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Effect of Age and Single Versus Multiple Dose Pharmacokinetics of Letrozole (Femara®) in Breast Cancer Patients

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ABSTRACT: Letrozole (trademark Femara®) is a new orally active, potent and selective aromatase inhibitor for the hormonal treatment of advanced breast cancer in postmenopausal women. The pharmacokinetics of letrozole and the suppression of peripheral estrogens were studied in 28 breast cancer patients after a single dose and at steady state. The pharmacokinetics of two distinct age groups ($\geqslant$50, $\leqslant$65, $N = 15$ and $\geqslant$70 years old, $N = 9$) were compared. There were no significant differences in area under the curve (AUC) or terminal half-life between the two age groups neither after a single dose nor at steady state. However, when comparing steady state to single dose kinetics, half-life and AUC increased significantly by 42% (90% CI: 1.13, 1.78) and 28% (90% CI: 1.12, 1.47), respectively. This deviation from linearity was probably due to a partial saturation or auto-inhibition of the dominant metabolic clearance mechanism of letrozole. At steady state, approximately 70% of the administered dose was excreted in urine as unchanged letrozole ($6.0 \pm 3.8\%$) or as the glucuronide of the major, pharmacologically inactive metabolite CGP44645 ($64.2 \pm 22.7\%$). A single dose of letrozole caused suppression of serum estrogen levels close to the quantification limit of the assay. No difference between single dose suppression and suppression at steady state could be detected. Copyright © 2001 John Wiley & Sons, Ltd.

Key words: Femara; aromatase inhibitor; pharmacokinetics; breast cancer patients

Introduction

The primary mechanism of action in hormonal therapy of breast cancer is estrogen deprivation. This can be achieved either by blocking the estrogen receptor with an antiestrogen such as tamoxifen or by inhibition of estrogen biosynthesis. In postmenopausal women the aromatase enzyme system generates estrone (E1) and estradiol (E2) from androgenic precursors. Inhibition of the aromatase enzyme inhibits the production of estrogens.

Letrozole (CGS 20267) belongs to a new generation of highly potent and selective aromatase inhibitors. It has been registered (trademark Femara®) for the treatment of advanced breast cancer in postmenopausal women after antiestrogens. Clinical studies have shown that single doses as low as 0.5 mg produce a potent long-lasting suppression of plasma levels of E1 and E2 without influencing plasma concentrations of cortisol or aldosterone [1,2]. The efficacy of

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aromatase inhibition is >99% at the registered daily dose of 2.5 mg [3], with substantial inhibition of intratumoral aromatase [4]. In Phase IIb/III comparative studies letrozole 2.5 mg daily showed an advantage in objective response (OR) and/or time to progression (TPP) and/or time to treatment failure (TTF) over megestrole acetate or aminogluthetimide [5,6]. Recently, letrozole also showed considerable advantage over tamoxifen (OR, TTP, TTF) in the treatment of postmenopausal women with advanced breast cancer in first line and preoperative settings [7,8].

The pharmacokinetics of letrozole have been characterized in various studies in healthy volunteers and in breast cancer patients. After oral administration, letrozole is rapidly and completely absorbed [9]. Protein binding is moderate (60%, mainly to albumin) and its plasma profile is characterized by a dominant terminal phase with a half-life of approximately 2 days in healthy volunteers [9,10,11]. Clearance is mainly via metabolism into a pharmacologically inactive alcohol metabolite [9,12]. *In vitro* experiments suggest that the cytochrome P450 isoenzymes 3A4 and 2A6 (CYP3A4, CYP2A6) are involved in the conversion of letrozole to this metabolite [12]. The contribution of each isoenzyme to the overall clearance is not known. However, *in vitro* experiments suggest that non-linearity in the pharmacokinetics of letrozole via an auto-inhibition or saturation of the CYP2A6 pathway may occur. The affinity to CYP2A6 seems to be high and saturation occurs in the low micromolar range. Letrozole was also found to be a strong inhibitor of CYP2A6 ($K_i = 0.12\,\mu M$ coumarin 7-hydroxylation) [12]. However, the affinity for CYP3A4 is apparently low and saturation could not be achieved at concentrations (100 μM) far exceeding physiological levels in plasma. In clinical studies, a slight deviation from linearity has been observed at high single doses ($\geqslant 30$ mg) or after daily doses of 2.5 mg [13, Novartis data on file]. Systemic exposure increased more than proportional to the dose. The present study was thus performed to assess the degree of non-linearity in a typical clinical setting by comparing the within-subject pharmacokinetics of letrozole after a single and repeated once-daily doses of 2.5 mg to patients with advanced breast cancer. In addition, the effect of age on the pharmacokinetics of letrozole was also investigated.

Subjects and Methods

Trial population and sample size

This study was performed in accordance with the Declaration of Helsinki, concerning medical research in humans ('Recommendations Guiding Physicians in Biomedical Research Involving Human Patients', Helsinki 1964, amended Tokyo 1975, Venice 1983, Hong Kong 1989, Somerset West, 1996), the Directive 91/507/EEC: The Rules Governing Medicinal Products in the European Community, and the US 21 Code of Federal Regulations dealing with clinical studies, parts 50 and 56, concerning Informed Patient Consent and IRB approval in order to ensure adherence to Good Clinical Practice.

Postmenopausal women of any ethnic group $\geqslant 70$ or $\geqslant 50$ and $\leqslant 65$ years of age, with either progressive locally advanced breast cancer, locoregional recurrence not curable by surgery or radiotherapy, or progressive metastatic disease, and with a WHO performance status of 0–2 were to be included. Patients were excluded when they had rapidly progressive metastases, uncontrolled cardiac disease and/or uncontrolled diabetes mellitus, significant hepatic dysfunction, and other concurrent malignant disease. Patients were also excluded if they had not recovered from toxicity caused by previous cancer therapies. Any concomitant anti-cancer treatment or treatment with steroids for indications other than cancer were also excluded except bisphosphonates or aerosol for obstructive airways diseases.

Based on the pharmacokinetic variability observed in previous trials, a sample size of 12 completed patients per age group was required to provide 90% power to detect a 25% difference at the 5% level.

Study design

This was an open-label, multi-center, non-randomized between-group phase II trial to compare single and multiple dose pharmacokinetics in

two different age groups of postmenopausal breast cancer patients. Each patient received a single dose of 2.5 mg letrozole (Day 1) followed by a treatment–free period of 10 days. Daily doses of 2.5 mg letrozole were then given for 8 weeks (until Day 66) to attain steady state and treatment was stopped for a second washout phase of 10 days (Day 75). Since E1 or E2 levels were not expected to return to normal until 14 days after administration of letrozole, the two treatment-free periods were not expected to have any harmful effect on the tumors. After the core part of the study, patients were allowed to continue treatment until progression of disease or any other event requiring discontinuation. Patients who prematurely discontinued the core trial were to be replaced.

Blood sampling and analytical methods

Sample times, handling. For the pharmacokinetic assessment, patients were hospitalized from Day 0 to Day 4 and from Day 65 to Day 69. Additional blood samples were taken on Days 7, 10, 24, 38, 52, 72, and 75 for which the patient had to visit the hospital. The following blood samples were taken following the single dose (Day 1) and at steady state (Day 66): before drug administration, and 15, 30 min and 1, 2, 4, 6, 8, 12, 24, 48, 72, 144, and 216 (Day 10) h after drug intake. In addition, trough level samples were collected on days 24, 38, and 52.

For each sample 3 ml blood was collected and the plasma was separated and frozen at $\leqslant -18^{\circ}C$. The exact time and date of sample taking were recorded. Urine was collected in selected patients during a dosing interval (24 h) on Day 66, starting just before drug administration and ending on Day 67.

For the measurement of estrogen levels blood samples were taken on Days 1 and 66 before drug administration and at 12, 24, 72, 144, and 216 h as well as trough level samples on Days 24, 38, and 52. Five milliliters of blood were collected and serum was prepared and frozen at $\leqslant -18^{\circ}C$.

Analyses for letrozole in plasma and urine were performed by means of high performance liquid chromatography (HPLC) with fluorescence detection as described in [14]. The limits of quantification (LOQ) for letrozole in plasma and urine were 1.4 and 2.8 nmol/l, respectively, and for the main metabolite (CGP 44645) in urine after enzymatic hydrolysis it was 0.109 μmol/l. Estrogen concentrations in serum were determined by radio immunoassay. The limits of quantification for estrone and estradiol were 5 and 1 pg/ml, respectively.

Pharmacokinetics, pharmacodynamics, and statistical methods

Pharmacokinetic parameters were determined using standard non-compartmental methods. AUC was calculated by the log-linear trapezoidal rule and renal clearance by the formula $CL_R = Ae_\tau / AUC_\tau$, Ae_τ representing the amount of letrozole excreted at steady state. The software package WinNonlin® from Pharsight Corporation, CA, USA, was used for calculation of most of the pharmacokinetic parameters. Log-transformed AUC and half-life were analyzed to test for age related differences using a general linear model. Age was considered as a factor variable on two levels or as a continuous variable and the 95% CIs for the differences due to age were calculated. Body weight and center were used as co-variates. To compare single dose to multiple dose kinetics, the difference in half-life and in AUC_τ at steady state to $AUC_{0-\infty}$ after a single dose was assessed using a general linear model. The model included subject-within-group and group as secondary covariates, while treatment (single dose versus steady state) was the main effect.

Estrogen suppression at time t was calculated as the percentage of suppression from baseline ($100 - 100 \times E1(t)/E1(0\,h)$ or $100 - 100 \times E2(t)/E2(0\,h)$). The sample drawn prior to administration of letrozole ($0\,h$) was taken as baseline. Concentrations in samples after dosing which were below the LOQ were set to LOQ−0.01 (in pg/ml) in order to calculate the estrogen suppression from baseline.

Clinical evaluations

Anti-tumor activity was measured according to UICC criteria at baseline and every 3 months thereafter. Performance status was assessed

according to the WHO scale. Safety assessments included the monitoring and recording of all adverse events and severe adverse events, regular checks of routine blood chemistry, hematology and urine values, measurements of blood pressure, pulse rate, vital signs, and physical examinations. Adverse events were recorded in 2–4 week intervals during the core phase of the trial and then at 3 monthly intervals. Laboratory evaluations were performed every 3 months.

Results

Patient exposure and clinical response

A total of 28 patients were enrolled into the study. Table 1 shows the number of patients who completed or discontinued the PK part of the study. The median age in the younger and elderly patients was 61 (range: 52–66) and 71 (70–76) years, respectively. In both groups the dominant site of disease involvement was soft tissue (18%), bone (61%) and viscera (21%). The median time since the original diagnosis of breast cancer was 56 months in the younger patients and 32.5 months in the elderly.

The mean duration of treatment was slightly more than 6 months. The majority of the discontinuations were due to disease progression. No complete response was observed. Four patients in the elderly group experienced a partial response, two of the elderly and 5 of the younger patients showed no change, four patients (1 younger, 3 elderly) were not assessable for tumor response, and 13 patients showed progressive disease.

Pharmacokinetics

Pharmacokinetic samples were obtained from 27 patients, 16 in the group of younger patients and 11 in the group of elderly. Fifteen of the younger and 9 of the elderly patients completed the pharmacokinetic part. Urine samples were obtained from 8 younger and from 6 elderly patients.

Table 1. Patient exposure during PK part of the study

Total no. of patients	Young	Elderly
Enrolled	16	12
Started PK sampling	16	11
Completed PK sampling	15	9

There was a tendency to a higher exposure in the elderly. However, none of the tested parameters (AUC and half-life at steady state and after a single dose) showed a statistically significant difference (*P*-values in the range 0.63–0.97). Figure 1 displays the mean plasma concentrations of letrozole of the two groups after a single dose and at steady state.

Since no difference between the age groups was evident, the data were pooled for further analysis (Table 2). Previous studies have indicated that the pharmacokinetics of letrozole are non-linear at higher single doses (30 mg) or after multiple dosing at $\geqslant 2.5$ mg/day [13, Novartis data on file]. The present study confirms these observations via the comparison of single and multiple dose pharmacokinetics. On pooled data of both age groups, AUC and half-life at steady state increased significantly by 28% (90% CI: 1.12, 1.47) and 42% (90% CI: 1.13, 1.78), respectively, when compared to the single dose data (note that these ratios represent the estimates of the analysis of variance on log-transformed data and are not derived from the arithmetic means provided in Table 2).

Approximately 70% of the dose was excreted in urine during a dose interval at steady state,

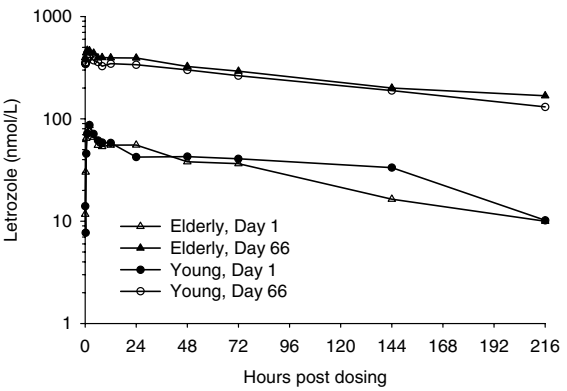

Figure 1. Mean plasma levels of letrozole

Table 2. Pharmacokinetic parameters of letrozole after a single dose and at steady state

Parameter	Single dose ($N = 27$)			Steady state ($N = 24$)		
	Mean	CV (%)	Median	Mean	CV (%)	Median
C_{max} (nmol/l)	107	36.6	100	467	52.2	408
t_{max} (h)	8.10	235.1	1.98	3.20	153.4	1.95
AUCτ (h nmol/l)	1372	39.4	1281	8926	54.5	7347
AUC_{inf} (h nmol/l)	7387	55.0	6194			
CL/F (L/h)	1.52	48.3	1.41	1.20	41.6	1.20
$t_{1/2}$ (h)	82.2	66.9	61.2	118	57.1	90
V_z/F (l)	152	48.1	147	183	65.0	142
Aeτ (letrozole)[a] (% of dose)				6.0	3.8	5.5
Aeτ (metabolite)[a] (% of dose)				64.2	22.7	59.1

[a] $N = 15$.

mainly as the glucuronide of the major metabolite CGP 44645. This is in good agreement with the data from an absolute bioavailability study performed in healthy postmenopausal women [9]. Also, after a single dose of 14C-labeled letrozole to healthy postmenopausal women, approximately 90% of the dose was recovered in the urine within 336 h [Novartis data on file]. Direct renal clearance of letrozole thus represents a minor pathway of drug elimination. Most of the drug is cleared via metabolism to the inactive alcohol metabolite CGP 44645 which is subsequently glucuronidated and excreted via the kidneys.

Hormone levels

Twenty-six patients had estrogen samples taken, 15 in the group of younger patients and 11 in the elderly. For 3 patients in the younger group the baseline E1 levels were below the LOQ of the assay, although letrozole dosing was at or shortly after the sample collection according to the case report forms. Estrone suppression was not calculated for these patients and their values were not considered for summary statistics.

The geometric mean for the two hormones is shown below in Figure 2. Mean suppression was around 50–60% for E1 and 60–70% for E2. Most of the patients had the majority of their E1 levels below the LOQ during treatment and all 26 patients had at least one sample below LOQ. For E2, 12 of 26 patients had at least one sample below the LOQ of the assay. Maximally detectable suppression was observed even after a single dose. Since for samples below LOQ, the value LOQ−0.01 pg/ml was used to calculate E1 or E2 suppression, the true suppression was

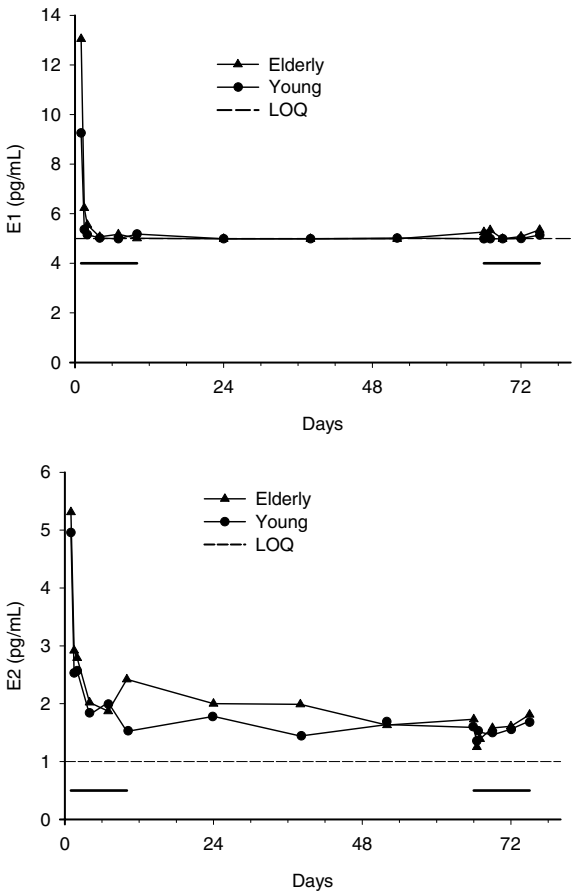

Figure 2. Geometric means of estrogen levels after a single dose of letrozole and at steady state. The thick bars indicate treatment free periods

certainly under-estimated in these samples. No relevant difference in serum estrogen suppression between the age groups was evident.

Discussion

This study was performed to assess the effect of age on the pharmacokinetics of letrozole and to compare single dose with steady state pharmacokinetics/pharmacodynamics. Age related changes in drug clearance, in particular hepatic metabolism, may require dose adjustments in the elderly populations. Since letrozole is indicated for the treatment of advanced breast cancer in postmenopausal women, a significant proportion of patients who may potentially receive treatment with letrozole is in the elderly population. European and FDA guidelines recommend an age greater than 65 years for the study of an elderly population. In this trial elderly patients with $\geqslant$70 years of age were compared to a group of younger patients between 50 and 65 years old in order to ensure a postmenopausal status. A difference of at least 5 years in age between populations was chosen to avoid any overlap in age.

The results of this study suggest that age does not affect the PK of letrozole. The sample size calculated for this trial was based on PK data from healthy volunteers. However, the variability in this patient study was higher than assumed from the healthy volunteer data. Furthermore, less than the originally planned 12 patients per group had completed their PK part. Thus, it cannot be concluded with certainty whether an influence of age exists on the pharmacokinetics of letrozole. However, based on the 95% confidence interval for AUC at steady state (0.68, 1.51), the influence of age is probably not substantial and not of clinical significance. Letrozole has been given to breast cancer patients at doses of up to 10 mg/day for three months without any sign of increased toxicity [4].

Earlier studies indicated that the PK of letrozole showed some deviation from linearity at higher single doses ($\geqslant$30 mg) or at daily doses of $\geqslant$2.5 mg. The within patient comparison in the present study confirmed this finding, although the deviation from linearity was rather small: AUC over a dose interval at steady state was on average 28% higher compared to the AUC extrapolated to infinity after a single dose. The trough level concentrations collected on Days 24, 38, 52, and 66 indicated that steady state was achieved at approximately Day 40, i.e. about 4 weeks after the start of daily dosing (Figure 3). Previous studies also indicated that steady state is achieved within 2–6 weeks and is maintained over long periods of time [15, Novartis data on file]. Thus, continuous drug accumulation seems not to occur. Letrozole has also been given at daily doses of 5 or 10 mg and the analyses of trough level samples indicated that steady state was achieved within approximately the same time frame [4, Novartis data on file].

As outlined in the introduction, the non-linearity in letrozole's PK is probably a consequence of an auto-inhibition or saturation of metabolic degradation via CYP2A6 which starts to become apparent at a daily dose of about 2.5 mg. Whether higher doses result in a further deviation from linearity is not clear. *In vitro* experiments suggest that a similar behavior is not to be expected for the metabolic pathway via CYP3A4. Although the contribution of each isoenzyme to the overall metabolic clearance is unknown, the non-linearity may be restricted to an exposure range where saturation/auto-inhibition of CYP2A6 occurs but the PK might again follow linearity when CYP3A4 becomes the dominant metabolizing enzyme.

The $AUC_{0-\infty}$ and terminal half-life values after a single dose were higher in this study in breast

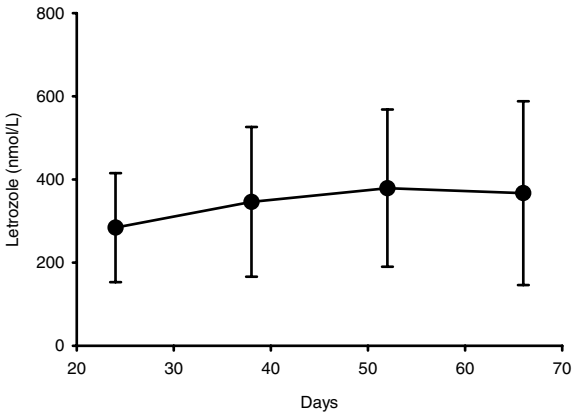

Figure 3. Trough level

cancer patients when compared to data from healthy volunteers [9,11]. In healthy volunteers the half-life is usually in the order of two days and the AUC around 4–5 h μmol/l with variation coefficients of about 35%. Letrozole has been shown to have an absolute bioavailability of 100% after oral administration to healthy volunteers [9]. It can thus be assumed that the higher exposure in patients is mainly due to a reduced elimination rate, most likely metabolic clearance. Extrapolation from healthy volunteer single dose data to the steady state situation would thus underestimate the exposure in patients.

The pharmacodynamic measurements (E1 and E2 serum levels) in this study were designed to investigate whether any fluctuation in peripheral estrogen suppression occurs at steady state. However, since even a single dose resulted in estrogen levels close to the LOQ, fluctuations at steady state were not detectable despite the good sensitivity of the analytical methods.

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