Cancer Chemother Pharmacol (1994) 34: 89–95

Cancer Chemotherapy and Pharmacology

The importance of tamoxifen metabolism in tamoxifen-stimulated breast tumor growth

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Received: 16 July 1993/Accepted 17 November 1993

Abstract. The acquired ability of tamoxifen to stimulate tumor growth has been suggested as one mechanism for the development of treatment failure in breast cancer. We have reported that tamoxifen-stimulated MCF-7 breast tumors in nude mice display reduced tamoxifen levels as compared with tamoxifen-inhibited tumors and an altered metabolite profile with isomerization of *trans*-4-hydroxytamoxifen to a weak antiestrogen and the production of metabolite E, an estrogenic metabolite. To investigate further the importance of tamoxifen metabolism in this model, we quantified levels of tamoxifen and major metabolites in tamoxifen-stimulated as compared with tamoxifen-inhibited MCF-7 tumors growing in nude mice and employed tamoxifen analogs resistant to metabolism. Tamoxifen-stimulated tumors have a relative abundance of *cis*-4-hydroxytamoxifen and metabolite E. However, in vivo treatment of mice carrying tamoxifen-stimulated tumors with fixed-ring nonisomerizable tamoxifen analogs or with nafoxidine, a nonsteroidal antiestrogen with a different structure, nonetheless resulted in tumor growth stimulation. Tumors were also stimulated by a deoxytamoxifen analog resistant to conversion to metabolite E. Growth of tamoxifen-stimulated tumors was inhibited by a pure steroidal antiestrogen, ICI 182,780, suggesting the need for clinical trials of this drug in patients with tamoxifen resistance. Growth of tamoxifen-stimulated tumors was further stimulated by estrogen replenishment, and this estrogen stimulation could be blocked by tamoxifen indicating that tamoxifen has both agonist and antagonist properties in these tumors. This study suggests that tamoxifen-stimulated tumor growth in this model is not due to isomerization or metabolism of tamoxifen to less antiestrogenic or more estrogenic metabolites. The mechanisms by which tamoxifen acquires more potent in vivo agonist properties, resulting in tumor growth stimulation over time, remain to be defined.

Introduction

The antiestrogen tamoxifen is the most frequently used drug for the treatment of breast cancer. Antagonism of estrogen-induced growth, thought to be its primary mode of action, is mediated by competitive blockade of the estrogen receptor. Although tamoxifen is effective in delaying recurrence and prolonging survival in the adjuvant setting and in inducing remission of patients with advanced breast cancer, its use is limited by the inevitable development of tamoxifen resistance [3, 17]. Selection of an estrogen receptor-negative and, thus, hormone-independent clone of cells occurs in some patients, but the mechanisms for the development of tamoxifen resistance in most patients remain obscure [4].

We have previously reported the development of an in vivo experimental model of tamoxifen resistance using estrogen receptor-positive MCF-7 human breast cancer cells growing as solid tumors in athymic mice [13, 14]. Tamoxifen suppresses tumor growth in this model for 4–6 months and then tumor growth resumes despite continued treatment. Resistance to the drug is not due to the emergence of a receptor-negative clone or to alterations in tamoxifen serum levels. Studies by Gottardis and Jordan [5], in addition to our own [14], have shown that this acquired tamoxifen "resistance" is related to the acquired ability of tamoxifen after several months of treatment to stimulate rather than to inhibit tumor growth. Pure steroidal antiestrogens inhibit this tamoxifen-stimulated tumor growth, suggesting that it is somehow related to the estrogenic effects of tamoxifen or its metabolites [6].

To investigate further the mechanism for tamoxifen-stimulated tumor growth in this model, we have previously

This work was supported in part by Cancer Center Support grants P30 CA 54174, P50 CA58183, and RO1 CA 30251 from the National Cancer Institute

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measured and compared levels of tamoxifen and several of its metabolites in both tamoxifen-inhibited and tamoxifen-stimulated tumors [14]. Tumor extracts from the latter are characterized by a 10-fold reduction in tamoxifen concentration and by isomerization of the potent antiestrogenic metabolite *trans*-4-hydroxytamoxifen, resulting in a relative increase in the less potent *cis* isomer in both cytosol and nuclear fractions of the tumor. Studies using a non-isomerizable analog indicate that *cis*-4-hydroxytamoxifen is a weak antiestrogen that has estrogen agonist properties at low concentrations in MCF-7 cells [10]. Furthermore, two estrogenic metabolites of tamoxifen, metabolite E and bisphenol (both lacking the dimethylaminoethoxy side chain necessary for antiestrogenic properties), have been found in extracts from these tumors [21]. A similar profile of tamoxifen and its metabolites has been reported by us in extracts from tamoxifen-resistant tumors from patients [15]. These data have led us to propose that one potential mechanism for tamoxifen resistance is a reduction in intracellular tamoxifen concentration together with isomerization and metabolism of the parent drug to less potent antiestrogenic or frankly estrogenic metabolites, resulting in tamoxifen-stimulated growth.

To investigate this hypothesis further, we quantified the levels of tamoxifen and several key metabolites in tumors from our nude mouse model. In addition, we employed analogs of tamoxifen that are resistant to isomerization and side-chain cleavage to determine the importance of tamoxifen metabolism in this experimental model of acquired resistance.

Materials and methods

Breast-cancer cell line. MCF-7 human breast cancer cells, passages 100–200, were used in these experiments. Tissue-culture methods have been described previously [12].

Athymic nude mouse model. Ovariectomized female BALB/c-nu+/nu+ mice aged 4–5 weeks were purchased from Harlan Sprague-Dawley (Madison, Wis.). Methods for housing and maintenance of the animals and for growing tumors from MCF-7 cell suspensions have been described in detail elsewhere [12, 13]. Tumors were grown s.c. in the axillary region of nude mice. Treatment with tamoxifen citrate (Zeneca Pharmaceuticals, Wilmington, Del.), 500 μg/mouse per day (Monday through Friday) s.c. in peanut oil, was begun when tumors had reached 8–12 mm in diameter. After 2–3 weeks, when tumors had stopped growing, some mice were killed by cervical dislocation, and tumors were harvested and frozen for later analysis. Tumors from these mice were considered "tamoxifen-inhibited." Other mice were killed 4–6 months later, when tumor progression was evident. These tumors were considered "tamoxifen-stimulated".

To determine the effect of tamoxifen analogs on tamoxifen-stimulated tumor growth, tumors were harvested at the time of tumor progression on tamoxifen and minced into 1-mm^3 fragments, which were transplanted s.c. into the axillary region of fresh recipient mice. Mice with transplanted tamoxifen-stimulated tumor fragments were then treated with the following: 0.25 mg 17β-estradiol pellet (Innovative Research, Rockville, Md.), *trans*-tamoxifen citrate given at 500 μg s.c. daily in peanut oil (Monday through Friday), one of the tamoxifen analogs given at the same dose and schedule, ICI 182,780 given at 5 mg s.c. weekly in oil, or peanut oil alone as a control. To determine if tamoxifen exhibits predominantly agonist versus antagonist properties in the tamoxifen-stimulated tumors, tumors were transplanted into recipient mice treated with maximal or submaximal doses of estrogen without or with tamoxifen. Tumor volumes were calculated twice weekly as described previously [13].

Antiestrogens. The structures of tamoxifen and the nonisomerizable seven-membered fixed-ring analogs used in these experiments are shown in Fig. 1. The seven-membered compounds, fixed in the *trans* configuration, were synthesized as described earlier [9]. They have a binding affinity for the estrogen receptor and an antiestrogenic potency in MCF-7 cells that is similar to that of the parent drug *trans*-tamoxifen [9–11]. A deoxytamoxifen analog (Fig. 1) that should be resistant to cleavage of the dimethylaminoethoxy side chain that produces metabolite E or bisphenol was provided by Zeneca Pharmaceuticals (Macclesfield, Cheshire, UK). Toremifene, a triphenylethylene antiestrogen similar to tamoxifen in structure and function, was a gift from Farmos (Turku, Finland) [18]. Nafoxidine, a diphenyldihydronaphthalene derivative structurally distinct from tamoxifen, was supplied by Upjohn (Kalamazoo, Mich.) [8]. The pure steroidal antiestrogen ICI 182,780, which appears to work through a mechanism different from that of tamoxifen by inhibiting DNA binding of the estrogen receptor, was also provided by Zeneca Pharmaceuticals [20].

High-performance liquid chromatographic assay of tamoxifen and metabolites. Tumors were weighed, homogenized, extracted with 2% hexane-butanol, and processed for high-performance liquid chromatography (HPLC) as previously described [14]. Conditions were optimized for sharp resolution of tamoxifen and various metabolites by changing the ionic strength with increasing concentrations of triethylamine. The sensitivity of the assay is approximately 2 ng/g tissue.

Results

Quantitative levels of tamoxifen and major metabolites in tamoxifen-inhibited versus tamoxifen-stimulated MCF-7 tumors

Similar to our previous report, levels of the parent drug tamoxifen were significantly reduced in tamoxifen-stimulated as compared with tamoxifen-inhibited tumors (Table 1) [14]. In general, tumor concentrations varied widely, but tamoxifen-stimulated tumors had a mean concentration of 998 ng/g tissue as compared with 15,780 ng/g tissue in tamoxifen-inhibited tumors (median values, 555 and 17,580 ng/g, respectively). The levels of *N*-desmethyltamoxifen and of the *cis* and *trans* isomers of 4-hydroxytamoxifen were also reduced in tamoxifen-stimulated tumors (Table 1). Confirming our previous report, the ratio of *cis* to *trans* was higher in stimulated tumors (1.2) than in inhibited tumors (0.6).

Metabolite E, an estrogenic metabolite lacking the dimethylaminoethoxy side chain, was also detected in both tumor groups, including tumors still being inhibited by tamoxifen (Table 1). We could not discriminate *trans* from *cis* metabolite E with this assay, and it cannot be excluded that the initially formed *trans* metabolite E generates some of the *cis* isomer under physiologic conditions, as can *trans*-4-hydroxytamoxifen but not *trans*-tamoxifen itself. However, the concentration of metabolite E relative to tamoxifen itself or to the potent antiestrogenic metabolite *trans*-4-hydroxytamoxifen was distinctly different in tumors that were being stimulated by tamoxifen treatment (Table 2). Although the absolute level of metabolite E was also reduced slightly in tamoxifen-stimulated tumors, it was relatively more abundant as compared with the parent drug

Table 1. Levels of tamoxifen and metabolites in tamoxifen-inhibited and tamoxifen-stimulated MCF-7 tumors

Metabolites	Tam-inhibited	Tam-stimulated	*P* value
Tam	15,780 ± 10,721	998 ± 1,099	0.001
N-des	280 ± 187	71 ± 41	0.066
cis-4-OH	104 ± 31	58 ± 24	0.016
trans-4-OH	162 ± 105	49 ± 23	0.001
Metabolite E	120 ± 92	49 ± 75	0.181

Mice with established MCF-7 tumors were started on treatment with tamoxifen (500 μg s.c. daily, Monday through Friday). When tumor growth had stopped 3 weeks later, 6 tumors were harvested for determination of tamoxifen and metabolite levels (tamoxifen-inhibited group). At 4–6 months thereafter, when tumor progression was evident, 10 tumors were harvested for assay (tamoxifen-stimulated group). Values are reported in ng/g tissue, mean ± SD
Tam, Tamoxifen; N-des, *N*-desmethyltamoxifen; 4-OH, 4-hydroxytamoxifen; values compared by the Wilcoxon rank-sum test

Table 2. Relative concentrations of tamoxifen, *trans*-4-OH-tamoxifen, and metabolite E

	Tam-inhibited	Tam-stimulated
Tam : metabolite E	132 : 1	20 : 1
trans-4-OH : metabolite E	1.4 : 1	1 : 1
N-des : metabolite E	2.3 : 1	1.4 : 1

The ratios of the mean values shown are derived from the tissue concentrations shown in Table 1

or the other metabolites. In fact, the tamoxifen-to-metabolite E ratio was 20: 1 in these tumors as compared with 132: 1 in tamoxifen-inhibited tumors. Very similar results were observed when the median of the individual tumor ratios was compared (33: 1 versus 136: 1). These differences were highly statistically significant ($P = 0.015$ by the Wilcoxon rank-sum test). Similar but less striking results were observed with *trans*-4-hydroxytamoxifen and *N*-desmethyltamoxifen. Whether these changes in the relative abundance of metabolite E are sufficient to reverse tamoxifen-inhibited growth is not clear from these data, but the recent observation that metabolite E is a full estrogen and is biologically active at a 100-fold lower concentration than tamoxifen suggests this possibility [7].

Effect of nonisomerizable tamoxifen analogs on in vivo growth of tamoxifen-stimulated tumors

The *cis* isomers of the parent drug tamoxifen and its metabolites display quite different biologic activities as compared with the *trans* isomers. *trans*-Tamoxifen is not known to isomerize under physiologic conditions, but since the *cis* isomer is a full estrogen with little or no antiestrogenic activity, any conversion to it could have adverse effects [16]. *trans*-4-Hydroxytamoxifen, a potent antiestrogen,

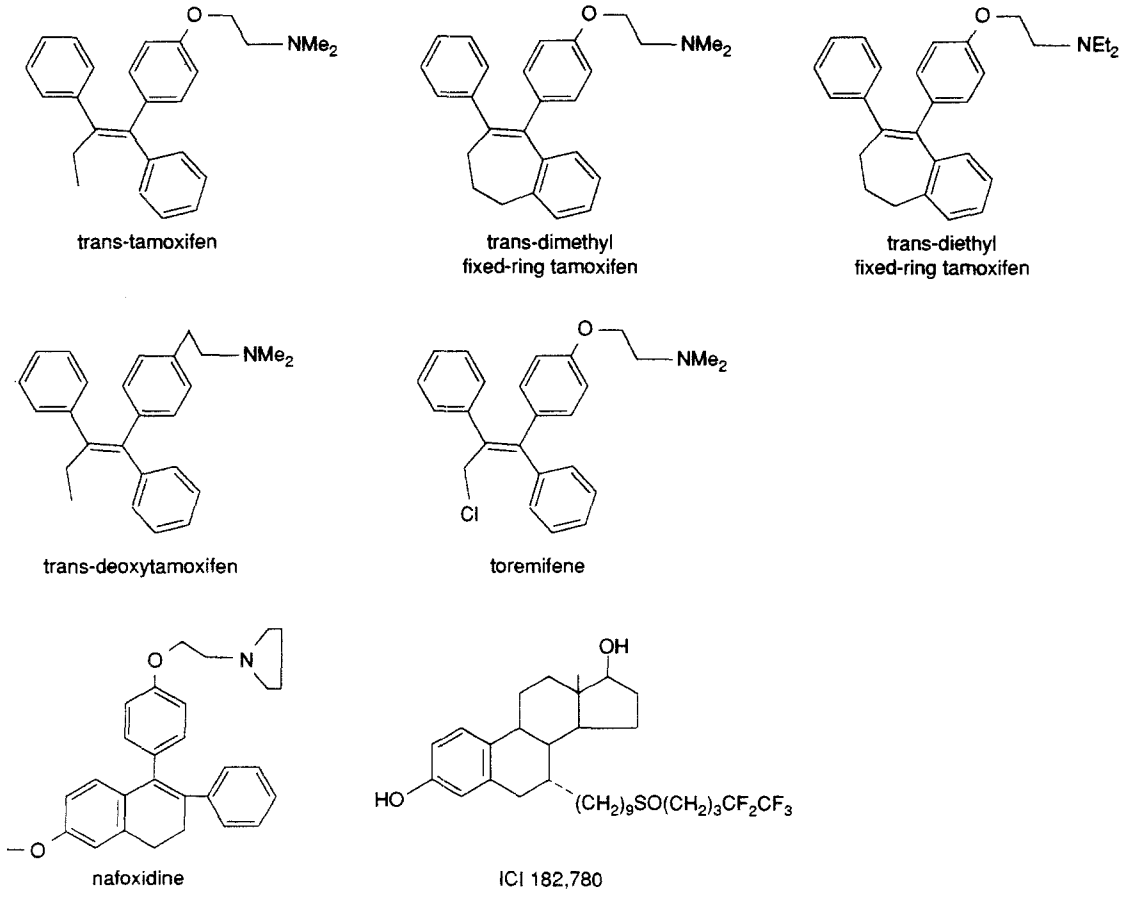

Fig. 1. Clinical structure of tamoxifen, tamoxifen analogs, and other antiestrogens

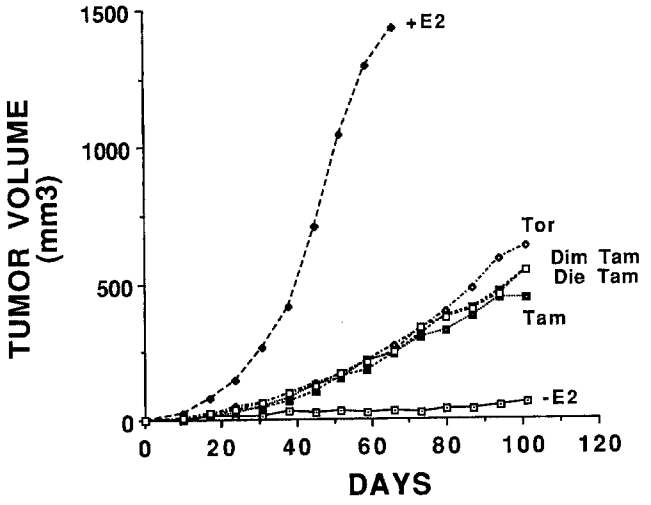

Fig. 2. Effect of nonisomerizable antiestrogens on growth of tamoxifen-stimulated tumors. Fragments from a tumor harvested during the tamoxifen-stimulated phase of tumor growth were transplanted s.c. behind the forelimb of female castrated nude mice. Groups of 10 mice each were treated with a 0.25-mg 17β-estradiol pellet s.c. ($+E_2$), toremifene (*Tor*), *trans*-dimethyl fixed-ring tamoxifen (*Dim*), *trans*-diethyl fixed-ring tamoxifen (*Die*), *trans*-tamoxifen (*Tam*), or peanut oil alone ($-E_2$). All antiestrogens were given at a daily dose of 500 μg s.c. in peanut oil on Monday through Friday. Values shown are the mean tumor volumes. The SE ranged from 5% to 15% of the mean

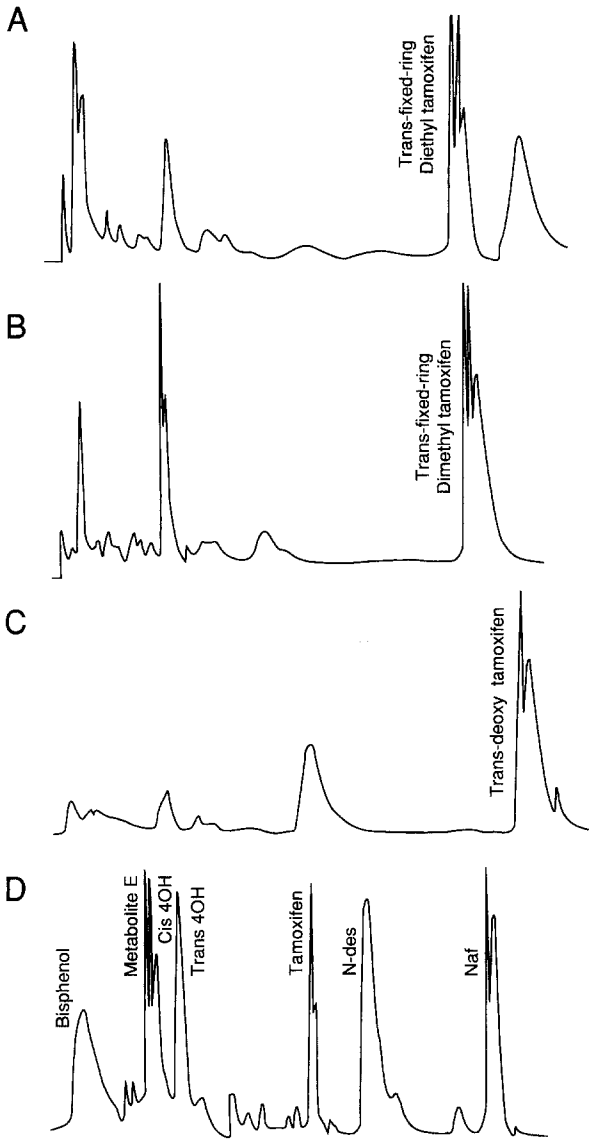

Fig. 3. Metabolites of tamoxifen analogs in tamoxifen-stimulated tumors. Extracts of tumors stimulated by *trans*-fixed-ring diethyl tamoxifen (**A**), *trans*-fixed-ring dimethyl tamoxifen (**B**), and *trans*-deoxytamoxifen (**C**) were analyzed by HPLC. **D** A tumor extract spiked with known standards

readily and spontaneously isomerizes to the *cis* isomer, a weak antiestrogen. To investigate further whether isomerization of tamoxifen or its metabolites is important for the development of tamoxifen-stimulated growth observed in our experimental model, we employed two fixed-ring nonisomerizable analogs of *trans*-tamoxifen (Fig. 1).

A tamoxifen-stimulated tumor from a mouse treated with tamoxifen for more than 6 months was excised and minced into 1-mm^3 fragments. Fragments were transplanted into recipient mice which were then divided into six treatment groups (Fig. 2). Fragments failed to form tumors when introduced into ovariectomized mice unless the mice received estrogen supplementation. As we have previously reported, tamoxifen treatment also stimulated tumor growth, although it was less effective than estrogen [14]. Toremifene, a triphenylethylene antiestrogen similar to tamoxifen, also stimulated tumor growth. Interestingly, both the dimethyl and diethyl fixed-ring analogs had a similar stimulatory effect on tumor growth. Under the same conditions, nafoxidine, another nonsteroidal antiestrogen that is structurally distinct from tamoxifen (Fig. 1), was just as potent as the other analogs in promoting growth of these tumors (not shown).

To ensure that the fixed-ring analogs remained intact in vivo, tumor extracts were analyzed by HPLC (Fig. 3A, B). A large peak corresponding to the parent drug was identified. In addition, several smaller, more polar peaks were also evident. The largest of these most likely represents the *trans*-4-hydroxymetabolite. In no case were doublet or two closely separated peaks identified that might suggest the presence of both the *cis* and the *trans* isomers, as we have previously reported with the 4-hydroxy metabolite of tamoxifen itself [14, 15]. Thus, the fixed-ring structure appeared to remain intact in vivo, preventing the formation of *cis* metabolites.

Effect of the deoxytamoxifen analog on in vivo growth of tamoxifen-stimulated tumors

Metabolite E and bisphenol are estrogenic metabolites of tamoxifen that are formed by cleavage of the dimethylaminoethoxy side chain. Due to the elimination of the oxygen atom and the presence of a carbon: carbon bond, the deoxytamoxifen analog (Fig. 1) should be relatively resistant to cleavage of this side chain. When mice transplanted with tamoxifen-stimulated tumors were treated with the deoxy analog, tumor growth stimulation nearly

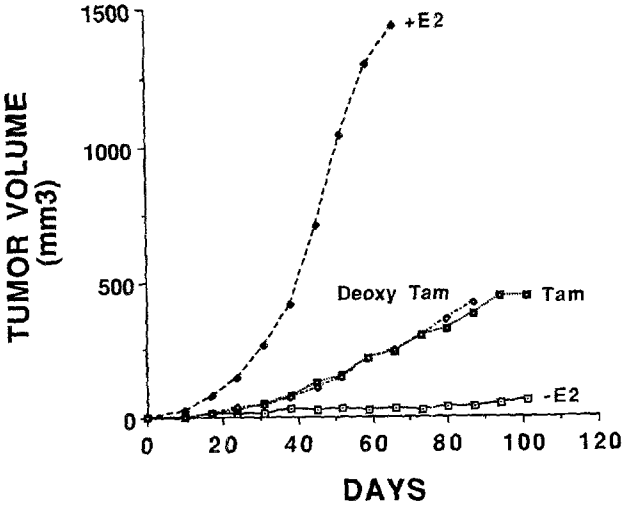

Fig. 4. Effect of deoxytamoxifen on tamoxifen-stimulated tumor growth. Mice (10/group) were treated as described in Fig. 2. Deoxytamoxifen (*Deoxy Tam*) and tamoxifen (*Tam*) were given at a dose of 500 μg/day s.c. on Monday through Friday. Values shown are the mean tumor volumes

identical to tamoxifen itself was observed (Fig. 4). When extracts of several of these tumors were analyzed by HPLC (Fig. 3C), no peak comigrating with the metabolite E or bisphenol standards could be detected, unlike our data obtained with tamoxifen itself [21]. Thus, the deoxytamoxifen analog, which is resistant to side-chain cleavage, was capable of stimulating growth of these tumors.

Effects of the pure steroidal antiestrogen ICI 182,780 on tamoxifen-stimulated tumor growth

Gottardis et al. [6] reported in their model that the pure steroidal antiestrogen ICI 164,384 blocked growth of a serially transplanted tamoxifen-stimulated tumor. We confirmed this observation using a new steroidal antiestrogen that is now in early clinical trial (ICI 182,780). Treatment of ovariectomized mice transplanted with a fragment from a tamoxifen-stimulated tumor with ICI 182,780 alone or with the oil vehicle alone ($-E_2$) failed to stimulate tumor growth (Fig. 5). Both tamoxifen and toremifene stimulated tumor growth, an effect that was inhibited by ICI 182,780. This drug also inhibited estrogen-induced tumor growth and growth stimulated by deoxytamoxifen (not shown). These data suggest that stimulation of tumor growth by tamoxifen and related analogs is mediated through the estrogen receptor and that by virtue of a different mechanism of action, pure steroidal antiestrogens may be useful in reversing this form of tamoxifen resistance.

To investigate further the effects of ICI 182,780 in reversing tamoxifen-stimulated growth, seven mice with MCF-7 tumors were treated with tamoxifen for 6 months. When tumor progression (tamoxifen-stimulated growth) was evident, mice were then begun on weekly injections of ICI 182,780. After 3 weeks, further tumor growth had ceased in six of the seven mice, and the mean tumor volume remained static for another 40 days, at which time the

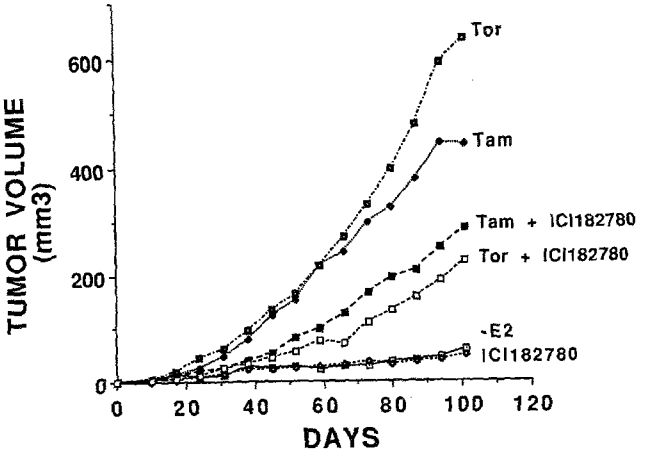

Fig. 5. Effect of ICI 182,780 on tamoxifen-stimulated tumor growth. Mice (10/group) were treated as described in Fig. 2 except that some mice received either ICI 182,780 at 5 mg/mouse per week s.c. alone or combined with tamoxifen or toremifene

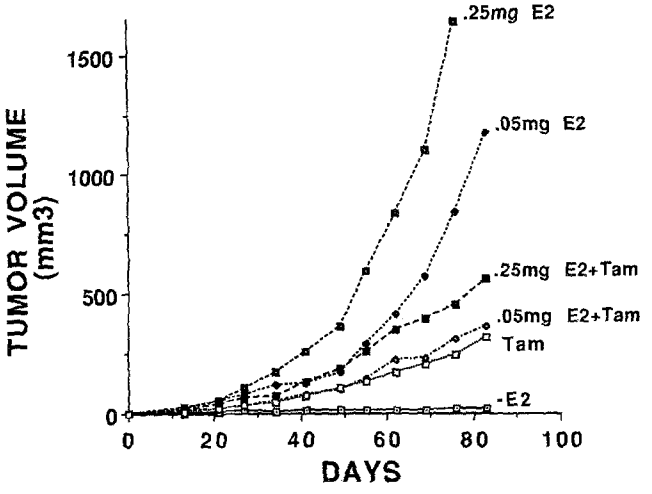

Fig. 6. Effect of estrogen with or without tamoxifen on growth of tamoxifen-stimulated tumors. Mice received transplants of tumor fragments from tamoxifen-stimulated tumors as described in Fig. 2. Groups of 10 mice each were then treated with either 0.25 or 0.05 mg estradiol pellets (E_2), tamoxifen alone (*Tam*; 500 μg/day), estradiol pellets plus tamoxifen (E_2+*Tam*), or vehicle alone ($-E_2$). Tumor volumes were determined on the days shown

experiment was stopped (data not shown). Tumor volumes continued to escalate in control animals maintained on tamoxifen alone.

Agonist and antagonist properties of tamoxifen in tamoxifen-stimulated tumors

If tamoxifen-stimulated growth is due to the conversion of tamoxifen, by whatever mechanism, to a pure estrogen agonist, then combinations of tamoxifen with a suboptimal concentration of estrogen might be expected to have an additive effect on stimulating tumor growth. To test this hypothesis, tamoxifen-stimulated tumor fragments were transplanted into fresh recipient castrated mice treated with

vehicle alone or with optimal or suboptimal doses of estrogen without or with tamoxifen (Fig. 6). Again, tumors failed to grow without hormone supplementation. Tamoxifen also stimulated tumor growth, albeit less effectively than either dose of estrogen. Interestingly, when tamoxifen was combined with either dose of estrogen antagonistic rather than additive agonistic properties were observed. Thus, tamoxifen has a dual effect: it is capable of stimulating tumor growth when used alone yet can antagonize estrogen when the drugs are used together.

Discussion

These data confirm our previously reported results [14] and those of Gottardis and Jordan [5], demonstrating that tamoxifen resistance in our model is associated with the acquired ability of tamoxifen to stimulate tumor growth. Furthermore, we confirmed our previous observation that this tamoxifen-stimulated growth is associated with a marked reduction in tumor tamoxifen levels. We have now extended these observations to show that levels of all major metabolites, including metabolite E, are also reduced in tamoxifen-stimulated tumors and that there is a relative increase in the *cis* as compared with the *trans* isomer of 4-hydroxytamoxifen.

Metabolite E, an estrogenic metabolite, was also quantified in tumor extracts and was detected in both tamoxifen-inhibited as well as tamoxifen-stimulated tumors. Relative to the parent drug and other metabolites, metabolite E was more abundant in tamoxifen-stimulated tumors, leading to the possibility that a relative accumulation of this estrogenic metabolite coupled with a relative decline in the potent antiestrogenic metabolite *trans*-4-hydroxytamoxifen contributed to tamoxifen-stimulated growth as a mechanism for acquired tamoxifen "resistance." Even at ratios of tamoxifen to metabolite E of 20: 1, if the latter has significantly higher affinity in vivo for the estrogen receptor, as has been suggested by previous reports [7, 16], then the receptor may be preferentially occupied by metabolite E, resulting in an estrogenic growth-stimulatory signal.

The present data obtained using analogs of tamoxifen that are resistant to isomerization and/or cleavage of the side chain necessary for its antagonist properties do not support the hypothesis that metabolism of tamoxifen is responsible for tamoxifen-stimulated growth. The two seven-membered ring analogs fixed in the *trans* configuration were just as potent as tamoxifen itself in stimulating MCF-7 tumor growth. The stability of these compounds in vivo is supported by the HPLC studies of tumor extracts in which we could identify only a single peak, presumably the *trans* isomer, in the region of the 4-hydroxylated metabolite. Thus, isomerization of tamoxifen to its estrogenic *cis* configuration, production of the potent estrogen *cis*-metabolite E, or conversion of the potent antagonist *trans* 4-hydroxytamoxifen to the weak *cis* isomer seems unlikely to contribute to this form of tamoxifen resistance. We cannot exclude the possibility from our data, however, that the fixed-ring compounds are nonetheless flexible enough to have altered receptor-binding profiles or biologic activities under certain in vivo conditions.

The deoxytamoxifen analog studies also reject the idea that metabolism to metabolite E is responsible for the estrogenic effects of tamoxifen in our model. Unlike the results obtained with tamoxifen treatment, we found no evidence of metabolite E in tumors from mice treated with the deoxy analog. However, this compound was as effective as tamoxifen in promoting growth of a tamoxifen-stimulated tumor transplant. Taken together, our studies implicate mechanisms other than metabolism for tamoxifen-stimulated tumor growth in this model. The altered metabolite profile may simply be a marker for the development of this tumor phenotype.

Another reproducible observation in this model of tamoxifen resistance is that tumors harvested during the phase of tamoxifen-inhibited growth contain 10–15 times as much of the parent drug as do tumors harvested when progression is evident. The mechanism responsible for this reduction in tumor tamoxifen concentration remains obscure. These tumors do not overexpress P-glycoprotein (DeGregorio and Osborne, unpublished observations), but an active efflux mechanism is an interesting possibility in view of the persistently high serum concentrations of tamoxifen observed in these mice. Whether the reduced tumor tamoxifen level is related somehow to the development of tamoxifen-stimulated growth is not clear. Modest stimulation of breast-cancer cell proliferation by low concentrations of tamoxifen has been reported [19]. Supportive evidence, however, is provided by a recent preliminary report demonstrating that a significant proportion of patients with acquired tamoxifen resistance have a marked reduction in tamoxifen concentration in their tumors as compared with those with de novo resistance [2]. This report confirms and extends our previously published clinical data demonstrating reduced tumor tamoxifen levels in patients with acquired resistance [15]. The phenomenon of reduced tumor tamoxifen levels, reproduced in our experimental model and in patients, and its possible clinical relevance deserve further investigation.

The data presented herein confirm and extend a recent publication using a fixed-ring tamoxifen analog in a serially transplanted model of tamoxifen-stimulated tumor growth [22]. This model differs from ours in that it uses a serially transplanted MCF-7 tumor and a different method of tamoxifen administration that results in significantly lower serum and tumor tamoxifen concentrations. These methodologic differences may explain the inability of this group to detect low levels of metabolite E or differences in tumor tamoxifen concentrations in tamoxifen-inhibited as compared with tamoxifen-stimulated tumors. Nevertheless, similar to the present data, a nonisomerizable tamoxifen analog was as effective as tamoxifen in stimulating tumor growth. Taken together, the observation that nonisomerizable antiestrogens such as nafoxidine and the fixed-ring tamoxifen analogs as well as the deoxytamoxifen analog resistant to side-chain cleavage are capable of enhancing in vivo growth of tamoxifen-stimulated tumors suggests that isomerization and/or metabolism of tamoxifen are not key elements for the development of resistance in this model.

The data presented herein do support the idea that tamoxifen-stimulated growth in our model is mediated by the estrogen receptor. These tumors continue to express estro-

gen and progesterone receptors [14], and preliminary sequenc studies demonstrate that the A/B and E regions of the receptor, those domains involved in ligand binding and transcriptional activation, are normal in tamoxifen-stimulated tumors (Fuqua and Osborne, unpublished observation). Finally, pure steroidal antiestrogens such as ICI 182,780 are effective inhibitors of tamoxifen-stimulated growth. Clinical investigation of these antiestrogens in patients with tamoxifen resistance will be of interest.

The mechanisms by which tamoxifen acquires the ability to stimulate tumor growth after a period of inhibition remains a mystery. It has long been known that tamoxifen has dominant estrogen agonist properties in some tissues or in some species, whereas antagonistic properties dominate in others such as the breast [1]. In our MCF-7 tumor model, estrogen-antagonistic properties dominate initially and tumor growth is inhibited. Thereafter, tumor progression occurs as the agonist activity of the drug increases, although antagonistic properties of the drug are preserved. It is interesting to speculate that changes in accessory proteins that affect transcriptional activation through the estrogen receptor contribute to tamoxifen-stimulated growth, a possibility that will require further study.

Acknowledgements. The HPLC analyses were performed by Dr. Michael W. DeGregorio. The secretarial assistance of Pamela Render is greatly appreciated.

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