ($P < 0.02$) by the Mann-Whitney rank test.

produce similar results for the continuous action of TAM on the delay of tumor appearance and reduction in tumor yield (1, 15, 19).

A primary aim of current pharmaceutical research is to develop high-affinity, nonestrogenic antiestrogen to eliminate the estrogenic side effects that may be seen during long-term TAM therapy (6, 12, 13). The antiestrogen, keoxifene, was chosen as a likely candidate because of its weak estrogenic activity and potent antiuterotrophic activity in rat uterine wet weight tests (11, 20). Unfortunately, keoxifene proved to be only as effective as TAM in inducing regression of established DMBA tumors (14, 16). Similarly in our experience, keoxifene proved to be less effective than TAM in preventing tumor appearance at equivalent doses.

Notably, we observed that some tumors which appeared during keoxifene therapy regressed after ovariectomy and thus were hormone dependent. One possible explanation for this finding may be the shorter biological half-life of the keoxifene compared to TAM. The failure of high-potency antiestrogens has also been seen previously in the DMBA model with MHT (21). MHT and keoxifene (22, 23) both have a shorter biological half-life than TAM. However, despite this shorter half-life, keoxifene appears to occupy and block binding of [^{3}H]estradiol in our ligand binding assays for a duration similar to TAM.

Another plausible explanation for the failure of hydroxylated antiestrogens such as MHT and keoxifene may be because of a weaker binding affinity to the AEBS compared to TAM (24, 25). The AEBS, by sequestering antiestrogens inside cells, may influence concentrations of antiestrogens within cells. Thus, the increased capacity of TAM for the AEBS gives it a distinct advantage in maintaining intracellular drug concentrations over other hydroxylated antiestrogens, thereby contributing to its efficiency as an antitumor agent.

Recently, an antiestrogen with structural similarity to keoxifene, LY117018, was shown to increase serum prolactin levels in immature female rats by increasing the turnover of dopamine in the medial basal hypothalamus (26). In contrast, TAM in this system did not increase prolactin levels. Although this might contribute to keoxifene's inability to stop the appearance of rat mammary tumors, it should be noted that other studies have not seen a rise in serum prolactin levels in tumor-bearing animals treated with keoxifene (14).

It must therefore be conceded that the mechanism of action of keoxifene and TAM as antitumor agents is not known. Further studies are needed both *in vitro* and *in vivo* using human-derived mammary tumor cells to evaluate the antitumor effects of keoxifene fully.

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REFERENCES

1. Jordan, V. C., Allen, K. E., and Dix, C. J. Pharmacology of tamoxifen in laboratory animals. Cancer Treat. Rep., *64:* 745–749, 1980.
2. Sterental, A., Dominques, J. M., Weissman, C., and Pearson, O. H. Pituitary role in the estrogen dependency of experimental mammary cancer. Cancer Res., *23:* 481–484, 1963.
3. Arafah, B. M., Manni, A., and Pearson, O. H. Effect of hypophysectomy and hormone replacement on hormone receptor levels and the growth of 7,12-dimethylbenz(a)anthracene-induced mammary tumors in the rat. Endocrinology, *107:* 1364–1369, 1980.
4. Osborne, C. K., Boldt, D. H., Clark, G. M., and Trent, J. M. Effects of tamoxifen on human breast cancer cell cycle kinetics: accumulation of cells in early G_1 phase. Cancer Res., *43:* 3583–3585, 1983.
5. Sutherland, R. L., Green, M. D., Hall, R. E., Reddel, R. R., and Taylor, I. W. Tamoxifen induces accumulation of MCF-7 human mammary carcinoma cells in the G_0/G_1 phase of the cell. Eur. J. Cancer Clin. Oncol., *19:* 615–621, 1983.
6. Tormey, D. C., and Jordan, V. C. Long-term tamoxifen adjuvant therapy in node positive breast cancer: a metabolic and pilot clinical study. Breast Cancer Res. Treat., *4:* 297–302, 1984.
7. Baum, M., and other members of the "Nolvadex" Adjuvant Trial Organization. Controlled trial of tamoxifen as an adjuvant agent in the management of early breast cancer cycle. Lancet, *1:* 257–261, 1983.
8. Rose, D. P., and Noonan, J. J. Hormone dependence of rat mammary tumors induced by *N*-nitrosomethylurea. Eur. J. Cancer Clin. Oncol., *7:* 1347–1358, 1981.
9. Arafah, B. M., Finegan, H. M., Roe, J., Manni, A., and Pearson, O. H. Hormone dependency in *N*-nitrosomethylurea-induced rat mammary tumors. Endocrinology, *111:* 584–588, 1982.
10. Rose, D. P., Fischer, A. H., and Jordan, V. C. Activity of the antioestrogen trioxifene against *N*-nitrosomethylurea-induced rat mammary carcinomas. Eur. J. Cancer, *17:* 893–898, 1981.
11. Black, L. J., Jones, C. D., and Falcone, J. F. Antagonism of estrogen action with a new benzothiopene-derived antiestrogen. Life Sci., *32:* 1031–1036, 1983.
12. Helgason, S., Wilking, N., Carlstrom, K., Camber, M. D., and von Schoultz, B. A comparative study of the estrogenic effects of tamoxifen and 17β-estradiol in post-menopausal women. J. Clin. Endocrinol. Metab., *54:* 404–408, 1982.
13. Sakai, F., Cheix, F., Clavel, M., Colon, J., Mayer, M., Pommatau, E., and Saez, S. Increases in steroid binding globulins induced by tamoxifen in patients with carcinoma of the breast. J. Endocrinol., *76:* 219, 1978.
14. Clemens, J. A., Bennet, D. R., Black, L. J., and Jones, C. D. Effects of a new antiestrogen, keoxifene (LY 156578), on growth of carcinogen-induced mammary tumors and on LH and prolactin levels. Life Sci., *32:* 2869–2875, 1983.
15. Jones, C. D., Jevnikor, M. G., Pike, A. J., Peters, M. K., Black, L. J., Thompson, A. R., Falcone, J. F., and Clemens, J. A. Antiestrogens. 2. Structure-activity studies in a series of 3-aroyl-2-(4-hydroxyphenyl)benzo-[*b*]thien-3-yl][4-[2-(1-piperidinyl)ethoxy]phenyl]methanone hydrochloride (LY 156578), a remarkably effective estrogen antagonist with only minimal intrinsic estrogenicity. J. Med. Chem., *27:* 1057–1066, 1984.
16. Wakeling, A. E., and Valcaccia, B. Antioestrogenic and antitumor activities of a series of nonsteroidal antioestrogens. J. Endocrinol., *99:* 455–464, 1983.
17. Rose, D. P., Pruitt, B., Stauber, P., Erturk, E., and Bryan, G. T. Influence of dosage schedule on the biological characteristics on *N*-nitrosomethylurea induced rat mammary tumors. Cancer Res., *40:* 235–239, 1980.
18. Moon, R. C., Grubbs, C. J., Sporn, M. B., and Goodman, D. G. Retinyl acetate inhibits mammary carcinogenesis induced by *N*-methyl-*N*-nitrosourea. Nature (Lond.), *267:* 620–621, 1977.
19. Wilson, A. J., Tehrani, F., and Baum, M. Adjuvant tamoxifen therapy for early breast cancer: an experimental study with reference to oestrogen and progesterone receptors. Br. J. Surg., *69:* 121–125, 1982.
20. Jordan, V. C., and Gosden, B. Differential antiestrogenic action in the immature rat uterus: a comparison of hydroxylated antiestrogens with high affinity for the estrogen receptor. J. Steroid Biochem., *19:* 1249–1258, 1983.
21. Fromson, J. M., Pearson, S., and Bramah, S. The metabolism of tamoxifen (ICI 46,474). I. In laboratory animals. Xenobiotica, *3:* 693–709, 1973.
22. Jordan, V. C., and Allen, K. E. Evaluation of the antitumor activity of the nonsteroidal antioestrogen, monohydroxytamoxifen, in the DMBA-induced rat mammary carcinoma model. Eur. J. Cancer Clin. Oncol., *16:* 239–251, 1980.
23. Gallick, N. G., and Lindstrom, T. D. Disposition and metabolism of a new antiestrogen, LY 156758, in rats, dogs, and monkeys. Fed. Proc., *42:* 376, 1983.
24. Lyman, S. D., and Jordan, V. C. Possible mechanisms for the agonist actions of tamoxifen and the antagonist actions of MER-25 (ethamoxytriphetol) in the mouse uterus. Biochem. Pharmacol., *34:* 2795–2806, 1985.
25. Miller, M. A., and Katzenellenbogen, B. S. Characterization and quantitation of antiestrogen binding sites in estrogen receptor-positive and -negative human breast cancer cell lines. Cancer Res., *43:* 3094–3100, 1983.
26. Toney, T. M., and Katzenellenbogen, B. S. Antiestrogen action in the medial basal hypothalamus and pituitary of immature female rats: insights concerning relationships among estrogen, dopamine, and prolactin. Endocrinology, *119:* 2661–2669, 1986.