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Pharmacokinetics of toremifene and its metabolites in patients with advanced breast cancer*

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Summary. A multicenter phase I pharmacokineticstudy of a new triphenylethylene antiestrogen, toremifene, was examined in 70 patients with advanced breast cancer. Patients were randomized to receive single daily oral doses of either 10, 20, 40, 60, 200, or 400 mg for 8 weeks. Plasma toremifene and its major metabolites, *N*-desmethyltoremifene and 4-hydroxytoremifene, were determined weekly during therapy and at 0, 7, 14, and 21 days after the discontinuation of therapy. The time to reach steady-state plasma concentrations was between 1 and 5 weeks, with steady-state being achieved earlier (1–2 weeks) at daily doses of 200 and 400 mg. The time to peak concentration following oral doses of toremifene ranged from 1.5 to 4.5 h. The terminal half-life of elimination was 5.0, 6.0, and 5.0 days for toremifene, desmethyltoremifene, and 4-hydroxytoremifene, respectively. Plasma concentrations of 4-hydroxytoremifene were detectable only at high doses (200 and 400 mg/day) of toremifene. The results of this phase I pharmacokinetic study show that toremifene has metabolic and kinetic patterns that are similar to those previously reported with tamoxifen.

Introduction

Antiestrogen therapy is a form of hormonal therapy that has been used in the treatment of breast cancer since 1974. In recent years, numerous clinical studies have combined antiestrogen therapy with chemotherapy in an attempt to improve response rates in advanced breast cancer. Currently, the most commonly used antiestrogenic agent for the treatment of patients with advanced breast cancer is tamoxifen. Although many patients with estrogen-receptor-positive tumors may benefit from tamoxifen therapy, there remains a patient population that fails to respond to antiestrogen therapy.

Toremifene is a new triphenylethylene antiestrogen compound that is structurally similar to tamoxifen (Fig. 1). The efficacy of toremifene and tamoxifen in patients with estrogen-receptor-positive breast cancer appears to be comparable, but effective daily doses of toremifene are higher (60 mg/day) than those of tamoxifen (20 mg/day) [7]. Following oral administration, toremifene undergoes metabolic conversion by both the *N*-desmethylation and 4-hydroxylation pathways. The two major active metabolites, *N*-desmethyltoremifene and 4-hydroxytoremifene are detected in plasma soon after the start of therapy. In the present phase I study, the steady-state pharmacokinetics of toremifene and its major metabolites were examined following oral administration of the drug.

Patients and methods

Patient characteristics. A total of 70 patients with advanced breast cancer were entered for evaluation of the pharmacokinetics of toremifene. Postmenopausal patients refractory to previous cytotoxic, hormonal, and radiation therapy were eligible. Patients with prior hysterectomies, oophorectomies, or salpingoophorectomies were included. Eligibility was independent of estrogen receptor status. Patients receiving prior therapies were eligible in the absence of myelosuppression (WBC $\geq 3{,}500$; granulocytes, $\geq 1{,}500$; platelets, $\geq 100{,}000$). A washout period of at least 3 weeks was required following previous therapy. Patients were required at study entry to have an ECOG performance status of 0–2. Adequate renal function, defined as a serum creatinine level of <2.0 mg/dl and a 24-h creatinine clearance of >50 ml/min, were required. Patients were required to have serum bilirubin levels of <2.0 mg/dl as well as SGOT and SGPT values <1.5 times the upper limit of normal at each institute.

Toremifene administration. Patients were randomly assigned to one of six (10, 20, 40, 60, 200, or 400 mg/day) treatment groups. Each patient received single daily doses of toremifene by oral administration. Doses were given as 10-, 20-, 60-, and 200-mg tablets each morning, 30 min prior to breakfast. Treatment was continued for 8 weeks to enable the attainment of steady-state blood levels of toremifene and its major metabolites. On days requiring blood samples, patients were instructed to delay tablet ingestion until after blood was drawn. Patient compliance was documented by weekly tablet counts.

Blood sampling and drug assay. Blood samples were collected in pre-iced heparinized tubes at various time points during and after the cessation of toremifene therapy. The

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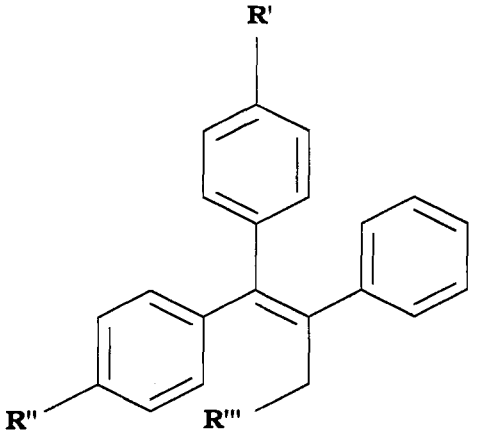

Toremifene
R' = $OCH_2CH_2N(CH_3)_2$
R'' = H
R''' = CL

Tamoxifen
R' = $OCH_2CH_2N(CH_3)_2$
R'' = H
R''' = H

N-desmethyltoremifene
R' = $OCH_2CH_2NHCH_3$
R'' = H
R''' = CL

4-hydroxytoremifene
R' = $OCH_2CH_2N(CH_3)_2$
R'' = OH
R''' = CL

Fig. 1. Chemical structure of toremifene and its metabolites

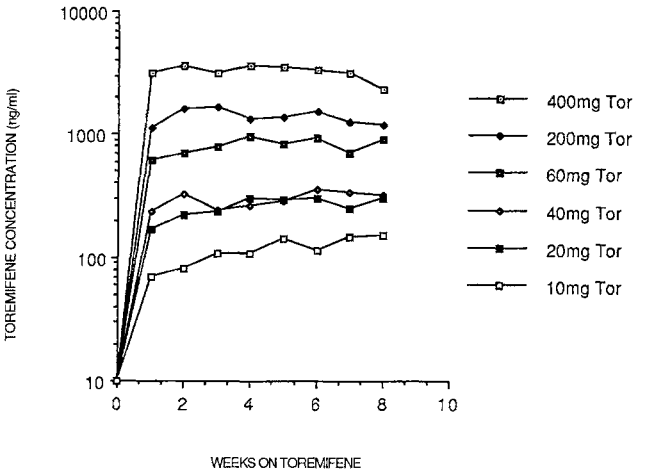

Fig. 2. Plasma concentrations of toremifene after doses of 10, 20, 40, 60, 200, and 400 mg/day vs time. Standard deviations are noted in Table 1

exact time of sampling was recorded in each case, and this value was used for pharmacokinetic analysis. Blood levels of toremifene and its metabolites were determined at 0, 1.5, 3.0, 4.5, 6.0, and 24 h and at 7, 14, and 21 days following the last dose. Plasma was separated from whole blood by centrifugation and was immediately frozen (−20° C) until analysis.

Quantitation of toremifene and its metabolites in plasma samples was carried out using photoactivation and HPLC analysis as previously described [5]. Briefly, plasma samples (1 ml) were thawed at room temperature and spiked with an internal standard (nafoxidine). Each sample was extracted with 2% *n*-butanol in hexane and irradiated with high-intensity UV light (254 nm). Samples were analyzed by HPLC using a C-18 reverse-phase column and eluded isocratically with a mobile phase of water and triethylamine in methanol. Fluorescence of all compounds were measured at a wavelength of 266 nm. The sensitivity of this assay was 8.0, 15.0, and 5.0 ng/ml for toremifene, *N*-desmethyltoremifene and 4-hydroxytoremifene, respectively. Linearity was measured through a concentration range of 25–750 ng/ml for all compounds, with a correlation coefficient of >0.98.

Pharmacokinetic analysis. Blood samples were drawn weekly for steady-state analysis of toremifene, *N*-desmethyltoremifene, and 4-hydroxytoremifene. Steady-state concentrations, time to steady state, and AUC over the

Table 1. Summary of toremifene and desmethyltoremifene pharmacokinetic parameters

	Toremifene			N-Desmethyltoremifene		
Dose (mg/day)	AUC (ng/ml·week)	T_{ss} (weeks)	C_{ss} (ng/ml)	AUC (ng/ml·week)	T_{ss} (weeks)	C_{ss} (ng/ml)
10 (n = 7)	841 (441)	3.5	135 (77)	2,517 (1,518)	4.0	468 (302)
20 (n = 8–9)	1,901 (755)	3.0	281 (112)	6,035 (1,970)	4.0	988 (394)
40 (n = 6–10)	2,098 (1,316)	1.5	308 (208)	7,866 (4,331)	2.0	1,348 (839)
60 (n = 8–12)	5,701 (1,817)	2.0	879 (400)	18,902 (6,391)	3.5	3,058 (1,153)
200 (n = 4–8)	9,880 (2,104)	1.0	1,413 (717)	34,868 (8,661)	2.0	5,942 (2,770)
400 (n = 9–10)	25,677 (13,542)	1.0	3445 (1,867)	81,397 (36,250)	2.0	11,913 (5,611)

AUC and C_{ss} values represent mean values; T_{ss} represents median values; standard deviations are in parentheses

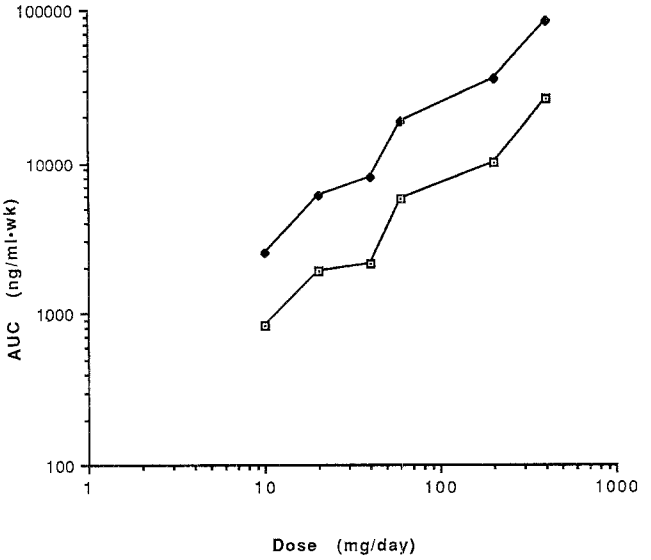

Fig. 3. AUC of toremifene *(open symbols)* and desmethyltoremifene *(closed symbols)* vs dose. Standard deviations are noted in Table 1

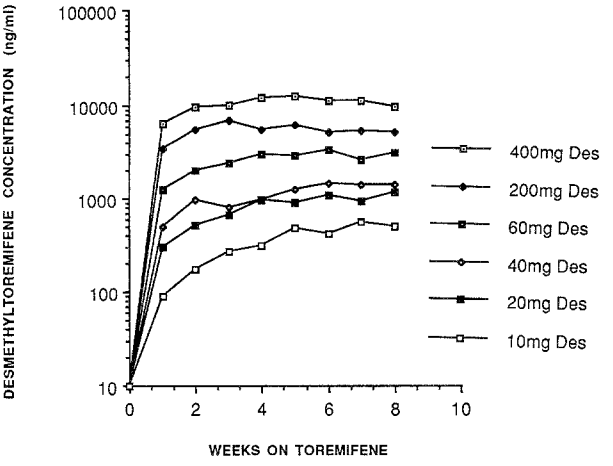

Fig. 4. Plasma concentrations of desmethyltoremifene after doses of 10, 20, 40, 60, 200, and 400 mg/day vs time. Standard deviations are noted in Table 1

8-week study period were determined. Following the completion of 8 weeks of therapy, seven patients were examined for absorption kinetics (time to peak, peak concentrations) and elimination kinetics (half-life, elimination rate constant) following the last dose. Pharmacokinetic analyses were done using compartment- and model-independent estimations of pharmacokinetic parameters based on statistical moment theory [4, 9]. The areas under the plasma time curves were calculated using linear and logarithmic trapezoidal methods [1, 13]. The half-life and elimination-rate constant were estimated by fitting three or more data points to a monoexponential equation [8]. Time to steady-state levels were defined as the time it takes to reach 90% of the steady-state plasma concentration.

Results

Steady-state plasma concentrations following low doses of toremifene (10–40 mg/day) were in the range of 135–308 ng/ml, whereas higher doses (60–400 mg/day) achieved steady-state concentrations in the range of 879–3,445 ng/ml (Table 1). The time to steady state following high doses (200 and 400 mg/day) of toremifene occurred within 1–2 weeks, whereas at lower doses (<40 mg) steady-state concentrations were achieved within 3–4 weeks (Fig. 2). Total area under the concentration vs time curve (AUC) during the first 8 weeks was also dose-proportional (Fig. 3).

Metabolites

Plasma concentrations of *N*-desmethyltoremifene and 4-hydroxytoremifene are shown in Figs. 4 and 5. Steady-state *N*-desmethyltoremifene concentrations were achieved between 2 and 4 weeks and were 3.5–4.5 times higher than toremifene steady-state concentrations (Table 1). The AUC for desmethyltoremifene ranged between 2,517 and 81,397 ng/ml·week. Both the AUC and steady-state *N*-desmethyltoremifene concentrations showed dose dependency (Table 1).

Plasma concentrations of 4-hydroxytoremifene were detected in patients receiving doses of 200 and 400 mg. Steady-state levels were achieved after 2–3 weeks and accounted for approximately 25%–30% of steady-state toremifene concentrations (Table 2). Total AUCs over the 8-week treatment period were 2,967 and 6,277 ng/ml·week at daily doses of 200 and 400 mg, respectively.

Plasma concentrations of toremifene were determined at three dosing levels following discontinuation of therapy. Toremifene concentrations were evaluated at 1.5, 3.0, 4.5, and 6.0 h after the last dose. Peak serum toremifene concentrations of 3,031 and 4,370 ng/ml were achieved in two patients 4.5 h after a 400-mg dose. Peak toremifene concentrations in four patients receiving a 60-mg dose were 1,117–1,270 ng/ml, with peak levels occurring between 1.5 and 3.0 h. The time to peak toremifene concentrations in three patients after a 20-mg dose was more variable (1.5–4.5 h), with peak concentrations in the range of 198–669 ng/ml. Peak concentrations of desmethyltoremifene were reached between 3 and 6 h after the last dose, with concentration ranges of 538–2,622 (20 mg/day), 2,709–5,769 (60 mg/day), and 7,937–9,135 ng/ml (400 mg/day). The 4-hydroxymetabolite was measured in one patient with peak concentrations of 383–515 ng/ml, occurring 4.5–6.0 h after a 400-mg dose.

The elimination half-life of toremifene at each dosing level is shown in Table 3. The median toremifene half-life of elimination for all dosing groups was 5 days. The half-lives for *N*-desmethyltoremifene and 4-hydroxytoremifene were 6 and 5 days, respectively (Tables 2 and 3).

Concentrations of toremifene and its metabolites remained detectable up to 21 days following the discontinuation of toremifene. Toremifene concentrations were >24 ng/ml in 80% of patients at day 21. *N*-desmethyltoremifene concentrations were in the range of 48–1,323 ng/ml and were approximately 6.5 times the

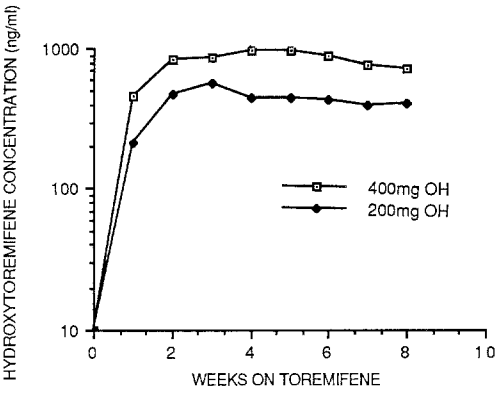

Fig. 5. Plasma concentrations of hydroxytoremifene vs time after doses of 200 and 400 mg/day. Standard deviations are noted in Table 2

toremifene concentration. 4-Hydroxytoremifene was only detected in a single patient, with levels of 10.7 ng/ml remaining at day 21; this was 10% of the toremifene concentration measured in this same patient.

Discussion

Toremifene is a new antiestrogen compound that is structurally and pharmacologically similar to tamoxifen. Preliminary studies have demonstrated that toremifene and tamoxifen have similar growth inhibitory effects in an estrogen-receptor-positive MCF-7 cell line [8]. Toremifene is similar to other antiestrogens in that it depletes cytosolic estrogen receptors by complexing and transferring them to the nucleus [6]. However, the half-life of the toremifene estrogen-receptor complex is short (3 days) compared with that of the tamoxifen-receptor complex (9 days) [7].

Although toremifene and tamoxifen show similar antiestrogenic activity at low doses, their mechanisms of action may differ [2, 12]. At high doses, toremifene exerts an antitumor effect that appears to be independent of its antiestrogenic effects. This biphasic action of toremifene may be mediated by specific proteins known as AEBS (antiestrogen binding sites) [10]. Watts et al. [11] have demonstrated a cytotoxic action of triphenylethylenes mediated by AEBS that is concentration-dependent. Structurally toremifene differs from tamoxifen by a single chloride ion at the R2 position. Chlorination of the R2 side-chain on other triphenylethylenes increases affinity for AEBS. Therefore, toremifene's affinity for AEBS may be increased, leading to its improved cytotoxic effects at high concentrations [2]. The mechanism underlying toremifene's increased in vitro cytolytic activity and its in vivo antitumor activity at high doses remains unresolved.

The present phase I study characterizes the plasma pharmacokinetics of toremifene after continuous oral dosing. Steady-state concentrations, AUCs, half-life, and time to peak concentrations after the last dose were evaluated for toremifene and its major metabolites *N*-desmethyltoremifene and 4-hydroxytoremifene at various dosing levels. Steady-state concentrations appeared to be more rapidly achieved at high doses of toremifene. Fluctuations in plasma levels noted at steady-state concentrations in all patients may be attributed to enterohepatic recirculation of the drug. Plasma kinetics demonstrated long half-lives for toremifene (5.0 days), desmethyltoremifene (6 days), and 4-hydroxytoremifene (5.0 days). The long half-life of toremifene may be due to both high plasma protein binding and enterohepatic recirculation. The kinetic behavior of toremifene following chronic

Table 2. Summary of 4-hydroxytoremifene pharmacokinetics

Dose (mg/day)	AUC (ng/ml·week)	T_{ss} (weeks)	C_{ss} (ng/ml)	$T_{1/2}$ (days)[a]	K ($days^{-1}$)
200 ($n = 5-8$)	2,967 (682)	1–2	438 (175)	–	–
400 ($n = 10$)	6,277.9 (2,397)	3–4	889 (421)	5.0	0.14

[a] Based on single patient

Table 3. Summary of toremifene and desmethyltoremifene pharmacokinetic elimination data

Dose	Toremifene		Desmethyltoremifene	
	$T_{1/2}$ (days)	K ($days^{-1}$)	$T_{1/2}$ (days)	K ($days^{-1}$)
20 ($n = 3$)	7.3 (5–10.4)	0.11	8.8 (4.5–15)	0.10
60 ($n = 3$)	4.5 (4–5)	0.16	5.2 (4.3–6.0)	0.14
400 ($n = 1$)	4.7[a] (–)	0.15[a]	6.3[a] (–)	0.11[a]

[a] Based on a single patient; range is noted in parentheses

dosing is very similar to that reported by Fabian et al. [3] for tamoxifen.

The *N*-desmethylation of both toremifene and tamoxifen appears to represent the primary metabolic pathway. Whereas 4-hydroxylation has been shown to be a minor metabolic pathway for tamoxifen, it appears to amplify at high doses of toremifene. Saturation of the *N*-desmethylation pathway with an associated increase in 4-hydroxylation may occur. Future studies examining the correlation between increased 4-hydroxylation and previously reported increased cytotlytic and antitumor action are needed.

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References

1. Chiou WL (1978) Critical evaluation of the potential error in pharmacokinetic studies using the linear trapezoidal rule method for the calculation of area under the plasma level time curve. J Pharmacokinet Biopharm 6: 539–546
2. Ebbs SR, Roberts JV, Baum M (1987) Alternative mechanism of action of "anti-oestrogens" in breast cancer. Lancet: (2) 621
3. Fabian C, Sternson L, Barnett M (1980) Clinical pharmacology of tamoxifen in patients with breast cancer: comparison of traditional and loading dose schedules. Cancer Treat Rep 64: 765–773
4. Gibaldi M, Perrier D (1982) Pharmacokinetics, 2nd edn. Marcel Dekker, New York, pp 409–417
5. Holleran WM, Gharbo SA, DeGregorio MW (1987) Quantitation of toremifene and its major metabolites in human plasma by high-performance liquid chromatography following fluorescent activation. Anal Lett 20 (6): 871–879
6. Horwitz KB, McGuire WL (1978) Nuclear mechanisms of estrogen action. Effects of estadiol and antiestrogens on estrogen receptors and nuclear receptor processing. J Biol Chem 253: 2185–2191
7. Kallio S, Kangas L, Blanco G, Johansson R, Karjalainen A, Perila M, Pippo I, Sundquist H, Sodervall M, Toivola R (1986) A new triphenylethylene compound, Fc-1157a: hormonal effects. Cancer Chemother Pharmacol 17: 103–108
8. Kangas L, Nieminen AL, Blanco G, Gronroos M, Kallio S, Karjalainen A, Perila M, Sodervall M, Toivola R (1986) A new triphenylethylene compound, Fc-1157a: antitumor effects. Cancer Chemother Pharmacol 17: 109–113
9. Riegelman S, Collier P (1980) The application of statistical moment theory to the evaluation of in vivo dissolution time and absorption time. J Pharm Sci 65: 509–535
10. Sutherland RL, Murphy LC, Hall RE, Reddel RR, Watts CK, Taylor IW (1984) Effects of antiestrogens on human breast cancer cells in vitro: interactions with high affinity intracellular binding sites and effects on cell proliferation kinetics. In: Bresciam F, Kinb RJB, Lippman ME, Namer M, Raynaud JP (Eds), Hormones and Cancer 2. Progress in Cancer Research and Therapy, vol XXXI. Raven Press, New York, pp 193–212
11. Watts CKW, Murphy LC, Sutherland RL (1987) Antioestrogen binding sites. In: Furr BJA, Wakeling AE (eds) Pharmacology and clinical uses of inhibitors of hormone secretion and action. Bailliere Tindall, London, pp 20–40
12. Wilson AJ, Baum M, Singh L, Kangas L (1987) Antioestrogen therapy of pure mesenchymal tumor. Lancet: 508
13. Yeh KC, Kwan KC (1978) A comparison of numerical integrating algorithms by trapezoidal, langrange, and spline approximation. J Pharmacokinet Biopharm 6: 79–98

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