Cancer Chemother Pharmacol (1997) 40: 185–188

SHORT COMMUNICATION

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Influence of age on toremifene pharmacokinetics

Received: 16 June 1996 / Accepted: 15 December 1996

Abstract Toremifene pharmacokinetics were compared in ten healthy young men (< 33 years) and elderly women (< 65 years). A single oral 120-mg dose of toremifene was given after an overnight fast and blood samples were collected over 28 days. Serum levels of the parent drug and the metabolites were determined; appropriate pharmacokinetic parameters were calculated and statistically evaluated. Toremifene peak concentrations (average 640 ng/ml) were achieved at 3.5 h. The area under the curve (AUC) and the apparent oral clearance were comparable in the young and elderly subjects. The half-life was prolonged (4.2 versus 7.2 days) and the apparent volume of distribution was increased (457 versus 627 l) in the elderly. The peak concentration of the main metabolite *N*-demethyltoremifene was lower (159 versus 233 ng/ml) and the half-life was prolonged (8.3 versus 19.1 days) in the elderly subjects, but the AUC values were comparable. The results suggest that toremifene is distributed more widely in the elderly but that its clearance is unaffected by age. It is concluded that the dosage requirement of the drug is unlikely to differ between young and elderly subjects.

Key words Toremifene · Pharmacokinetics · Elderly subjects

Introduction

Toremifene is a novel antiestrogen with clinically documented efficacy as an anticancer agent in breast cancer. It acts as a competitive antagonist at the estrogen receptor, blocking gene expression and cell proliferation [9], and displays an additional antitumor effect by inducing apoptosis [19]. Toremifene is mainly metabolized by CYP3A4 in the liver [5] to metabolites that are excreted predominantly in the feces [3]. The normal half-life, 6.2 days, is prolonged in patients with impaired hepatic function and shortened in patients who are taking drugs that have produced hepatic enzyme induction [4], indicating that liver metabolic activity should be considered when toremifene administration is contemplated.

Many patients with breast cancer are elderly; the mean age at the time of diagnosis is 62 years, although women of childbearing age may be susceptible to the malignancy [6]. Since age has an influence on liver metabolism [7, 11, 17], this study was undertaken to evaluate the effect of aging on toremifene kinetics for consideration as to whether dose adjustment might be necessary for breast cancer patients.

The drug was given orally to young men and elderly women, and multiple blood samples were drawn for up to 28 days. The CYP3A4 and CYP2A6 activity of the study subjects was also measured using probe drugs to test whether probe-drug metabolism could be useful in the estimation toremifene kinetics.

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Subjects and methods

Subjects

Toremifene pharmacokinetics was investigated in 20 healthy subjects, including 10 young men (19–33 years) and 10 elderly women (65–74 years; Table 1). The subjects were defined as "healthy persons" if their medical histories and physical examinations showed no evidence of hepatic, renal, or cardiovascular disease. Laboratory tests including complete blood counts, determinations of serum electrolytes and serum lipids, liver- and kidney-function tests, urinanalysis, and ECGs, revealed that the subjects were normal. The subjects were nonsmokers and consumed alcohol occasionally. They had no continuous medication. All subjects had a normal physical condition.

Protocol

This was an open study that received approval from the Ethics Committee of Oulu Deaconess Institute. The subjects gave written informed consent. They were investigated as inpatients. They fasted overnight and continued to fast until the 3-h blood sample had been

Table 1 Demographic characteristics of the subjects. Data represent mean values ± SD (n = 10)

	Young	Elderly
Gender	M	F
Age (years)	24.4 ± 4.4	66.9 ± 3.1
Weight (kg)	77.8 ± 11.6	67.3 ± 9.7
Height (cm)	180.4 ± 4.2	159.3 ± 4.7
Body mass index (kg/m²)	23.9 ± 3.2	26.7 ± 3.6

taken. A total dose of 120 mg toremifene was given as two 60-mg tablets to everyone. Venous blood samples for the assay of toremifene and its metabolites were taken before dosing and at 0.5, 1, 2, 3, 4, 6, 8, 12, and 24 h and 3, 5, 10, 21, and 28 days after dosing; the exact postdose sampling times were recorded. The sera were stored frozen (–20 °C) until analyzed. After the trial (on the 28th day) the routine liver-function tests were repeated.

Bioanalytical methods

Concentrations of toremifene and its metabolites, *N*-demethyltoremifene and (deaminohydroxy)toremifene, were determined in serum by a high-performance liquid chromatography (HPLC) method [4]. The quantitation limit for each analyte was 10 ng/ml. The intra- and interassay coefficient of variation generally ranged from 5% to 12% at the concentration range of 0.01–1.00 μg/ml.

To evaluate the role of age on the hepatic isozyme activities, probe drugs metabolized by specific isoenzymes were used. The CYP3A4 activity, reflected by the formation of monoethylglycinexylidide (MEGX) at 15 min after lidocaine (1 mg/kg) infusion, was analyzed [12]. The intra- and interassay coefficients of variation were below 3% at the concentration of 310 ng/ml. The oral coumarin test was used to measure the activity of another isozyme, CYP2A6 [15], for elucidation of the effect of age on the function of an isoenzyme that is not influenced by other drugs [14]. The excretion of the metabolite 7-hydroxycoumarin into urine after oral administration (5 mg) was measured. The intra- and interassay coefficients of variation for the duplicate analyses were below 2% at the 7-hydroxycoumarin concentration of 20 n*M*.

Serum albumin and total bilirubin content as well as the activities of aspartate and alanine aminotransferase and of alkaline phosphatase were measured with the use of standard automatic analyzer techniques. Serum procollagen aminoterminal propeptide (PIIINP), an indicator of the fibrotic activity, was measured by an equilibrium-type radioimmunassay as described elsewhere [16]. Intra- and interassay coefficients of variation were about 5% at all antigen concentrations.

Pharmacokinetic analysis

Noncompartmental pharmacokinetic parameters were calculated by standard methods. The peak concentration (C_{max}) and its time of occurrence (t_{max}) were taken by visual inspection of the data. The area under the serum concentration-time curve from time zero to infinity (AUC) was calculated with use of the linear trapezoidal rule to the last nonzero concentration and extrapolated to infinity. The terminal half-life ($t_{1/2}$) was calculated by ln 2 / elimination rate constant, which was determined by unweighted linear least-squares regression analysis from the linear segment of the log concentration-time data. The apparent oral clearance (CL/F) was calculated by division of the delivered dose by the AUC, and the apparent volume of distribution (V_z/F) was calculated by division of the clearance by the elimination rate constant.

Statistical analysis

Demographic and pharmacokinetic data were summarized as group mean values with standard deviations. One-way analysis of variance (ANOVA) was used to examine any differences found in logarithmically transformed pharmacokinetic parameters between the two age groups. However, non-transformed t_{max} values were compared by the Mann-Whitney nonparametric test. Statistical significance was accepted at $P < 0.05$.

Results

Clinical characteristics

The young men were robust as compared with the elderly women. The body mass index did not diverge (Table 1). Laboratory tests were normal in the subjects; however, the aging process was reflected as a higher level of serum total cholesterol (6.6 ± 1.1 versus 4.2 ± 1.0 mmol/l, $P < 0.001$), lower levels of serum albumin (38.9 ± 1.3 versus 42.7 ± 3.7 g/l, $P <$ 0.005) and hemoglobin (137.6 ± 6.2 versus 147.5 ± 7.7 g/l, $P < 0.005$), and a tendency toward higher level of serum PIIINP (4.0 ± 1.1 versus 3.3 ± 1.0 μg/l; difference not significant). Toremifene was well tolerated; no adverse event was reported. The poststudy laboratory tests (data not shown) revealed no change related to the drug. All participants completed the study.

Pharmacokinetic profiles

The main pharmacokinetic parameters of toremifene and the two metabolites are listed in Table 2, and the serum concentration-time profiles obtained for toremifene and *N*-demethyltoremifene are given in Fig. 1. The drug was equally absorbed in young and elderly subjects, the peak concentration in serum being achieved in 3 h, and the AUC values did not diverge. The terminal half-life was prolonged (young 4.2 days and elderly 7.2 days, $P < 0.01$). The apparent volume of distribution was increased (young 457 l and elderly 627 l, $P < 0.05$), and the apparent oral clearance was comparable.

The peak concentration of the major metabolite *N*-demethyltoremifene appeared in serum at 3 days (median) after administration, with considerable variation occuring from 8 to 144 h. The peak concentration was lower and the half-life was prolonged in the elderly subjects. AUC values were comparable.

The peak concentration of serum (deaminohydroxy)toremifene appeared within 2 h in both groups after toremifene administration. The peak and AUC values did not diverge in the groups. The half-life value could not be calculated.

Age, toremifene elimination, and CYP3A4 and CYP2A6 activities

The aging process has a reducing effect on toremifene, lidocaine, and coumarin metabolism. The toremifene half-life was prolonged with age (r = 0.674, $P < 0.001$), coumarin hydroxylation was reduced (r = –0.661, $P < 0.01$), and MEGX formation was delayed (r = –0.648, $P < 0.001$).

The toremifene half-life was related to 7-hydroxycoumarin excretion (r = 0.490, $P < 0.05$) and to MEGX

Table 2 Pharmacokinetic parameters of toremifene, *N*-demethyltoremifene, and (deaminohydroxy)toremifene. Data are given as mean values ± SD (C_{max} Peak concentration, t_{max}, time to reach C_{max}, *AUC* area under the serum concentration-time curve, $t_{1/2}$ half-life, *CL/F* apparent oral clearance, V_z/F apparent volume of distribution)

Parameter	Young	Elderly	One-way ANOVA[a]
Toremifene:			
C_{max} (ng/ml)	630 ± 192	652 ± 125	$F_{1,18}$ = 0.3; P = 0.60
t_{max} (h)	3.1 ± 1.9	3.9 ± 2.1	$F_{1,18}$ = 0.8; P = 0.39
AUC (μg h ml^{-1})	40.8 ± 14.7	49.5 ± 17.8	$F_{1,18}$ = 1.6; P = 0.23
$t_{1/2}$ (days)	4.2 ± 1.2	7.2 ± 2.3	$F_{1,18}$ = 12.9; P = 0.002
CL/F (l/h)	3.3 ± 1.0	2.7 ± 0.9	$F_{1,18}$ = 1.6; P = 0.23
V_z/F (l)	457 ± 176	627 ± 146	$F_{1,18}$ = 5.9; P = 0.03
N-Demethyltoremifene:			
C_{max} (ng/ml)	233 ± 65	159 ± 47	$F_{1,18}$ = 9.8; P = 0.006
t_{max} (h)	77 ± 35	64 ± 55	$F_{1,18}$ = 0.4; P = 0.56
AUC (μg h ml^{-1})	95.6 ± 32.4	109.5 ± 55.0	$F_{1,18}$ = 0.3; P = 0.60
$t_{1/2}$ (days)	8.3 ± 2.2	19.1 ± 2.2	$F_{1,18}$ = 11.8; P = 0.003
(Deaminohydroxy)toremifene:			
C_{max} (ng/ml)	52 ± 16	48 ± 21	$F_{1,18}$ = 0.5; P = 0.48
t_{max} (h)	1.8 ± 0.6	2.0 ± 0.5	$F_{1,18}$ = 0.7; P = 0.41
AUC (μg h ml^{-1})	0.76 ± 1.01	0.38 ± 0.32	$F_{1,18}$ = 1.4; P = 0.24

[a] Nonparametric Mann-Whitney test was performed for t_{max} values

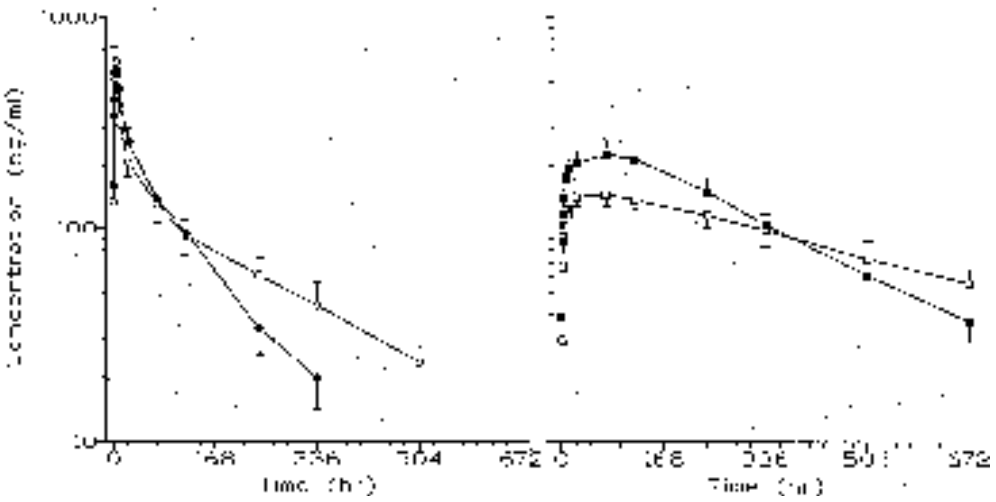

Fig. 1 Serum toremifene (*circles*) and *N*-demethyltoremifene (*squares*) concentration-time curves generated for young men (*black symbols*) and elderly women (*white symbols*) after a single oral 120-mg dose of toremifene. Data represent mean values ± SE

formation (r = 0.467, $P < 0.05$), demonstrating that although toremifene and lidocaine are metabolized by the same isozyme, CYP3A4, the metabolic interrelationship between toremifene and lidocaine was no better than that between toremifene and coumarin, which is metabolized by a different isoenzyme (CYP2A6).

Discussion

Pharmacokinetics studies of a new drug should include subjects of the age and sex targeted by the drug [11]. We investigated the effect of age on the pharmacokinetics of toremifene in young men and elderly women. The drug was given orally because it is poorly water-soluble and, therefore, not suitable for intravenous administration. The data show no age-dependent change in toremifene absorption. The distribution volume was increased with age, and the apparent oral clearance was comparable. The half-life was prolonged (4.2 versus 7.2 days). The peak concentration of the main metabolite *N*-demethyltoremifene was decreased, and the elimination was delayed. The prolongation of toremifene half-life observed in the elderly was due to the increased volume of distribution because oral clearance was unaltered. The decrease in peak concentration with unaltered AUC values noted for the main metabolite, which is known to have anticancer activity, was associated with delayed elimination in the elderly. These results suggest that the elimination rate of toremifene at steady-state would be comparable in young and elderly subjects.

In vitro, toremifene is mainly hydroxylated by the CYP3A4 isoform [5], and the change in liver metabolism is reflected in the drug pharmacokinetics [4]. Our subjects were healthy persons who were not taking any medication or smoking and whose alcohol consumption was scant. They had clinically normal liver size and function and no sign of heart failure. Metabolism of the probe drugs lidocaine by CYP3A4 and coumarin by CYP2A6 were

delayed with age. This suggests a diminished functional capacity of the isoenzymes in vivo with age. Although the laboratory tests were normal, elderly subjects had lower serum albumin and hemoglobin values, a high serum cholesterol content, and a tendency toward a higher PIIINP concentration. This suggests that the associated "physiological" alterations in oxygen supply, liver parenchyma, protein synthesis, and other metabolic processes [11, 17], together with age-induced changes in liver blood flow [18] and protein binding [2], must be noted in comparisons of toremifene metabolism among young and elderly subjects. Other factors such as the differences in life-style, diet, and physical activity between young and elderly may further influence the elimination of toremifene and its metabolites with age.

A major problem in the investigation of drugs that have potentially harmful effects on the reproductive organs of young women is selection of the subjects. In general, young and healthy men have been used instead of women. Care is needed in the interpretation of results obtained from subjects of opposite sex for the basic pharmacokinetic evaluation.

The interaction between gender and drug metabolism in the elderly is a complex process. Experimental studies indicate that drug metabolism is more extensive in men than in women [8, 10]. Testosterone induces more enzyme protein synthesis than estrogen, which corresponds to a variation in the activity of metabolizing enzymes. Thus, the sex difference in drug metabolism in the postmenopausal phase is probably small [1]. Sex-related differences in the metabolism of some but not all drugs have also been reported in humans [1,13]. The CYP3A subfamily participates in metabolism the of steroids testosterone and estradiol [20]. In vitro studies show that testosterone interacts more with toremifene metabolism by CYP3A4 than does estrogen [5]. This phenomenon may also occur in vivo in humans. Thus, toremifene metabolism in young men was dependent on testosterone-induced CYP3A4 activity and competition for the enzyme-binding sites with the testosterone blood levels. We tested CYP3A4 activity by the MEGX test and found a significant prolongation with age. The correlation between toremifene and lidocaine metabolism was not good, indicating that the probe-drug metabolism, although related to age, was not changed to the same extent as that of toremifene. On the basis of this observation, we may assume that CYP3A4 activity in young women is comparable with or slower than but not faster than that in young men. In young women, toremifene would compete with natural estrogen levels with CYP3A4 activity, which is less pronounced than testosterone competition in young men. Consequently, toremifene elimination in young women would be within the ranges noted for young men and faster than those found in elderly women. Thus, a close follow-up of the clinical response to the therapy in young patients is important for determination as to whether they require a larger drug dose because of this active metabolism.

In conclusion, we can say that the aging process has an effect on toremifene pharmacokinetics. The increase in the volume of drug distribution with age contributes to the increased half-life of the drug. However, since the oral clearance did not differ significantly between the two groups, dose adjustments based on age alone do not appear to be necessary in elderly patients.

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