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ANTIESTROGENIC AND ANTITUMOR PROPERTIES OF THE NEW TRIPHENYLETHYLENE DERIVATIVE TOREMIFENE IN THE RAT

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Summary—The effects of toremifene, a new triphenylethylene derivative, on the uterus and DMBA-induced mammary tumors in rats were compared to tamoxifen. The ability of toremifene to compete with [^{3}H]estradiol for cytoplasmic estrogen receptor from rat uterus was similar to tamoxifen, the IC_{50} being 26 and 23 μM respectively.

In immature intact rats the two compounds, administered orally for three consecutive days, had similar intrinsic partial estrogenic efficacy, at 50 mg/kg, about 40% of that of estradiol benzoate (EB). However, at doses $\leq$ 10 mg/kg, the estrogenic effect of toremifene was seen at doses about 40 times higher than that of tamoxifen. The two compounds, administered together with a standard dose of EB, expressed the same maximal antiestrogenic efficacy (about 65% inhibition) at 50 mg/kg. However, the minimal effective antiestrogenic dose of toremifene was about 10 times that of tamoxifen and the ratio between antiestrogenic/estrogenic properties was favourable to toremifene.

The duration of the antiestrogenic (antiuterotrophic) effect of a single oral dose (10 mg/kg) of the two compounds proved similar: at least 4 days in intact rats and 3 days in ovariectomized rats.

In DMBA-induced tumor bearing rats toremifene was administered p.o., 6 times/week for 4 weeks at 0.08, 0.4, 2, 10 and 50 mg/kg. It was effective at the doses of 2, 10 and 50 mg/kg, inducing 39, 35 and 46% tumor regressions. The activity of toremifene at the minimal effective dose of 2 mg/kg was then compared with that of tamoxifen given at the same dose level. The compounds had comparable activity (47 vs 44% tumor regressions).

INTRODUCTION

The primary event in target organ response to estrogens is the formation of a cytosol estrogen receptor (ER) complex. Antiestrogens are thought to antagonize the action of estrogens in cells by direct competition for binding to ER [1]. Tamoxifen, the standard antiestrogen agent in breast cancer therapy, displays various degrees of estrogenic agonism, depending upon the target organ, the species and the estrogen milieu [2, 3]. The search for antiestrogens with a better antagonist/agonist ratio was based on these limitations.

A number of antiestrogens (4-hydroxytamoxifen, trioxifen, LY117018 and LY139481) with significantly less partial agonist activity than tamoxifen on the rat uterus have been described [4]. Surprisingly, when evaluated in dimethylbenz(α)anthracene (DMBA)-induced mammary carcinoma in rats, these new antiestrogens were less effective than tamoxifen in inhibiting tumour growth [4–6]. The tumoral and uterine cells did not show different sensitivity to the new antiestrogens and tamoxifen; from *in vitro* studies the order of potency for the growth inhibition of human breast cancer cells (MCF 7) was the same as the order of relative binding affinity (RBA) for ER of rat uterus or DMBA tumors [4]. The fact that these new antiestrogens have lower antitumoral activity than tamoxifen has been partly attributed to a shorter biological "half-life". In fact, tamoxifen was the longest-acting antiestrogen (antiuterotrophic) agent after a single oral dose to rats [4, 7]. The lower efficacy of high doses of antiestrogens (e.g. trioxifen and LY117018) in the DMBA model after a 4-week oral treatment schedule has been attributed to induction of metabolic enzymes, reducing the effective drug concentration [4]. These data are consistent with the view that the pharmacokinetic properties of antiestrogens, particularly of compounds with a free-hydroxyl group like 4-hydroxytamoxifen, LY117018 and LY139481, are of predominant importance in the expression of antitumor activity.

Toremifene (Fc-1157a) (4-chloro-1,2-diphenyl-1-{4-[2(*N*,*N*-dimethylamino)ethoxy]phenyl}-1-butene) is a new triphenylethylene derivative with antiestrogenic [8] and antitumor (DMBA-induced tumor) properties in rats [9]. After subcutaneous administration to immature rats the compound showed potent antiestrogenic but very weak partial intrinsic

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estrogenic activity; no comparison with tamoxifen was reported [8].

In this study we compared the estrogenic/antiestrogenic properties of oral toremifene and tamoxifen in immature rats. The duration of the antiestrogenic effect of a single oral dose of toremifene, the binding to rat uterus ER and the effect on DMBA-induced rat mammary carcinoma were also investigated in comparison with tamoxifen.

ESTROGENIC/ANTIESTROGENIC ACTIVITY IN RATS

Immature (21-day-old) female Sprague–Dawley rats were used. Toremifene and tamoxifen were supplied as citrate salts by Farmos Group (Turku, Finland) and dispersions for oral administration were prepared in 0.5% aqueous Methocel. Doses are given as the salt. To determine estrogenic (uterotrophic) activity, various doses of toremifene and tamoxifen were administered orally on three consecutive days. As positive standard subcutaneous estradiol benzoate (EB, in sesame oil) was used. To determine antiestrogenic (antiuterotrophic) activity a standard dose of EB (0.03 mg/kg s.c.) was injected on three consecutive days with various oral doses of toremifene and tamoxifen. On the fourth day the animals were killed, uteri were removed and wet weights recorded.

Both test compounds acted as partial estrogen agonists, their maximal uterotrophic efficacy being about 40% that of EB (Fig. 1). However, from the dose–response curve, the estrogenic potency of toremifene was about 40 times lower than tamoxifen. The two compounds, administered with a standard dose of EB, expressed similar maximal antiestrogenic efficacy (about 65% inhibition—Fig. 2). In terms of potency, toremifene in the dose-range up to 1 mg/kg, was 10 times less potent than tamoxifen as

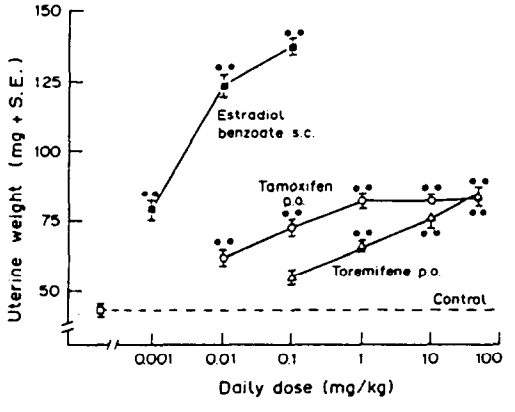

Fig. 1. Estrogenic effect of estradiol benzoate (■), tamoxifen (○) and toremifene (△) in the uterine weight test for immature intact rats. Compounds were administerd daily for 3 days and the rats were killed on the 4th day. Each point is the mean and standard error of 2 separate assays with 6 rats per group in each assay. $^{**}P < 0.01$ vs control group (□).

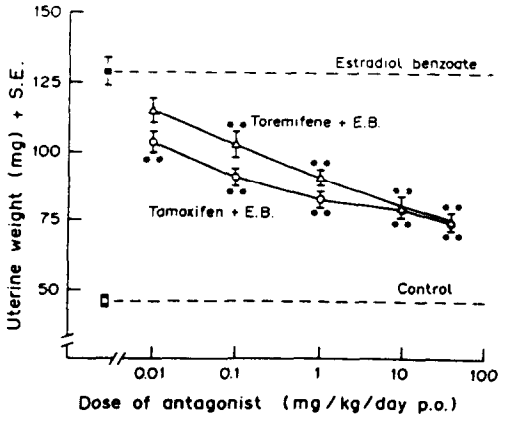

Fig. 2. Antiestrogenic effect of toremifene (△) and tamoxifen (○) in the uterine weight test of the immature intact rats. Compounds were administered daily for 3 days with a standard dose of estradiol benzoate (EB) (0.03 mg/kg s.c.) and the rats were killed on the 4th day. Each point is the mean and standard error of 2 separate assays with 6 rats per group in each assay. $^{**}P < 0.01$ vs estradiol benzoate group (■).

an antiestrogen and the ratio between antiestrogenic/estrogenic properties was favourable to toremifene. The duration of the antiestrogenic effect of a single oral dose (10 mg/kg) of both compounds was determined in immature intact rats treated for three consecutive days with a standard dose of EB and killed on the fourth day. The antiestrogen dose was administered 1, 2, 3 or 4 days before the first dose of EB. A highly significant reduction in uterine weight was observed with both compounds at each time (Fig. 3).

With the aim of extending the interval between the antiestrogen dose and the uterine stimulation test up to six days ovariectomized rats were used. In a

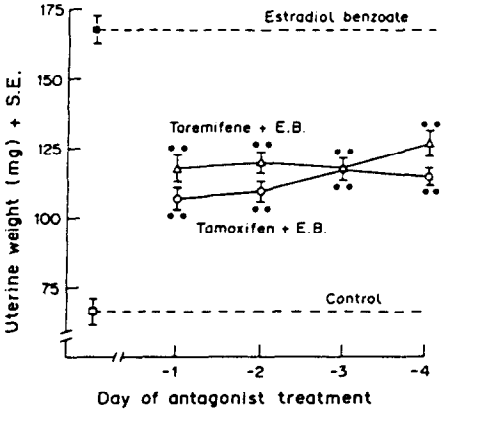

Fig. 3. Residual antiestrogenic effect of a single dose (10 mg/kg p.o.) of toremifene (△) and tamoxifen (○) administered 1–4 days before the first dose of estradiol benzoate (EB) in immature intact rats. EB (0.03 mg/kg s.c.) was administered for three consecutive days and the animals were killed on the 4th day. Each point is the mean and standard error of 2 separate assays with 8 rats per group in each assay. $^{**}P < 0.01$ vs estradiol benzoate group (■).

preliminary study (data not shown) and in a further experiment (Fig. 4) a significant antiestrogen effect was seen with both compounds (at 10 mg/kg p.o.) only up to 3 days, indicating a shorter duration of action and/or lower sensitivity to antiestrogens in ovariectomized compared to intact rats.

BINDING TO UTERINE ESTROGEN RECEPTORS

The ability of toremifene, tamoxifen and unlabeled estradiol to compete with [^{3}H]estradiol for cytoplasmic ER from uteri of adult ovariectomized rats was determined by an adaptation of the dextran-coated charcoal adsorption technique [10].

Various concentrations of test compounds were incubated with [^{3}H]estradiol (10^{-9} M) at 4°C for 18–20 h. The concentration of each compound required to reduce specific [^{3}H]estradiol binding by 50% (IC_{50}) was determined. The relative binding affinity (RBA) of each compound (as a percentage of that of estradiol) was calculated as follows: RBA = (IC_{50} of estradiol/IC_{50} of test compound) × 100.

Toremifene was bound to ER with high affinity, its IC_{50} being 2.6×10^{-7} M (Fig. 5, Table 1). Similar results were obtained with tamoxifen. Compared to estradiol, the RBA were 0.54 and 0.61% respectively for toremifene and tamoxifen.

ANTITUMOR ACTIVITY IN RATS

The antitumor effects of toremifene and tamoxifen were tested on DMBA induced mammary tumors in rats. The hormone-dependency of this experimental tumor makes it the most widely used model for testing hormonal compounds.

Tumor responses to toremifene and tamoxifen given p.o., 6 days a week for 4 weeks, are reported

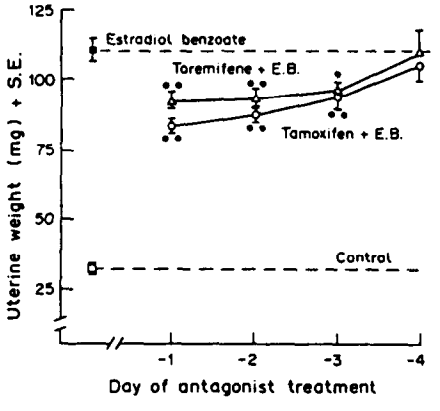

Fig. 4. Residual antiestrogenic effect of a single dose (10 mg/kg p.o.) of toremifene (△) and tamoxifen (○) administered 1–4 days before the first dose of estradiol benzoate (EB) in immature ovariectomized rats. EB (0.03 mg/kg s.c.) was administered for three consecutive days and the animals were killed on the 4th day. Each point is the mean and standard error of 2 separate assays with 8 rats per group in each assay. $^*P < 0.05$; $^{**}P < 0.01$ vs estradiol benzoate group (■).

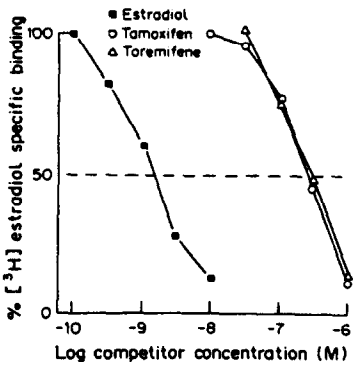

Fig. 5. Inhibition of the binding of [^{3}H]estradiol (10^{-9} M) to estrogen receptor from rat uteri by toremifene (△), tamoxifen (○) and estradiol (■).

in Table 2. The percentage of spontaneous tumor regressions (% CR + % PR) in the control group was 23%. Tumor regressions induced by toremifene at the doses of 0.08, 0.4, 2, 10 and 50 mg/kg amounted to respectively 20, 21, 39, 35 and 46%. Tamoxifen induced 23% tumor regression at the dose of 0.08 mg/kg, 50% at 0.4 mg/kg, 15% at 2 mg/kg and 47% at 10 mg/kg. The number of new tumors was low in the control group and neither compound had any effect on their development.

The activity of toremifene and tamoxifen was not dose-related; the highest percentage of tumor regressions induced by toremifene (46%) was similar to that induced by tamoxifen (50%). The lack of activity of tamoxifen at the dose of 2 mg/kg was unexpected, in contrast with our previous data and with data from Wakeling and Valcaccia[11]. We therefore further investigated tamoxifen's activity at this dose in a second experiment using "late" tumors (i.e. those that develop more than 110 days after DMBA treatment). Both compounds were administered at the dose of 2 mg/kg p.o., 6 days a week for 4 weeks. Toremifene induced 47% tumor regressions and tamoxifen 44% (Table 3). Development of new tumors was inhibited in both groups.

CONCLUSION

The relative binding affinity of toremifene to uterine ER was similar to tamoxifen, confirming previous data from Kallio *et al.*[8]. The meaning of toremifene's *in vitro* interaction with ER was investigated *in vivo* in the estrogenic/antiestrogenic test

Table 1. Inhibition of the binding of [^{3}H]estradiol (10^{-9} M) to estrogen receptor from rat uteri by toremifene, tamoxifen and estradiol

Compound	IC_{50} (M)	RBA (%)
Estradiol	1.4×10^{-9}	100
Toremifene	2.6×10^{-7}	0.54
Tamoxifen	2.3×10^{-7}	0.61

RBA = relative binding affinity (estradiol = 100%).

Table 2. Effect of toremifene and tamoxifen, administered orally, on DMBA-induced tumors in rats

Compound[1]	Dose (mg/kg) p.o.	No. of rats	No. of tumors[2] evaluated	Effect at the end of treatment %CR[3]	%PR[4]	%CR + PR	New tumors/ rat
—	—	12	13	8	15	23	0.4
Toremifene	0.08	11	15	13	7	20	0.4
	0.4	11	14	7	14	21	0.6
	2	11	13	31	8	39	0.4
	10	11	14	21	14	35	0.4
	50	9	11	9	37	46	0.8
Tamoxifen	0.08	11	13	15	8	23	0.3
	0.4	11	18	22	28	50	0.4
	2	11	13	15	0	15	0.5
	10	11	15	14	33	47	0.3

[1]Compounds were administered daily, 6 days/week for 4 weeks. [2]Tumors were induced by a single gastric intubation of 20 mg DMBA to 50-day-old female Sprague–Dawley rats. [3]CR-complete remission. [4]PR-partial remission (reduction in tumor weight > 50% of initial).

Table 3. Effect of toremifene and tamoxifen, administered orally, on DMBA-induced tumors in rats ("late" tumors).

Compound[1]	Dose (mg/kg) p.o.	No. of rats	No. of tumors[2] evaluated	Effect at the end of treatment %CR[3]	%PR[4]	%CR + PR	New tumors/ rat
—	—	9	15	0	0	0	0.8
Toremifene	2	10	15	40	7	47	0.2
Tamoxifen	2	10	16	25	19	44	0

[1]Compounds were administered daily, 6 days/week for 4 weeks. [2]Tumors were induced by a single gastric intubation of 20 mg DMBA to 50-day-old female Sprague–Dawley rats. [3]CR = complete remission. [4]PR = partial remission (reduction in tumor weight > 50% of initial).

(uterine weight) in immature rats. The compound, given orally, showed partial estrogen agonism with antiestrogenic activity. The ratio between its anti-estrogenic/estrogenic properties was better than tamoxifen.

The biological half-life of the antiestrogenic effect of a single oral dose of toremifene proved similar to tamoxifen and, according to various authors [4, 7], is considered an important feature for the expression of the antiestrogens' antitumor activity.

In the DMBA tumor model toremifene showed interesting antitumor activity, its maximum effect (47% regressions) being comparable to tamoxifen.

In conclusion toremifene is a promising novel antiestrogen agent. In clinical phase I studies in postmenopausal women the compound proved safe, and has now entered further clinical studies.

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