J. steroid Biochem. Vol. 36, No. 3, pp. 191–195, 1990
Printed in Great Britain.

0022-4731/90 $3.00 + 0.00

REVIEW OF THE PHARMACOLOGICAL PROPERTIES OF TOREMIFENE

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Summary—New compounds were synthesized with the aim to develop new anti-estrogenic antitumor drugs. The biological properties of the molecules were screened by (1) estrogen receptor (ER) binding, (2) effect on MCF-7 cells, (3) uterotrophic effect and inhibition of estradiol induced uterotropic effect and (4) antitumor effect in DMBA induced rat mammary cancer. One of the molecules, Fc-1157a = toremifene, exhibited the following characteristics: competitive inhibition of [^{3}H]estradiol binding to ER (IC_{50} = 0.3 μmol/l), inhibition of MCF-7 cell growth in a concentration-dependent manner and cell-killing effect at higher than 3 μmol/l concentrations. Minimal estrogenic dose of toremifene on rat uterus weight was about 40 times higher than that of tamoxifen. Toremifene had statistically significant effect against DMBA-induced rat mammary cancer. Further screening consisted of antitumor, pharmacokinetic and safety studies. Toremifene inhibited the growth of ER-negative, glucocorticoid sensitive, mouse uterine sarcoma in a dose-dependent manner. Pharmacokinetics and metabolism of toremifene resembled closely those of tamoxifen, but since the chlorine atom of the toremifene molecule was not metabolically cleaved tamoxifen and toremifene did not have chemically similar metabolites. Toremifene was well tolerated in animal toxicity studies. No hyperplastic or neoplastic nodules, which were seen in almost all high-dose (48 mg/kg for 24 weeks) tamoxifen-treated rats, were found in toremifene-treated rats (dose 48 mg/kg). In clinical phase I studies in healthy voluntary postmenopausal women, no side effects were reported, at doses $\leqslant$460 mg, neither after a single dose nor after five daily doses. At the dose of 680 mg two out of five persons experienced vertigo and headache. Toremifene, at the dose of 68 mg daily, had antiestrogenic effect on estradiol-induced human vaginal epithelial cells. Clinical phase II studies have confirmed that toremifene has a promising antitumor effect.

INTRODUCTION

The development of toremifene was started in 1979. At that time the cytostolic estrogen receptors were the best biochemical properties available to predict the response of individual breast cancers to hormonal, especially antiestrogen, therapy. About 80% of ER positive cancers and only about 20% of ER negative cancers responded to tamoxifen treatment [1]. The target of the development was a more effective compound than tamoxifen which still affects the tumor cells by a specific mechanism of action, in this case, as antiestrogens through estrogen receptors. A screening strategy and methodology were developed. The new compounds to be synthesized should bind to ER and inhibit the growth of ER positive breast cancer cell lines *in vitro*. These two assays were able to discriminate clearly ineffective compounds from probably effective ones. However, these assays were not able to separate pure estrogens from estrogen antagonists (e.g. diethylstilbestrol would give a positive result in these assays). The uterotrophic assay using immature mice or rats was therefore the next screening test. After the three assays about 90% of the new compounds (altogether 210 new chemical entities were synthesized) were rejected. The remaining 10% went into antitumor tests *in vivo*. DMBA-induced rat mammary cancers were used as the tumor model. Although these tumors are quite heterogeneous they are sensitive to antiestrogen treatment. Four compounds were highly effective in this model. The final selection of the target compound was based on preliminary subacute toxicity assays in rats and on activity in other tumor models. Toremifene was selected as the most promising compound for clinical trials. The chemical structure of toremifene is presented in Fig. 1 in comparison to that of tamoxifen.

The first human studies were started late 1982–early 1983. As toremifene was considered to be a nontoxic compound, healthy postmenopausal women volunteers were included in the first pharmacoclinical trials. Two different dosing schedules were used: either a single dose or 5-day regimen in which toremifene was given once daily. The starting dose was 3 mg and it was gradually increased up to 680 mg which was the final dose level. The anti-estrogenicity was evaluated by the maturation index of vaginal superficial cells after estrogen priming. As these studies showed that toremifene is a safe antiestrogenic compound in humans, clinical phase II studies

Proceedings of the Toremifene Satellite Symposium held at the *UICC World Cancer Congress*, Budapest, Hungary, 1986.

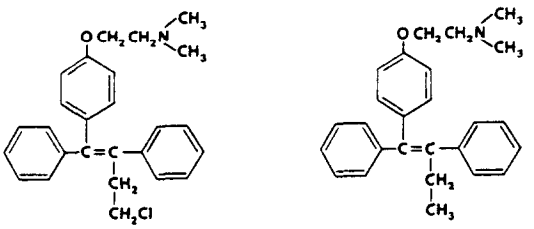

Fig. 1. Chemical structure of toremifene and tamoxifen.

were started in breast cancer patients. In these and in phase III studies toremifene has proven to be an effective and safe antitumor compound in the treatment of postmenopausal breast cancer.

Although toremifene resembles tamoxifen in chemical structure and in pharmacological properties at low doses, toremifene had very interesting antitumor effects especially at high doses: toremifene was active in a transplantable mouse uterine sarcoma, which is a tamoxifen resistant ER negative tumor, and in aggressively growing DMBA-induced mammary cancers. This antitumor effect could not be explained by classical ER theory. On the other hand, during toremifene development, the estrogen receptor theory had been partially revised: ER had been shown to be nuclear only [2], the primary structure of ER had been completely elucidated by gene technology [3], tamoxifen adjuvant therapy had been shown to be as effective in ER positive as in ER negative patients [4] and ER had been shown to have marked homology to erb-A protein which is an important growth factor for oncogenic avian erythroblastosis virus [3]. At the same time the predictive value of ER determinations had decreased clinically; only about 50% of ER positive tumors were expected to respond to antiestrogen therapy [5] in spite of the improved ER determination methodology. However, regardless of this progress in understanding the biochemistry of the estrogen receptor, the mechanism of action of antiestrogens as hormone antagonists and antitumor agents remained unclarified. As high doses of toremifene had an oncolytic effect which was not explained by ER mediated pathways, it is an interesting compound when investigating the nonreceptor-mediated growth inhibitory effects.

PHARMACODYNAMICS OF TOREMIFENE

Effects of toremifene on ER and PgR

The binding of toremifene to ER was determined by a dextran-coated charcoal method (DCC) [16] using cytosol from immature or ovariectomized rat uteri. Toremifene was bound to the receptors with high affinity. The concentration which displayed 50% of [^{3}H]labeled estradiol was 0.5 μmol/l. The dissociation constant was about 1 nmol/l, which was identical to tamoxifen under similar conditions [7].

Toremifene was administered to rats as a single dose (3 mg/kg) or as repeated doses. Cytosolic receptors were determined as above by a DCC method and nuclear ER by a hydroxylapatite method [8]. Long-term retention of ER in the nuclear compartment was evident (Table 1). Like tamoxifen, toremifene induced a statistically significant increase in the synthesis of progesterone receptor (PgR).

Estrogenic and antiestrogenic effect of toremifene

The uterine size of immature rats and mice is a commonly used indicator of estrogenicity of different compounds. Estrogens increase the size when given to the animals for 3 days. Antiestrogens inhibit the

Table 1. Effect of toremifene and tamoxifen on the cytosolic and nuclear ER concentrations in rat uterus

Time after single dose (h)	Control animals	Toremifene (3 mg/kg i.p.)	Tamoxifen (3 mg/kg i.p.)	Estradiol (40 μg/kg s.c.)
	Concentration of cytosolic ER fmol/mg protein			
1	331 ± 105	109 ± 32	72 ± 29*	199 ± 95
2	352 ± 71	118 ± 7*	87 ± 13*	324 ± 155
24	365 ± 112	150 ± 24	76 ± 19*	245 ± 75
48	288 ± 98	348 ± 32	182 ± 24	320 ± 9
	5 daily doses (1 mg/kg)			
	349 ± 52	141 ± 23*	31 ± 11*	284 ± 34
	Concentration of nuclear ER (fmol/mg DNA)			
1	315 ± 40	1071 ± 310*	793 ± 282*	867 ± 193
2	523 ± 80	2334 ± 493*	1353 ± 464	1581 ± 890
24	605 ± 173	1504 ± 658	1478 ± 720	999 ± 411
48	899 ± 313	2385 ± 342	3321 ± 588	1846 ± 542
	5 daily doses (1 mg/kg)			
	1669 ± 77	3225 ± 251*	2550 ± 224*	1244 ± 177
	Concentration of PgR (fmol/μg DNA)			
1	145 ± 11	218 ± 55*	374 ± 124*	323 ± 142*
24	197 ± 33	344 ± 75*	362 ± 98*	458 ± 115*

Dose of anti-estrogens in single dosing 3 mg/kg i.p. and in five daily dosing 1 mg/kg i.p. Progesterone receptors were measured after the single dose. ER and PgR were measured by DCC method. Each value (mean ± SE) has been obtained from 2–6 separate test series, each containing at least 8 pooled uteri.
* = Statistically significant difference ($P < 0.05$ or smaller) to control (Student's t-test).

uterotrophic effect [9]. Most known antiestrogens have an intrinsic and species-specific estrogenic effect in this model [10]. As reported earlier [7] toremifene was partially estrogenic in mouse uterus but an almost pure antiestrogen on the uterus of 21-day-old rats which apparently already secrete estrogen and consequently show fairly high uterine wet weight values. In later studies with 18-day-old rats estrogenic properties were also found with toremifene (Fig. 2). These results indicated that toremifene is a weaker estrogen than tamoxifen at low and moderate doses (statistically significant difference at doses 30 μg/kg–3 mg/kg). The maximal estrogenic activity (at doses 10 and 30 mg/kg) was, however, not statistically significantly different with these two compounds.

Effect of toremifene on MCF-7 cells in vitro

The effect of toremifene on MCF-7 cells (kindly donated by Dr Charles M. McGrath, Michigan Cancer Foundation) was studied in the presence of different concentrations of toremifene and for different culture times. For the assays toremifene was dissolved in 70% ethanol (10^{-2} mol/l) and diluted with growth medium to the intended concentrations (from 1 nmol/l to 10 μmol/l). The growth of the cells was followed by a bioluminescence method based on the determination of intracellular adenosine triphosphate (ATP). This method is technically very simple and rapid, gives a good estimate of the number of living cells and correlates well with the vital staining assay and [^{3}H]thymidine incorporation [11]. Toremifene inhibited the growth of MCF-7 cells in a concentration-dependent manner. In the presence of Phenol Red and 1% of unstripped FCS no net growth was observed when the toremifene concentration was 1 μmol/l. At concentrations of 5 μmol/l or higher toremifene killed all the cells within a couple of days. These data, which closely resemble those of tamoxifen, show that toremifene had a biphasic action on the MCF-7 cells: growth inhibition at low concentrations and oncolytic activity at high concentrations.

Toremifene in DMBA-induced rat mammary cancer

Mammary adenocarcinomas were induced by a single p.o. dose of dimethylbenzanthracene, 12 mg/animal dissolved in sesame oil, to 50 ± 2-day-old Sprague–Dawley female rats. The induction was carried out in an isolator. The animals were kept in the isolator for 3 weeks and then transferred to chambers in the animal house. Tumors became palpable at about 6 weeks after the induction. Treatment with antiestrogens was started when the largest tumors were about 1 cm in diameter. The treatment was given p.o. and was continued daily for 5 weeks. As the tumors grew very heterogenously, the tumors were classified into three groups: (1) actively growing (tumor volume increased during the treatment more than 4-fold), (2) stable and (3) regressing (tumor volume decreased to less than ¼ of the pretreatment volume). Different dose levels from 0.1 to 50 mg/kg were given. The overall results are shown in Table 2. Toremifene and tamoxifen had almost similar antitumor efficacy. At a high dose, 45 mg/kg, however, toremifen also had an antitumor effect in a series of very aggressively growing tumors which were resistant to a small dose, 1 mg/kg (Table 3). At the dose of 45 mg/kg tamoxifen was lethal to the rats, but toremifene at the same dose was well tolerated. In a transplantable and ER-negative mouse uterine sarcoma tamoxifen had no antitumor effect at any

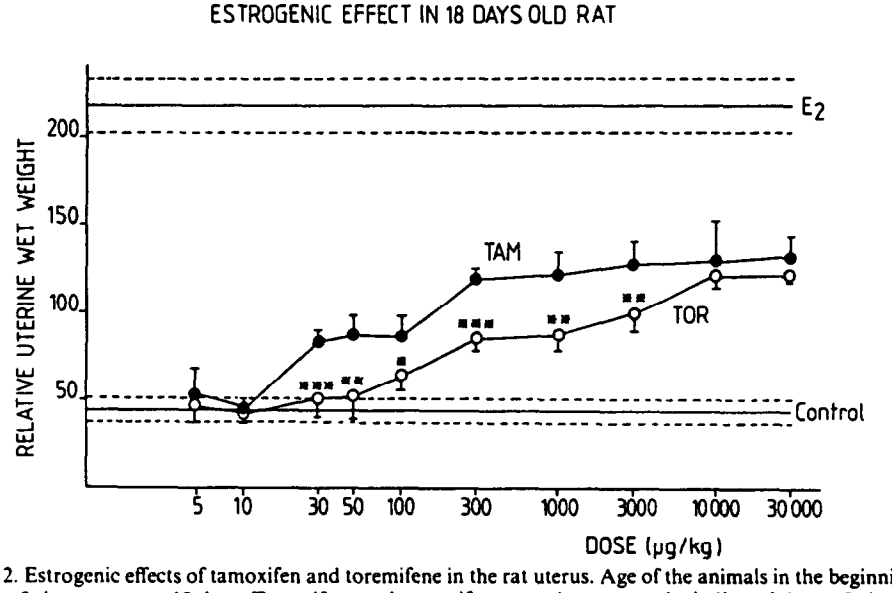

Fig. 2. Estrogenic effects of tamoxifen and toremifene in the rat uterus. Age of the animals in the beginning of the 3-day assay was 18 days. Toremifene and tamoxifen were given s.c. at the indicated doses. Relative uterine wet weights (mg/g body wt × 100) have been recorded and presented as means ± SD. Number of animals is 5–10 in each group. Statistical significance (t-test) between tamoxifen and toremifene: * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$.

Table 2. Antitumor effect of toremifene and tamoxifen against DMBA-induced rat mammary cancer at the dose levels of 0.3–30 mg/kg

Group	n	Number of: Growing tumors	Stable tumors	Regressing tumors	Change of tumor number/animal	Difference to control (P)
Control	40	181	101	7	3.7 ± 3.1	—
Toremifene	74	120	163	51	1.1 ± 1.7	<0.001
Tamoxifen	63	105	130	39	1.4 ± 1.5	<0.001

The antiestrogens were given p.o. for 5 weeks in vehicle containing NaCl 8.65 g, polyethylene glycol 3000 28.8 g, Tween 80 1.92 g, methyl-p-hydroxybenzoate 1.73 g and propyl-p-hydroxybenzoate 0.19 g in 1.00 l of distilled water.
Statistial analysis: χ^2-test based on the number of growing, stable and regressing tumors, which have been defined in the text.

tolerable dose level but toremifene inhibited the growth of the tumors in a dose-dependent manner at high doses (100 and 200 mg/kg for 5 days) [12]. These results refer to an ER-independent antitumor mechanism at high doses of toremifene.

SAFETY OF TOREMIFENE

Toremifene was found to be a well tolerated compound. The acute LD_{50} value was higher than 2000 mg/kg in mice. In 10-day subacute studies, when tamoxifen and toremifene were given to rats at increasing doses, tamoxifen killed the animals at 2–4-fold lower doses than toremifene. The cause of death was similar with both compounds: acute gastric dilatation. In 6 and 12 months comparative chronic toxicity studies in rats (administration route p.o.) toremifene had no biologically significant effects on the non-endocrine organs. It caused similar or weaker atrophy than tamoxifen of the uterus and ovaries and clearly less atrophy of the testis of rats. In a 5 months' comparative toxicity study tamoxifen induced at a high dose, 48 mg/kg, microscopical (apparently benign hypertrophic) nodules in the livers of all rats (males and females). With the same dose toremifene had no effect on the liver. No hematological or clinical chemistry toxicities were found with either compound. When the treatment was continued to 1 yr, tamoxifen had induced large tumors in the livers of all rats at the highest dose level (45 mg/kg). The tumors did not disappear during the 3 months recovery period; on the contrary the tumors had grown further and many of the tumors were malignant. No tumors were found in toremifene-treated animals (highest dose 48 mg/kg).

Toremifene did not show any mutagenic potential in the Ames test, the sister chromatic exchange assay—with or without metabolic activation—and in the micronucleus test.

The secondary pharmacological effects of toremifene were few and mild: in a comprehensive battery of tests consisting of e.g. cardiovascular, immunological, hematological and CNS testing, the only clearly demonstrable effect was analgesic activity in mice at pharmacological i.p. doses (3 mg/kg or more).

PHARMACOKINETICS AND METABOLISM OF TOREMIFENE

Toremifene was well absorbed from the gastrointestinal tract. In a comparative study in rats and dogs, toremifene was given p.o. and i.v. The serum and tissue concentrations were similar after both administration routes as soon as 2–3 h after the administration. In animal and human studies there were no signs of dose-dependent pharmacokinetics over a wide dose range.

As toremifene is a lipophilic compound it was distributed throughout the body. The highest toremifene derived radioactivities in rats were found in the lungs, the lowest in bone, eye and red blood cells.

The metabolism of toremifene has been extensively studied in rats, dogs, monkeys and humans. There are only small species-specific differences. The chlorine atom in the molecule is stable and is not cleaved from

Table 3. Antitumor activity of toremifene and tamoxifen on DMBA-induced rat mammary cancer in one induction series growing aggressively

Group	n	Number of: Growing tumors	Stable tumors	Regressing tumors	Change of tumor number/animal	Difference to control (P)
Control	3	19	9	0	6.0 ± 5.2	—
Toremifene						
1 mg/kg	6	23	12	0	3.2 ± 2.9	NS
45 mg/kg	5	8	10	1	1.2 ± 1.1	<0.05
Tamoxifen						
1 mg/kg	6	18	12	0	2.8 ± 1.7	NS
45 mg/kg	6	Toxic to all animals				

Small and high doses were compared. Half of the control animals died on progressive disease before the end of the 5 weeks treatment period.
Dosing and statistics: see Table 2.

the molecule in the metabolic reactions. Therefore toremifene and tamoxifen have no identical metabolites, although the compounds are chemically related and are obviously metabolized through similar pathways. The metabolic reactions are as follows: the nitrogen containing side chain is *N*-demethylated and further oxidated to an alcohol and finally to a carboxylic acid; independently 4-hydroxylation and 4′-hydroxylation may occur.

Toremifene obviously undergoes enterohepatic circulation and is excreted mainly via the feces as metabolites. The details of its metabolism and pharmacokinetics have been described by Anttila *et al.*[13] and Sipilä *et al.*[14] in this issue.

CLINICAL PHARMACOLOGY OF TOREMIFENE

Clinical phase I and II studies establishing that toremifene is a well-tolerated antiestrogen with clearly demonstrable antitumor activity in advanced breast cancer have been described in detail in other articles of this issue. In particular, the clinical results of high-dose toremifene studies have indicated antitumor activity after tamoxifen relapses and after other hormonal, as well as chemo- and/or radiotherapies.

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