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Comparative pharmacodynamic analysis of TAT-59 and tamoxifen in rats bearing DMBA-induced mammary carcinoma

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Abstract TAT-59 suppressed the growth fo DMBA-induced mammary tumors in rats earlier and more strongly than tamoxifen (TAM). After oral administration of the drugs, DP-TAT-59, one of the main metabolites of TAT-59, was found in 10- to 15-fold higher concentrations in both the tumor and blood compared to 4-OH-TAM, an active metabolite of TAM. In a 3-day antiuterotrophic test, every detected metabolite of TAT-59 showed stronger antiestrogenic activity than did TAM. In a competition assay, the affinity of the metabolites for estrogen receptors ranged from that of estradiol to that of TAM. These results suggest that the superior antiestrogenic activity of TAT-59 compared to TAM was either due to its higher penetration into tumor tissue or to the stronger antiestrogenic activity of its metabolites.

Key words TAT-59 · Pharmacodynamics · Anti-estrogenic activity · DMBA-induced mammary tumor

Introduction

TAT-59, a nonsteroidal antiestrogenic drug, was synthesized and screened at our institute [1]. This compound has been subjected to basic studies performed in parallel with tamoxifen (TAM), a very well-known antiestrogenic drug [21, 22]. Since the efficacy of TAM depends on the number of estrogen receptors (ERs) in breast cancer cells [4], its efficacy appears to be low against breast tumors possessing a small number of ERs. In contrast, TAT-59 suppresses the growth of dimethylbenzanthracene (DMBA)-induced mammary carcinoma at a dose level ten times lower than that required for TAM, and also shows superior activity against tumors containing a low number of ERs (10–20 fmol/mg protein) [21].

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The mechanism(s) of the antitumor action of triphenylethylene derivatives has been reported to be due to competitive antagonism at the ERs of tumor cells [10]. In the case of TAM, 4-OH-TAM shows the highest affinity for ERs, but neither TAM itself nor *N*-desmethyl TAM (DM-TAM), the main metabolite of TAM in humans, show a strong affinity for ERs [3]. For TAT-59, the dephosphorylated metabolite, DP-TAT-59, is one of the main metabolites in humans, and shows affinity similar to estradiol for ERs. However, in order to display antiestrogenic activity, TAT-59 or its active metabolite(s) must penetrate into the tumor tissue and remain there at high concentrations for a prolonged period.

To find an explanation for its superior activity as compared to TAM, we studied the antiestrogenic activities of the metabolites of TAT-59 and their tissue distribution.

Materials and methods

Materials

Tamoxifen citrate and 17β-estradiol were purchased from Sigma Chemicals. [2,4,6,7-^{3}H]-estradiol (4.255 TBq/mmol) and (*Z*)-4-[*N*-methyl-^{3}H]-hydroxytamoxifen (3.219 TBq/mmol) were obtained from New England Nuclear. 7,12-DMBA was obtained from Tokyo Chemicals Co. 4-OH-TAM, DM-TAM, TAT-59, DP-TAT-59 and 1-en-DP-TAT-59 were synthesized at our institute [15].

DMBA (20 mg/ml in sesame oil, 1 ml/body) was administered orally to 50-day-old Sprague-Dawley (SD) rats to induce tumors as previously described by Huggins et al. [9]. The antiestrogenic drugs were first dissolved in dimethylsulfoxide to prepare a stock solution. The stock solution was then diluted in 0.5% carboxymethylcellulose (CMC) for pharmacodynamic studies, in 0.5% CMC containing 0.5% Tween 80 for the antitumor test and the determination of ER content in the uterus, or in saline for the antiuterotrophic test. Female SD rats were purchased from Japan SLC (Shizuoka, Japan). They were housed under specific pathogen-free conditions. Food and water were available ad libitum and a 12-h light/dark schedule was maintained.

Antitumor test

Using DMBA-induced rat mammary carcinoma, the antitumor activities of TAM and TAT-59 were compared. Drug treatment was started when the tumors had reached a diameter of about 10 mm. Tumor-

bearing rats were divided into groups of 19 or 20. Drugs were orally administered at 0.3 mg/kg per day six times per week for 4 weeks. The size of the tumors was measured using calipers and the tumor volume was estimated from the following expression, using perpendicular diameters: tumor volume (mm^3) = ($width^2$) × (length)/2. The percentage change in the tumor volume was calculated on the basis of the initial tumor volume. Student's *t*-test was used for statistical analysis.

Determination of ER content in the uterus

Rats at the age of 11 weeks were weighed and divided into groups of seven or eight. TAT-59 or TAM was administered orally at 3 or 0.3 mg/kg per day for 1, 2, 4, 8 or 16 days. The day after the last administration, the rats were killed and the uteri were excised and stored at −80 °C until use. The stored uteri were homogenized to obtain a cytosol. The cytosol was incubated in various concentrations of [^{3}H]-estradiol and then treated with dextran-coated charcoal (DCC). The number of ERs was determined by Scatchard methods [19].

Pharmacodynamic study

DMBA-induced mammary tumor-bearing rats were given 0.3 mg/kg per day of antiestrogens orally for 1 day or for 14 days. Plasma and tumor tissue were collected at the indicated times after treatment. The plasma was extracted with *n*-hexane/ethyl acetate (1 : 1) for TAT-59 and its metabolites and with 2% butanol/*n*-hexane for TAM and its metabolites. The tissue samples were homogenized and sonicated in ice-cold methanol. The homogenates were then centrifuged and their supernatants were collected. Both the supernatants of tissue homogenate and plasma extracts were dried under a stream of nitrogen gas and dissolved in a solution of 90% methanol in 10 m*M* PBS (pH 6.3), and then subjected to HPLC analysis as described by Brown et al. [2].

Antiuterotrophic test

Seven or eight female SD rats (4 weeks old) per group were used to determine the antiuterotrophic activity of the metabolites of TAT-59 or TAM. In order to avoid the influence of endogenous estrogen, the animals were ovarectomized under light ether anesthesia. Compounds were administered i.p. for 3 days and estradiol was administered s.c. at 0.3 mg/body (3.0 mg/ml of sesame oil) simultaneously. One day after the final administration, the animals were killed, and wet weights of the uteri were determined. The percentage inhibition of uterine growth was calculated based on the uterine weight using the following equation: inhibition (%) = (control − tested)/(control − ovarectomized) × 100. The value of ED_{50} was calculated from linear regression lines of the logit-log plot.

Competition analysis

Uteri obtained from SD rats (4 weeks old) were stored at −80 °C until use. Tissues were thawed, minced and homogenized in buffer (20 m*M* Tris-HCl, 5% glycerol, 12 m*M* monothioglycerol, pH 7.4) using a Biotron homogenizer. The homogenate was then centrifuged at 105,000 *g* for 1 h at 2 °C to obtain the cytosol. The cytosol was incubated on ice for 16 h with metabolites in the presence of [^{3}H]-estradiol (5×10^{-9} *M*). After incubation, the cytosol was treated with DCC suspension [11]. The fraction of [^{3}H]-estradiol bound was calculated using the following equation, based on the radioactivity present in the supernatant:

$$[^3\mathrm{H}]\text{-estradiol bound } (\%) = \frac{\text{test} - \text{nonspecific}}{\text{whole} - \text{nonspecific}} \times 100$$

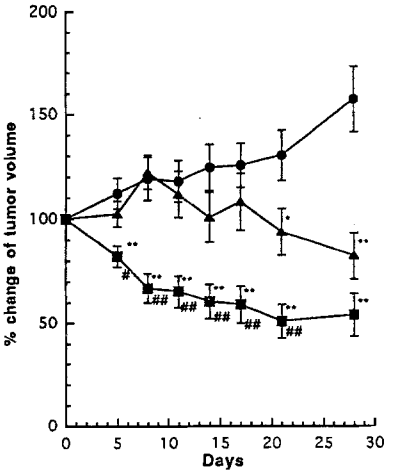


Fig. 1 Antitumor effect of antiestrogenic drugs on DMBA-induced rat mammary carcinoma. *Points* and *bars* are the means and SE of the percentage changes in tumor volume, respectively (● control, ▲ tamoxifen, ■ TAT-59). $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, versus the group treated with tamoxifen; $^{*}P < 0.05$, $^{**}P < 0.01$, versus control

Results

Antitumor test

TAT-59 was able to suppress the tumor growth even after administration for 1 week, whereas TAM did not show any suppressive ability after the same period of administration. There was a significantly different percentage change in tumor volume between the two treatment groups ($P < 0.01$). TAT-59 suppressed tumor growth until the final date of measurement (Fig. 1).

In vivo ER suppressive activity of TAT-59

Since TAT-59 showed a growth suppressive effect earlier than TAM, we determined the ER levels after administration using rat uteri. TAT-59 was able to inhibit the binding of estrogen to ERs (Table 1). ER levels decreased in a time-dependent manner following treatment with TAT-59 or TAM. After treatment with 0.3 and 3 mg/kg TAT-59, ERs were not observed in one of eight and five of eight rats, respectively, even after 1 day of administration, whereas ERs were measurable in all of the TAM-treated rats. After treatment with 3 mg/kg TAT-59 for 8 days and 16 days, ERs could not be detected in any rats and in seven of eight rats, respectively, while in the case of TAM treatment, ERs were detectable in all rats.

Pharmacodynamic analysis

To examine the tissue penetration of TAT-59-related compounds and to detect metabolites, we performed pharmacodynamic studies using DMBA-induced mammary carcinoma-bearing rats. After oral administration of TAT-59,

Table 1 Effect of oral TAT-59 and tamoxifen treatment of uterine ER levels in mature rats. Rats were killed 24 h after the final administration, and the ER contents of the uteri were determined by radioreceptor assay. Values are means ± SD

Administration period	Treatment		No. of animals	Uterine ER content (fmol/mg protein)	Complete lack of ER[a]
	Drug	Dose (mg/kg)			
	Untreated		8	324.2±97.7	–
1 day	TAT-59	3.0	8	2.1± 0.3	5/8
		0.3	8	3.1± 3.3	1/8
	Tamoxifen	3.0	8	7.2± 6.8	0/8
		0.3	8	78.4±32.1	0/8
2 days	TAT-59	3.0	8	2.1± 1.2	3/8
		0.3	8	2.8± 2.2	5/8
	Tamoxifen	3.0	7	7.5± 3.2	0/7
		0.3	8	77.7±26.1	0/8
4 days	TAT-59	3.0	8	2.0± 0.3	5/8
		0.3	8	2.7± 0.6	3/8
	Tamoxifen	3.0	8	5.3± 2.3	0/8
		0.3	7	16.7± 7.9	0/7
8 days	TAT-59	3.0	8	0	8/8
		0.3	8	5.8± 3.1	4/8
	Tamoxifen	3.0	8	10.7± 6.8	0/8
		0.3	8	31.9±10.3	0/8
16 days	TAT-59	3.0	8	1.7	7/8
		0.3	8	3.4± 0.9	3/8
	Tamoxifen	3.0	8	4.4± 0.8	0/8
		0.3	8	27.9±14.8	0/8

[a] Ratio of the ER-negative uteri to the total uteri in the group

three main metabolites were isolated. These metabolites, DP-TAT-59, DM-DP-TAT-59 and 1-en-DP-TAT-59, were *Z*-isomers. The corresponding *E*-isomers were not detected.

After a single administration, DP-TAT-59, an active metabolite, reached a peak plasma concentration of 4.01 ng/ml 12 h after administration. Detectable concentrations were found for 24 h. The peak concentrations of the other two metabolites, DM-DP-TAT-59 and 1-en-DP-TAT-59, were 2.25 and 1.17 ng/ml, and were reached 12 and 3 h, respectively, after TAT-59 administration. The peak plasma concentrations of TAM, DM-TAM and 4-OH-TAM were 2.79, 1.33 and 0.27 ng/ml, and were reached at 3, 12 and 12 h, respectively. A comparison of the peak concentrations of the main active metabolites revealed that the concentration of 4-OH-TAM was 15-times lower than that of DP-TAT-59 (Table 2).

In tumor tissue, peak levels of DP-TAT-59, DM-DP-TAT-59 and 1-en-DP-TAT-59 were reached 12, 12 and 14 h after administration and were 57.86, 17.48 and 10.00 ng/g, respectively. These metabolites were still detected 96 h after administration. In the case of TAM, DM-TAM and 4-OH-TAM, peak concentrations of 46.68, 25.51 and 5.78 ng/g were observed at 192, 96 and 48 h, respectively, after administration. Although the AUC value of TAM was similar to that of DP-TAT-59, the affinity of TAM for ERs is ten times lower than that of DP-TAT-59 [21]. The AUC value of 4-OH-TAM, the TAM metabolite with the highest affinity for ERs [21], was nine times lower than that of DP-TAT-59, which has a similar ER affinity (Table 3). DM-TAM, a main metabolite in humans, possessing an affinity for ERs similar to that of TAM, generated an AUC of about half that of DP-TAT-59.

After the multiple administration, peak concentrations of DP-TAT-59, DM-DP-TAT-59 and 1-en-DP-TAT-59 in the plasma reached 6.19, 4.96 and 1.96 ng/ml, and in the tumor 156.57, 150.17 and 37.20 ng/g, respectively. The concen-

Table 2 Plasma concentrations (ng/ml) of TAT-59 metabolites, tamoxifen and its metabolites following a single oral administration of 0.3 mg/kg TAT-59 or tamoxifen to DMBA-induced mammary tumor-bearing rats. Each value is the mean ± SD from three rats (*ND* not detected)

Hours after administration	After TAT-59 administration			After Tamoxifen administration		
	DP-TAT-59	DM-DP-TAT-59	1-en-DP-TAT-59	Tamoxifen	DM-tamoxifen	4-OH-tamoxifen
3	3.39±0.83	0.39±0.34	1.17±1.03	2.79±0.74	0.36±0.31	0.22±0.38
6	3.07±1.94	0.54±0.61	1.05±0.98	2.37±0.83	1.05±0.64	0.25±0.43
12	4.01±1.77	2.25±1.50	0.63±0.73	2.51±1.33	1.33±0.40	0.27±0.24
24	0.63±0.74	0.58±1.00	0.54±0.47	ND	ND	ND
48	ND	0.35±0.61	ND	ND	ND	ND
AUC (ng h/ml)	63.86	38.49	17.15	26.57	9.80	2.60

Table 3 Intratumor concentrations (ng/g) of TAT-59 metabolites, tamoxifen and its metabolites following a single oral administration of 0.3 mg/kg TAT-59 or tamoxifen to DMBA-induced mammary tumor-bearing rats. Each value is the mean ± SD from three rats (*ND* not detected)

Hours after administration	After TAT-59 administration			After tamoxifen administration		
	DP-TAT-59	DM-DP-TAT-59	1-en-DP-TAT-59	Tamoxifen	DM-tamoxifen	4-OH-tamoxifen
3	16.35± 3.43	2.23± 0.20	3.20±3.02	34.35± 5.76	7.29± 1.01	3.03±0.94
6	25.32± 9.23	2.71± 1.46	6.95±6.03	37.89± 1.23	14.43± 2.00	4.59±1.73
12	57.86±36.21	17.48±15.82	5.36±6.50	46.68±15.56	25.51±12.95	5.78±0.11
24	31.02±24.76	14.38±17.47	10.00±7.31	32.90± 3.13	20.25± 1.47	4.36±0.37
48	8.96±11.99	11.60±17.43	1.28±1.51	10.72± 4.22	5.99± 2.57	1.47±0.52
96	0.98± 1.70	0.51± 0.45	0.33±0.58	2.05± 0.03	0.26± 0.44	ND
192	ND	ND	ND	0.88± 1.52	ND	ND
AUC (ng h/ml)	1588.17	864.89	323.12	1861.64	902.78	177.89

trations of these metabolites were 1.5- to 2.2-fold higher in the plasma and 2.7- to 8.6-fold higher in the tumor than after the single administration. TAM, DM-TAM and 4-OH-TAM reached peak concentrations of 6.29, 1.94 and 0.94 ng/ml in the plasma, and 105.86, 60.70 and 18.56 ng/g, respectively, in the tumor. These levels were about 1.5- to 3.5-fold higher than those observed after the single administration. The peak concentration of the main metabolite, DP-TAT-59, was observed 12 h after the last administration both in the plasma and in the tumor tissue. DP-TAT-59 was still present in the plasma 48 h after administration, whereas in the tumor, its levels were measurable up to 192 h. This retention period was similar for other metabolites, with the exception of DM-DP-TAT-59 which was detected in the tumor tissue up to 384 h after administration (Tables 4 and 5).

Antiestrogenic activity of metabolites

In order to examine the antiestrogenic activity of the metabolites, 3-day antiuterotrophic tests were performed. The inhibition curves of uterine growth are shown in Fig. 2. The ED_{50} values of TAT-59, DP-TAT-59, DM-DP-TAT-59, 1-en-DP-TAT-59 and TAM were 614, 592, 4409, 1717 and 2663 mg/kg, respectively. The minimum doses necessary to inhibit uterine growth by TAT-59, DP-TAT-59, DM-DP-TAT-59 and 1-en-DP-TAT-59 were 8.0, 8.0, >1.6 and 40.0 mg/kg, respectively. These values were 25, 25, 100 and 5 times lower than the value for TAM, respectively. The structural isomer, DP-TAT-59(*E*), showed low antiestrogenic activity.

Competitive binding activities with estradiol

Since metabolites of TAT-59 were able to suppress the estrogen-induced uterine growth, their competitive activities were examined (Fig. 3). All of the TAT-59-related compounds showed a similar competitive activity with estradiol in binding to the ERs. Based on this assay, a relative binding affinity (RBA) was calculated from the ratio between the concentrations of test compound and non-

Fig. 2 Antiuterotrophic effect of TAT-59 and its metabolites after i.p. administration in ovarectomized sprague-Dawley rats. *Points* and *bars* are the means and SD of the values from more than six uteri. The mean uterine weights of the control and the group treated with estradiol were 29.25±3.17 and 131.25±7.38 mg, respecitively (● – – – ● TAT-59, ●——● DP-TAT-59, ▲ DP-TAT-59(*E*), ■ 1-en-DP-TAT-59, ◆ DM-DP-TAT-59, ○ tamoxifen)

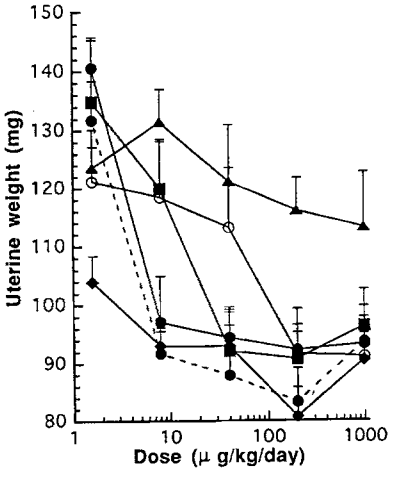


Fig. 3 The competitive binding of TAT-59 metabolites with [^{3}H]-estradiol to cytoplasmic ERs (● DP-TAT-59, ▲ DP-TAT-59(*E*), ■ 1-en-DP-TAT-59, ◆ DM-DP-TAT-59, □ 4-OH-tamoxifen, ○ tamoxifen)

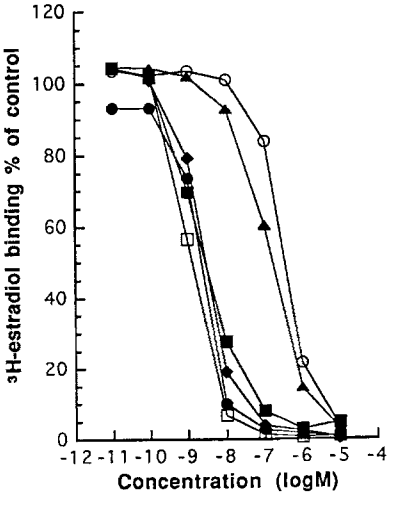


Table 4 Plasma concentrations (ng/ml) of TAT-59 metabolites, tamoxifen and its metabolites following 14 daily doses of 0.3 mg/kg TAT-59 or tamoxifen to DMBA-induced mammary tumor-bearing rats. Each value is the mean ± SD from three rats (*ND* not detected)

Hours after last administration	After TAT-59 administration			After tamoxifen administration		
	DP-TAT-59	DM-DP-TAT-59	1-en-DP-TAT-59	Tamoxifen	DM-tamoxifen	4-OH-tamoxifen
3	3.56±1.31	0.68±0.30	1.93±0.55	6.29±2.80	1.58±0.81	0.65±0.61
6	3.17±0.90	0.56±0.50	1.96±0.22	5.37±2.28	1.94±0.70	0.94±0.16
12	6.19±3.30	4.96±3.55	1.12±0.59	2.31±0.74	1.63±0.73	0.35±0.31
24	0.17±0.29	0.18±0.31	0.77±0.32	0.31±0.28	ND	ND
48	0.19±0.32	0.96±1.66	ND	ND	ND	ND
96	ND	0.32±0.55	ND	ND	ND	ND
AUC (ng h/ml)	86.00	94.68	29.31	65.69	18.36	7.23

radioactive estradiol necessary for a 50% reduction in ER-specific binding (Table 6). The RBAs of the metabolites of TAT-59 were similar to that of estradiol, and ranged from 52.1 to 153.9, while the RBA of TAM was very low at 0.6.

Discussion

We have shown that TAT-59 exerts antitumor activity against hormone-dependent mammary carcinoma at lower dose levels [21, 22] and earlier than TAM (Fig. 1), despite having a similar chemical structure. Such differences might be caused by different metabolism or by different pharmacodynamic characteristics. Regarding the metabolism of TAT-59, all known metabolites of TAT-59 in tumor tissue were *Z*-isomers, whereas those of TAM have been reported to be a mixture of both *Z*- and *E*-isomers [16]. As *Z*-isomers showed 100-fold higher affinity for ERs than *E*-isomers (Fig. 3) and as the metabolites of TAT-59 showed a high RBA for ERs (Table 6) and strong antiestrogenic activity against uteri (Fig. 2), metabolites of TAT-59 might inhibit estrogen-induced tumor growth by competition with endogenous estrogens. Based on the pharmacodynamic data, TAT-59 was easily converted to its metabolites and the level of these metabolites both in tumor tissue and in plasma remained high (Tables 2–5). The penetration of highly active metabolites of TAT-59 to target tissues might cause a loss of free ERs in target tissues (Table 1) and therefore might contribute to its superior antitumor activity.

As triphenylethylene derivatives show an antiestrogenic activity in humans as well as in rats [7], we may predict a clinical antiestrogenic activity of TAT-59, based on in vivo studies in rats. This prediction is based on the following observations: (1) TAT-59 seemed to suppress the hormone-dependent growth of mammary tumors more rapidly than TAM (Fig. 1); (2) TAT-59 decreased the levels of uterine cytoplasmic ERs more rapidly than TAM (Table 1); (3) TAT-59-related compounds suppressed uterine growth at lower dose levels than TAM (Fig. 2); and (4) these compounds reached much higher intratumor concentrations than TAM (Tables 3 and 5). These results suggest that TAT-59 shows stronger and earlier growth-inhibitory activity against human breast cancer than does TAM, even in premenopausal patients.

A necessary property in selecting a triphenylethylene derivative is a high affinity for ERs. A hydroxy group at the 4-position is important in this regard [12, 18]. Although TAM is metabolized in rats to 4-OH-TAM which has an affinity for ERs as high as estradiol, in humans TAM is mainly metabolized to DM-TAM, and not to 4-OH-TAM [5, 7, 13]. The affinity of DM-TAM for ERs is similar to that of TAM [23]. Moreover, most of the TAM remains in the unmetabolized form, both in the plasma and in the tumor [6]. Therefore, the clinical efficacy of TAM may depend on DM-TAM and TAM, both of which have a relatively low affinity for ERs, rather than on 4-OH-TAM [12]. Another

Table 5 Intratumor concentrations (ng/g) of TAT-59 metabolites, tamoxifen and its metabolites following 14 daily doses of 0.3 mg/kg TAT-59 or tamoxifen to DMBA-induced mammary tumor-bearing rats. Each value is the mean ± SD from three rats (*ND* not detected)

Hours after last administration	After TAT-59-administration			After Tamoxifen administration		
	DP-TAT-59	DM-DP-TAT-59	1-en-DP-TAT-59	Tamoxifen	DM-tamoxifen	4-OH-tamoxifen
3	58.92±19.03	18.99± 9.43	28.47± 3.20	86.82±11.27	45.32±11.45	8.69±4.23
6	70.27±18.48	22.19± 10.55	37.20± 2.39	105.86±13.27	55.59± 6.72	18.56±2.75
12	156.57±70.46	150.17±107.54	13.97±16.06	79.94±18.29	60.70±16.82	11.78±1.44
24	37.08±18.53	16.33± 13.56	29.81± 6.25	48.10±15.60	27.91± 9.57	6.00±4.25
48	43.34±43.17	68.16± 94.33	10.32± 9.09	17.38± 4.27	8.92± 2.58	1.77±0.65
96	4.54± 5.50	25.14± 40.50	0.24± 0.42	8.81± 7.85	10.33±13.60	0.25±0.43
192	0.36± 0.62	0.51± 0.48	ND	0.76± 0.90	ND	ND
384	ND	0.63± 1.09	ND	ND	ND	ND
AUC (ng h/ml)	4473.95	6200.06	1292.40	3618.57	2003.84	393.33

Table 6 Relative binding activity of TAT-59 metabolites to estrogen receptors (*RBA* = (IC_{50} of estradiol/IC_{50} of anti-estrogen) × 100)

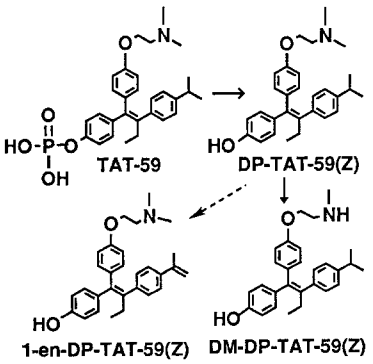
	IC_{50} (n*M*)	RBA
Estradiol	2.74	100
DP-TAT-59	1.78	153.9
1-en-DP-TAT-59	5.26	52.1
DM-DP-TAT-59	4.19	65.4
Tamoxifen	455	0.60
4-OH-tamoxifen	2.13	128.6

antiestrogen, toremifene (an analog of triphenylethylene), has similar metabolites and pharmacology to those of TAM [24]. On the other hand, TAT-59 was rapidly metabolized to DP-TAT-59, a compound showing a highly competitive activity against estradiol for ERs (Tables 2 and 4). Other metabolites of TAT-59, e.g. DM-DP-TAT-59 and 1-en-DP-TAT-59, also demonstrated a highly competitive activity against estradiol for ERs (Fig. 3). The rapid release of metabolites is due to a sufficient activity of phosphatase in both rats and humans. Based on the order of appearance of metabolites in the plasma after the single administration, the metabolic pathway of TAT-59 was assumed to be as shown in Fig. 4.

Other mechanisms of action of triphenylethylene derivatives have been studied in in vitro experiments using concentrations from 10^{-5} to 10^{-7} *M*. These mechanisms include calmodulin antagonistic activity [8], binding to nosteroidal antiestrogen binding sites [20] and protein kinase C inhibition [14]. Based on our pharmacodynamic data, the peak tissue level of the metabolites of TAT-59 after multiple administration was about 150 ng/g (Table 5), which is almost equivalent to 3.5×10^{-7} *M*. It was assumed that the total tissue level of metabolites of TAT-59 might be around 10^{-6} *M*. At such a high concentration, we may expect activities other than the antiestrogenic effect. At a concentration of approximately 10^{-6} *M*, triphenylethylene compounds are able to reverse multidrug resistance (MDR) [17]. As doxorubicin is a frequently used drug for the treatment of breast cancer, the good penetration of TAT-59 into tumor tissue and its MDR-reversing ability might be helpful in doxorubicin therapy.

These results suggest that TAT-59 may become a useful therapeutic agent for patients suffering from breast cancer.

Fig. 4 Proposed metabolic pathway of TAT-59



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