Cancer Chemother Pharmacol (1993) 32: 353–358

Cancer Chemotherapy and Pharmacology

Effect of toremifene on the growth, hormone receptors and insulin-like growth factor-1 of hormone-dependent MCF-7 tumors in athymic mice

Yuichi Iino, Yoshiki Takai, Tatsumasa Ando, Noritaka Sugamata, Michio Maemura, Takeshi Takeo, Susumu Ohwada, Yasuo Morishita

Second Department of Surgery, Gunma University School of Medicine, Showamachi, Maebashi, Japan

Received 3 December 1992/Accepted 12 March 1993

Abstract. Toremifene given in different sizes of silastic capsules was used to treat MCF-7 tumors in athymic mice. Toremifene inhibited the estradiol-stimulated growth of MCF-7 tumors in athymic mice. Average serum concentrations of toremifene obtained using a sustained-release preparation of the drug (in 0.5-, 1.0-, and 2.0-cm silastic capsules) increased gradually in a capsule-size-dependent fashion. Much higher levels of toremifene or *N*-demethyltoremifene were detected in tumors (target tissues of estrogen) as compared with muscles (non-target tissues of estrogen). The concentration of toremifene in serum (i.e., 10–30 ng ml^{-1}) was sufficient to inhibit the estrogen-stimulated growth of MCF-7 tumors at physiological (i.e., 200–400 pg ml^{-1}) serum estradiol concentrations in premenopausal women. No significant difference in estrogen receptor (ER) levels was found between the estradiol-alone group and the toremifene-treated groups. However, the ER levels in the toremifene-alone group and the no-treatment group (no toremifene or estradiol) tended to increase as compared with the estradiol-alone group. Toremifene blocked the estradiol-induced increase in progesterone receptor levels in a dose-dependent fashion. Insulin-like growth factor-1 (IGF-1) levels in the MCF-7 tumors significantly decreased in the toremifene-alone group as compared with the estradiol-alone group. These results show the antiestrogenic action of toremifene on hormone-dependent MCF-7 tumors in athymic mice.

Introduction

Toremifene is a triphenylethylene antiestrogen with characteristics similar to those of tamoxifen [13, 22, 26]. The structure of toremifene is a modification of the tamoxifen molecule [22]. It has a high affinity for estrogen receptors (ER) and an ability to induce progesterone receptors (PgR) [11]. Its antitumor effect has been shown in 7,12-dimethylbenz[a]anthracene (DMBA)-induced rat mammary carcinomas [12, 22] and in human breast tumors growing in athymic mice [22, 25]. The antitumor effect of the drug has also been clinically investigated in advanced breast cancer of postmenopausal women in several phase II [5, 25] and phase III studies [20]. The clinical usefulness of toremifene has been recognized internationally as being equivalent to that of tamoxifen in postmenopausal women with advanced breast cancer [20]. However, its clinical effectiveness in premenopausal patients has not been confirmed. One study has shown that either exogenous insulin-like growth factor-1 (IGF-1) or serum-free conditioned medium from MCF-7 cells containing IGF-1 activity was capable of transiently supporting the growth of these hormone-dependent cells in castrated nude mice in the absence of estrogen supplementation [2]. In the present study, the antitumor activity of the drug as well as its effect on hormone receptors and IGF-1 were examined in MCF-7 tumors in athymic mice so as to clarify the role of the drug and its similarity to or difference from tamoxifen.

Materials and methods

Tumors. A total of 2×10^6 cells of ER-positive MCF-7 were inoculated bilaterally into the thoracic mammary fat pads (1/side) of 6- to 7-week-old athymic mice. All animals were also implanted subcutaneously with a 1.0-cm silastic capsule containing 17 β-estradiol (described below) because tumors failed to grow in the absence of estrogen. Tumor measurements were performed weekly using slide calipers. Tumor sizes were obtained using the formula (length/2 × width/2) × π.

After 5 weeks of estrogen treatment, tumors had reached an average size of 0.5 cm^2. Animals were then randomized into six groups, and the estradiol capsules were removed. All animals in each group then received one of the following treatments: a 1.0-cm estradiol capsule (11 animals); a 2.0-cm toremifene capsule (8 animals); a 1.0-cm estradiol capsule plus a 0.5-cm (10 animals), 1.0-cm (12 animals), or 2.0-cm toremifene capsule (11 animals); or no treatment (7 animals). The size of each tumor was recorded weekly as a percentage of the initial tumor size at day zero, and mean values (± SE) for percentage changes at each time point were

Correspondence to: Yuichi Iino, Second Department of Surgery, Gunma University School of Medicine, 3-39-15, Showamachi, Maebashi 371, Japan

Table 1. Concentrations of estradiol, toremifene, and *N*-demethyltoremifene measured in the serum, tumor, and muscle of nude mice in each experimental group at the 5th week after the administration of estradiol and toremifene in silastic capsules

Group	Serum			Tumor		Muscle	
	E_2 (pg/ml)	TORE (ng/ml)	TORE-1 (ng/ml)	TORE (ng/g tissue)	TORE-1 (ng/g tissue)	TORE (ng/g tissue)	TORE-1 (ng/g tissue)
E_2[a] alone ($n = 6$)	372 ± 247[b, c]	–	–	–	–	–	–
E_2[a] + TORE, 0.5 cm ($n = 10$)	311 ± 235[b, d]	6.8 ± 2.1[e, f]	3.4 ± 3.0	278 ± 42*[g, h]	111 ± 28*[i, j]	73 ± 44[k]	7 ± 11
E_2[a] + TORE, 1.0 cm ($n = 12$)	241 ± 84[b]	12.9 ± 10.5[e, f]	4.0 ± 2.7	426 ± 276*[g, h]	196 ± 125*[i, j]	82 ± 33[k, l]	26 ± 24[m]
E_2[a] + TORE, 2.0 cm ($n = 11$)	182 ± 79[b]	24.5 ± 15.0	5.8 ± 3.6	918 ± 315*	423 ± 189*	148 ± 56	77 ± 68[m, n, o]
TORE 2.0 cm alone	33 ± 39	24.6 ± 9.3	5.6 ± 2.7	1104 ± 739*	352 ± 209*	120 ± 51	26 ± 25[m]
No treatment ($n = 7$)	–	–	–	–	–	–	–

Data represent mean values ± SD. E_2, Estradiol; TORE, toremifene; TORE-1, *N*-demethyltoremifene

[a] E_2 1.0-cm silastic capsule; the TORE or TORE-1 concentration detected in the tumor was significantly higher than that found in the muscle in each TORE-treated group (*$P < 0.01$)

[b] Significantly different from TORE, 2.0 cm alone ($P < 0.01$)

[c] Significantly different from E_2 + TORE, 2.0 cm ($P < 0.05$)

[d] Significantly different from E_2 + TORE, 2.0 cm ($P < 0.01$)

[e] Significantly different from TORE, 2.0 cm alone ($P < 0.01$)

[f] Significantly different from E_2 + TORE, 2.0 cm ($P < 0.01$)

[g] Significantly different from TORE, 2.0 cm alone ($P < 0.01$)

[h] Significantly different from E_2 + TORE, 2.0 cm ($P < 0.01$)

[i] Significantly different from TORE, 2.0 cm alone ($P < 0.01$)

[j] Significantly different from E_2 + TORE, 2.0 cm ($P < 0.01$)

[k] Significantly different from E_2 + TORE, 2.0 cm ($P < 0.01$)

[l] Significantly different from TORE, 2.0 cm alone ($P < 0.05$)

[m] Significantly different from E_2 + TORE, 0.5 cm ($P < 0.05$)

[n] Significantly different from E_2 + TORE, 1.0 cm ($P < 0.05$)

[o] Significantly different from TORE, 2.0 cm alone ($P < 0.01$)

calculated for each group. Tumors were measured for 5 weeks, after which all animals were killed and tissues were taken for determination of toremifene, for ER and PgR assays, and for IGF-1 measurement. Levels of ER and PgR in the cytosol of MCF-7 tumors were determined by enzyme-immunoassay using ER-EIA and PgR-EIA kits (Abbot Laboratories, Chicago, Ill.). Hormone-receptor assays were performed following the standard kit protocol. IGF-1 in the MCF-1 tumors was measured by radioimmunoassay using a supernatant of the homogenized MCF-7 tumor and somatomedin C (Ciba Corning) kits. Athymic mice were obtained from the Institute of Experimental Animal Research, Gunma University. Sterilized water and diet (MF; Oriental Yeast Co., Ltd., Tokyo) were given ad libitum.

Drug administration. Estradiol or toremifene was given by subcutaneous implantation of the respective silastic capsule. Silastic capsules were prepared by plugging one end of pieces of medical-grade silastic tubing of various lengths (inside diameter, 0.078 cm; outside diameter 0.125 cm; Dow Corning, Midland, Mich.) with silastic silicone type A medical adhesive (Dow Corning) and then filling them with either crystalline toremifene-free base (supplied by Nippon Kayaku Co., Ltd., Tokyo) or 17 β-estradiol (Sigma Chemical Co., St.Louis, Mo.) mixed 1:3 (w/w) with silastic 382 medical-grade elastomer (Dow Corning) without a catalyst. Capsules were completed by filling the open end of the pieces of tubing with adhesive and sterilizing them with radiation.

Estradiol and toremifene. Concentrations of circulating 17 β-estradiol, toremifene, and metabolites of toremifene were measured by a modification of the original determination method as described elsewhere [10, 15, 22] in serum samples taken from tumor-bearing mice. Blood samples were obtained by heart puncture using light ether anaesthesia at autopsy. After clotting for 2–3 h, samples were centrifuged at 2,000 *g*; the serum was removed and stored at –80° C until analysis. Toremifene measurements in tissues were made using normal-phase high-performance liquid chromatography (HPLC) with fluorescent detection of the parent compound and metabolites as previously described [10, 22, 23]. Duplicate assays were performed in each determination.

Statistical analyses. Statistical analyses were performed using the Wilcoxon test.

Results

Estradiol and toremifene concentrations in the serum and tissues of animals

Table 1 shows the concentrations of estradiol, toremifene, and *N*-demethyltoremifene measured in the serum, tumor, and muscle of nude mice after 5 weeks of toremifene treatment. 4-Hydroxytoremifene was not detectable at the doses used. Average circulating estradiol levels in each treatment group decreased in a (toremifene) capsule-size-dependent fashion. A significant difference in serum estradiol levels was found between the estradiol-only group and the estradiol +2.0-cm toremifen-capsule group. Average toremifene and *N*-demethyltoremifene concentrations in the serum increased in a capsule-size-dependent fashion in the toremifene-treated groups, and those in the tissues showed a similar increase. Toremifene and *N*-demethyltoremifene concentrations detected in the tumors (target tissue) were significantly higher than those found in the muscles (non-target tissue) in each treated group.

Growth-inhibitory action of toremifene on breast tumors and uterine tissue

MCF-7 tumors grew remarkably and continuously in the 1.0-cm estradiol-capsule group. Percentage changes in

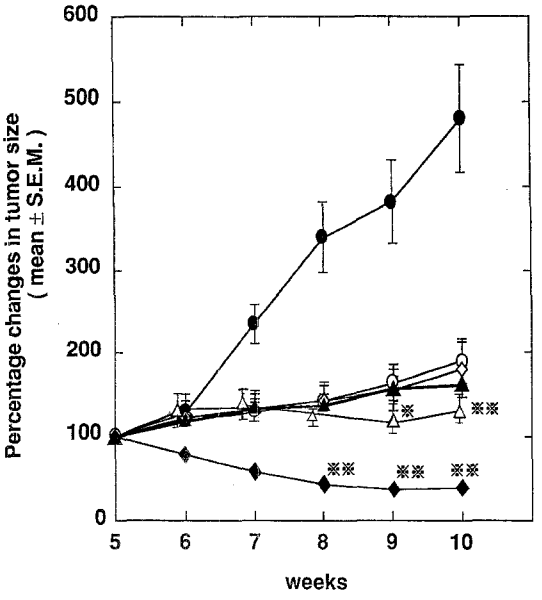

Fig. 1. Antitumor action of toremifene on the estradiol-stimulated growth of MCF-7 tumors in athymic mice. The tumor growth was significantly blocked in the no-treatment group (P <0.05–0.01) and in the toremifene (TORE)-alone group (P <0.01) as compared with the estradiol (E_2)-alone group. ●, 1.0-cm E_2-capsule group; ○, 1.0-cm E_2-capsule + 0.5-cm TORE-capsule group; ◇, 1.0-cm E_2-capsule + 1.0-cm TORE-capsule group; ▲, 1.0-cm E_2-capsule + 2.0-cm TORE-capsule group; ◆, 2.0-cm TORE-capsule group (n = 7); △, no-treatment group. ※ P <0.05, ※※ P <0.01

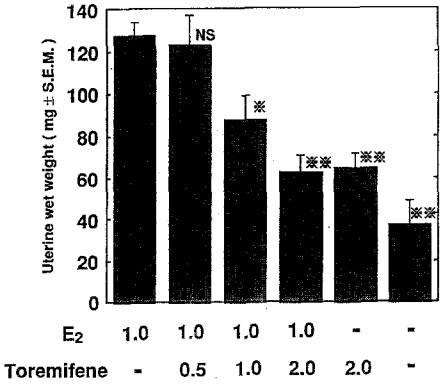

Fig. 2. Effect of toremifene on the uterine weights in athymic mice in each group. The uterine wet weights were significantly lower in the estradiol (E_2) + 1.0-cm toremifene (TORE)-capsule group (P <0.05), in the E_2 + 2.0-cm TORE-capsule group (P <0.01), in the 2.0-cm TORE-capsule-alone group (P <0.01) and in the no-treatment group (P <0.01) as compared with the E_2-alone group. E_2, Estradiol capsule size (cm); *Toremifene*, toremifene capsule size (cm). *NS*, Not significant. ※ P <0.05, ※※ P <0.01

tumor size at the 5th week were 190% in the 1.0-cm estradiol-capsule + 0.5-cm toremifene-capsule group, 180% in the 1.0-cm estradiol-capsule + 1.0-cm toremifene-capsule group, and 163% in the 1.0-cm estradiol-capsule + 2.0-cm toremifene-capsule group. Toremifene showed an augmented antitumor effect on the estradiol-stimulated growth of MCF-7 tumors in a capsule-size-dependent fashion. However, the differences in tumor

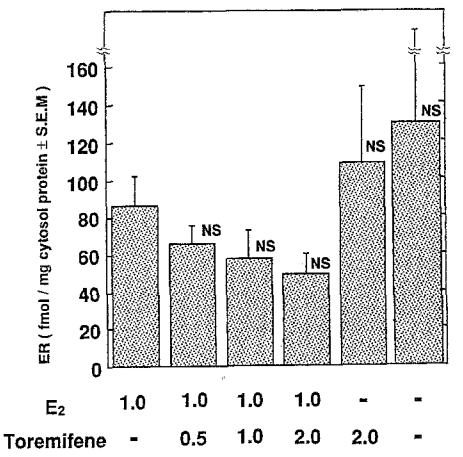

Fig. 3. ER levels in MCF-7 tumors in each group. Significant differences in ER levels were not found among the groups; however, ER levels tended to be elevated in the estrogen-withdrawal groups as compared with the estradiol (E_2)-alone group. E_2, Estradiol capsule size (cm); *Toremifene*, toremifene capsule size (cm). *NS*, Not significant

size observed among the three groups were not significant. A pronounced decrease in tumor size was recognized in the 2.0-cm toremifene-capsule group (Fig. 1).

Toremifene partially blocked the estrogen action; however, it did not completely stop the estrogen-stimulated MCF-7 tumor growth at physiological estradiol concentrations (i.e. 200–400 pg ml^{-1}). Toremifene did not completely block MCF-7 tumor growth in the toremifene-alone group, which showed low levels of serum estradiol. Uterine wet weights were also measured in all animals at the end of the experiment. Toremifene partially reversed the uterine growth induced by estrogen (Fig. 2).

Effect of an increased dose of toremifene on the steroid receptor content and IGF-1 levels in MCF-7 tumors

No significant difference in the ER levels in MCF-7 tumors was found among the estradiol-alone group, the toremifene-treated groups, and the no-treatment group (Fig. 3). Toremifene blocked the estradiol-induced increase in PgR in the tumors in a dose-dependent fashion (Fig. 4). The IGF-1 levels in the tumors were lower in the toremifene-alone group (P <0.05) and the no-treatment group (P <0.05) as compared with the estradiol-alone group (Fig. 5).

Discussion

The effectiveness of tamoxifen has been reported in many experimental studies [4, 8, 9, 19, 21] and clinical trials [3, 16]. Tamoxifen has provided benefits to premenopausal as well as postmenopausal patients [17, 24]. Robinson et al. [22] have reported that toremifene is effective in inhibiting the growth of human breast cancer cells grown either in culture or in athymic mice. Toremifene (10^{-10}–10^{-6} M) inhibited the growth of MCF-7 breast cancer cells in vitro

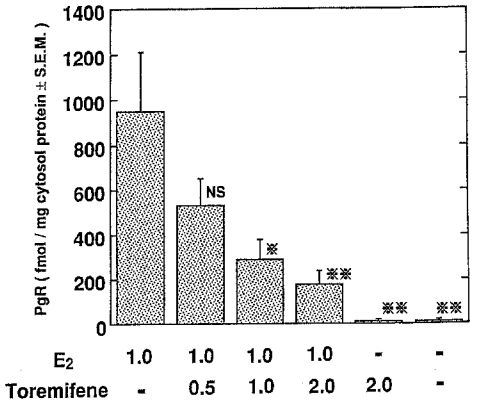

Fig. 4. PgR levels in MCF-7 tumors in each group. Significant differences in PgR levels were seen in the estradiol (E_2) + toremifene (TORE), 1.0 cm, group (P <0.05), the E_2 + TORE, 2.0 cm group (P <0.01), the TORE-alone group (P <0.01) and the no-treatment group (P <0.01) as compared with the E_2-alone group. E_2, Estradiol capsule size (cm); *Toremifene*, toremifene capsule size (cm). *NS*, Not significant. ※ P <0.05, ※※ P <0.01

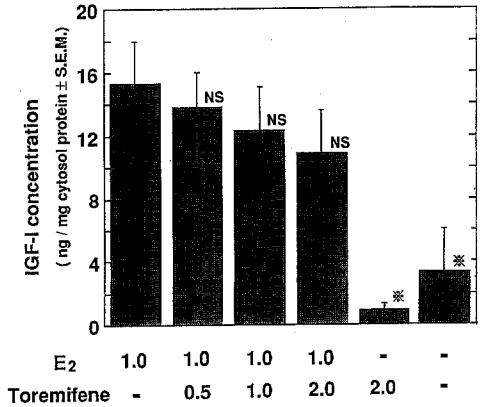

Fig. 5. IGF-1 concentrations in MCF-7 tumors in each group. The IGF-I concentrations were significantly lower in the 2.0-cm toremifene-capsule-alone group (P <0.05) and in the no-treatment group (P <0.05) as compared with the estradiol (E_2)-alone group. *NS*, Not significant. ※ P <0.05

but was ineffective against hormone-independent MDA-MB-231 cells. This activity was reproduced in vivo using the athymic mouse model. Maximal MCF-7 tumor growth was produced in athymic mice by circulating estradiol levels of approximately 200 pg/ml (from a 0.5-cm silastic capsule implanted s.c.). Toremifene (77 ± 44 μg/day from a 2-cm silastic capsule) inhibited estradiol (0.5-cm capsule)-stimulated growth by more than 70%. No tumor growth was observed in mice treated with toremifene alone, although toremifene acted as a weak partial agonist and a potent antagonist on the mouse uterus.

The uterine wet weight of ovariectomized mice was ordered into estradiol (0.5-cm silastic capsule), toremifene (2-cm silastic capsule), estradiol plus toremifene, and control groups, respectively. Prolonged toremifene treatment of athymic mice (3 months) with the same type of time-release preparation produced a change in the uteri, making them refractory to subsequent prolonged estrogen stimulation. The growth of MDA-MB-231 tumors was not influenced by either estradiol or toremifene. Toremifene (200 μg/day) was effective in preventing the development of 7,12-dimethylbenzanthracene-induced rat mammary tumors when it was given p.o. beginning on day 28 after carcinogen administration. The antitumor activity was reversed when the toremifene treatment was stopped. These findings indicate that toremifene is a tumoristatic agent rather than a tumoricidal agent.

In the present study, toremifene inhibited the estradiol-stimulated growth of MCF-7 tumors in athymic mice to the same extent reported by Robinson et al. [22]. Maximal MCF-7 tumor growth was produced in athymic mice by circulating estradiol levels of approximately 370 pg/ml (from a 1.0-cm silastic capsule implanted s.c.). The growth-inhibitory action of toremifene was shown. Toremifene (2.0-cm silastic capsule) inhibited estradiol (1.0-cm capsule)-stimulated growth by more than 65%. No tumor growth was observed in mice that received no treatment, and the observed decrease in MCF-7 tumor size, even in the toremifene-alone group, was not dramatic because the mechanism of action of toremifene is not tumoricidal but tumoristatic indicated by Robinson et al.'s report.

N-Demethyltoremifene levels were lower in the serum, tumor, and muscle as compared with toremifene levels. However, *N*-demethyltoremifene has been reported to have almost the same biological activity as toremifene [18]. Toremifene levels in both the serum and the tumor increased in a dose-dependent fashion; however, *N*-demethyltoremifene levels in the serum remained essentially unchanged, whereas those in the tumor increased in correlation with the toremifene dose. These findings suggest that the *N*-demethyltoremifene in the serum may have entered the cancer cells more easily than toremifene in all of the toremifene-treated groups and indicate a biological activity against cancer cells.

Serum concentrations of toremifene ranged from 3 to 65 ng ml^{-1}, depending on the capsule size used, and much higher levels of toremifene were detected in target tissues of estrogen as compared with non-target tissues.

These serum levels are sufficient to inhibit the estrogen-stimulated growth of MCF-7 tumors exposed to physiological (i.e., 200–400 pg ml^{-1}) serum estradiol concentrations in premenopausal patients. In addition, toremifene concentrations in the tumor (target tissues) might influence the antitumor activity as strongly as serum toremifene levels. The uterine growth induced by estrogen was reversibly controlled by toremifene. These findings are almost the same as the results reported by Robinson et al. [22].

Toremifene has been used in postmenopausal patients with advanced breast cancer as first-line treatment at dose levels of 20, 60, and 240 mg and as second-line or subsequent treatment at high-dose levels of 200–240 mg [5]. However, on the basis of the fundamental data obtained in the present study, we would expect that toremifene would be as effective as tamoxifen in premenopausal patients as well.

In our previous study [8], athymic animals were divided into three groups: an estradiol-alone group, an estradiol

plus a tamoxifen-capsule group, and an estradiol plus a tamoxifen-capsule and -injections group (1 mg given i.p. three times weekly; high-dose tamoxifen group). ER and PgR levels in tumors were determined by enzyme-immunoassay (EIA) using ER-EIA and PR-EIA kits. Tamoxifen caused a dose-dependent increase in ER levels in MCF-7 tumors and also blocked the estradiol-induced increase in PgR levels in a dose-dependent fashion. Although tamoxifen is known to increase PgR levels in some target tissues in vivo and in tumor cells in vitro [1, 6, 7, 14], it prevented estrogen-induced increases in PgR levels in MCF-7 tumors in vivo.

Welshons and Jordan [27] reported that estrogen withdrawal caused similar increases in ER levels and decreases in PgR levels in MCF-7 cells in vitro. In the present study, toremifene showed almost the same trends reported by Iino et al. [8] and by Welshons and Jordan [27] for PgR levels. An increase in ER levels was shown only by the toremifene-alone group and the no-treatment group because of estrogen withdrawal. However, since a high dose of toremifene was not given in the present study a pronounced difference in ER or PgR levels might not have been detectable among the groups as compared with our previous study using a high dose of tamoxifen.

Yee et al. [28] propose that the IGFs are important stimulators of breast cancer cells and that their growth-promoting effects may be mediated by autocrine, paracrine, or endocrine mechanisms. Breast cancer cells produce only IGF-II, whereas both IGF-I and IGF-II may be produced by the fibroblast. Breast cancer cells express both type I and type II IGF receptors, and IGF-binding proteins produced by the breast cancer cell may influence the interaction between the ligand and the receptor [28]. We could not determine serum IGF-I levels because all sera were used for the assays of circulating estradiol, toremifene, and toremifene metabolites. Toremifene decreased the IGF-I levels in the MCF-7 tumors in the absence of estrogen in our experiment. The IGF-I levels were too low to clarify the interaction between the mechanism of action of toremifene and the IGF system. However, there is a possibility that toremifene has a close relationship with the IGF system and that it may decrease both tissue and serum IGF-I levels because it inhibits the estrogen-stimulated growth of MCF-7 cells that acts to stimulate IGF expression in the fibroblast [27].

We conclude that the inhibitory effect on hormone-dependent tumor growth, the regulation of ER and PgR levels, the reversible effect on uterine growth and the inhibitory effect on IGF-1 induction observed in the present study following toremifene administration were caused by an antiestrogenic action of the drug and that toremifene is an effective agent for premenopausal as well as postmenopausal patients as extrapolated from data obtained in animal models.

Acknowledgements. We would like to thank Nippon Kayaku Co., Ltd. (Tokyo) for supplying the crystalline toremifene-free base and for its cooperation during this work.

References

1. Campen CA, Jordan VC, Gorski J (1985) Opposing biological action of antiestrogens in vitro and in vivo: induction of progesterone receptor in mouse uterus. Endocrinology 116: 2327
2. Dickson RB, McManaway ME, Lippman ME (1986) Estrogen-induced factors of breast cancer cells partially replace estrogen to promote tumor growth. Science 232: 1540
3. Furr BJA, Jordan VC (1984) The pharmacology and clinical uses of tamoxifen. Pharmacol Ther 25: 127
4. Gottardis MM, Robinson SP, Satyaswaroop PG, Jordan VC (1988) Contrasting actions of tamoxifen on endometrial and breast tumor growth in the athmic mouce. Cancer Res 48: 812
5. Hietanen T, Baltina D, Johansson R, Numminen S, Hakala T, Helle L, Valavaara R (1990) High dose toremifene (240 mg daily) is effective as first line hormonal treatment in advanced breast cancer. An ongoing phase II multicenter Finnish-Latvian cooperative study. Breast Cancer Res Treat 16 [Suppl]: S37
6. Horwitz KB, Koseki Y, McGuire WL (1978) Estrogen control of progesterone receptor in human breast cancer. Role of estradiol and estrogen, Endocrinology 103: 1742
7. Iino Y, Ishikawa H, Izuo M, Takikawa H (1989) Sequential hormone therapy with medroxyprogesterone acetate for 7,12-dimethylbenz[a]anthracene-induced rat mammary tumors. Jpn J Clin Oncol 19: 45
8. Iino Y, Wolf DM, Langan-Fahey SM, Johnson DA, Ricchio M, Thompson ME, Jordan VC (1991) Reversible control of oestradiol-stimulated growth of MCF-7 tumors by tamoxifen in the athymic mouse. Br J Cancer 64: 1019
9. Jordan VC, Allen KE, Dix CJ (1980) Pharmacology of tamoxifen in laboratory animals. Cancer Treat Rep 64: 745
10. Jordan VC, Fritz NF, Tormey DC (1987) Endocrine effects of adjuvant chemotherapy and long-term tamoxifen administration on node-positive patients with breast cancer. Cancer Res 47: 624
11. Kallio S, Kangas L, Blanco G, Johansson R, Karjalainen A, Perila M, Piippo I, Sundquist H, Soclervall M, Toivola R (1986) A new triphenylethylene compound, Fc-1157a: I. Hormonal effects. Cancer Chemother Pharmacol 17: 103
12. Kangan L, Nieminen A-L, Blanco G, Gronroos M, Kallio S, Karjalainen H, Perila M, Sodervall M, Toivola R (1986) A new triphenylethylene compound, Fc-1157a. II. Antitumor effects. Cancer Chemother Phamacol 17: 109
13. Kohler PC, Hamm JT, Wiebe VJ, DeGregorio MW, Shemano I, Tormey DC (1990) Phase I study of toremifene and the pharmacokinetics of toremifene in patients with cancer. Breast Cancer Res Treat 16 [Suppl]: S19
14. Koseki Y, Zava DT, Chamness GC, McGuire WL (1977) Progesterone interaction in the rat uterus: receptor effects. Steroids 30: 168
15. Langan-Fahey SM, Tormey DC, Jordan VC (1990) Tamoxifen metabolites in patients on long-term adjuvant tamoxifen therapy for breast cancer. Eur J Cancer 26: 883
16. Legha SS, Carter SK (1974) Antiestrogens in the treatment of breast cancer. Cancer Treat Rev 3: 205
17. Manni A, Pearson OH (1980) Antiestrogen-induced remissions in premenopausal women with stage IV breast cancer: effects on ovarian function. Cancer Treat Rep 64: 779
18. Nippon Kayaku Co., Ltd. (1986) Summary of toremifene. Nippon Kayaku Co., Ltd., Tokyo, p 20
19. Osbone CK, Mobbs SK, Clark GM (1985) Effects of estrogens and antiestrogens on growth of human breast cancer cells in athymic nude mice. Cancer Res 45: 584
20. Pyrhonen SO (1990) Phase III studies of toremifene in metastatic breast cancer. Breast Cancer Res Treat 16 [Suppl]: S41
21. Robinson SP, Jordan VC (1987) Reversal of the antitumor effects of tamoxifen by progesterone in the 7,12-dimethylbenzanthracene-induced rat mammary carcinoma model. Cancer Res 47: 5386
22. Robinson SP, Parker CJ, Jordan VC (1990) Preclinical studies with toremifene as an antitumor agent. Breast Cancer Res Treat 16 [Suppl]: S9

23. Robinson SP, Langan-Fahey SM, Johnson DA, Jordan VC (1991) Metabolites, pharmacodynamics and pharmacokinetics of tamoxifen in rats and mice compared to the breast cancer patient. Drug Dispos Metab 19: 36
24. Sawaka CA, Pritchard KI, Paterson AHG, Sutherland DJA, Thomson DB, Shelley WE, Myers RE, Mobbs BG, Malkin A, Meakin JW (1986) Role and mechanism of action of tamoxifen in premenopausal women with metastatic breast cancer. Cancer Res 46: 3152
25. Valavaara R (1990) Phase II trials with toremifene in advanced breast cancer: a review. Breast Cancer Res Treat 16 [Suppl]: S31
26. Valavaara R, Pyrhonen S, Heikkinen M, Rissanen P, Blanco G, Tholix E, Nordman E, Taskinen P, Holsti L, Hajba A (1988) Toremifene, a new antiestrogenic compound, for treatment of advanced breast cancer. Phase II study. Eur J Cancer Clin Oncol 24: 785
27. Welshons WV, Jordan VC (1987) Adaptation of estrogen-dependent MCF-7 cells to low estrogen (phenol red-free) culture. Eur J Cancer Clin Oncol 23: 1935
28. Yee D, Rosen N, Favoni RE, Cullen KJ (1990) The insulin-like growth factors, their receptors and their binding proteins in human breast cancer. In: Lippman ME, Dickson RB (eds) Regulatory mechanisms in breast cancer. Kluwer, Boston, pp 93–106