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Effects of the aromatase inhibitor anastrozole on serum oestrogens in Japanese and Caucasian women

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Abstract *Purpose*: Substantial differences in plasma oestrogen disposition have been reported between Japanese and Caucasian women, but there are currently few data available on the relative endocrinological effects of aromatase inhibitors in these two groups. Hence, the effects of the nonsteroidal aromatase inhibitor anastrozole on serum oestrogen concentrations were compared in 24 healthy postmenopausal Japanese women and 24 healthy postmenopausal Caucasian women. *Methods*: Anastrozole, 1 mg/day, was given once daily for 16 days. Serum oestradiol and oestrone sulphate levels were measured on three consecutive days beginning 2 days before the first dose, and on a further three consecutive days beginning on the penultimate day of dosing. Trough concentrations of anastrozole (measured 24 h after dosing) were also determined during the same periods. *Results*: There were no substantial differences in plasma oestrogen concentrations between the Japanese and Caucasian women at baseline. On average, anastrozole suppressed serum oestradiol and oestrone sulphate levels by approximately 87% and 93%, respectively, for both Japanese and Caucasian women, and minimum plasma anastrozole concentrations at steady-state (anastrozole C_{min}) were also similar in both groups. Statistical analysis of serum oestradiol and serum oestrone sulphate levels, and plasma anastrozole C_{min} showed that there were no statistically significant differences between the Japanese and Caucasian women. *Conclusion*: Neither the pharmacodynamic effects of anastrozole on serum oestrogens nor the pharmacokinetics of anastrozole differ between postmenopausal Japanese and Caucasian women. Hence, these findings suggest that the therapeutic benefits of anastrozole in Caucasians will be predictive of the drug's effect in Japanese women and support the use of anastrozole in postmenopausal Japanese women with breast cancer.

Key words Anastrozole · Aromatase inhibitor · Caucasian · Japanese · Oestradiol · Oestrone sulphate

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Introduction

Approximately one-third of human breast cancers are oestrogen-dependent and regress following oestrogen deprivation [19]. In postmenopausal women, the principal source of oestrogens is peripheral conversion of adrenal androgens (androstenedione and testosterone) by the enzyme aromatase. Inhibition of aromatase thus represents a rational approach to the endocrine therapy of breast cancer [6, 18]. Early clinical experience with the first aromatase inhibitor, aminoglutethimide, confirmed the feasibility of this approach [13], but the usefulness of this agent is limited by its lack of selectivity for the aromatase enzyme and by adverse events such as rash and lethargy [4]. As a result, a number of novel steroidal and nonsteroidal aromatase inhibitors have been developed for clinical use, and these are more potent, selective and better tolerated than aminoglutethimide [15].

Anastrozole (Arimidex) is a benzyltriazole derivative that has been shown in preclinical studies to be a potent and selective aromatase inhibitor [11, 22]. In preliminary trials in postmenopausal women with breast cancer,

anastrozole, 1 mg/day, inhibited in vivo aromatization by 96.7%, and suppressed plasma concentrations of oestradiol, oestrone and oestrone sulphate by approximately 84–94% [12]. Further studies have shown that anastrozole has no effect on adrenal steroid synthesis, or on the steroidogenic response to adrenocorticotrophic hormone (ACTH) challenge [11, 24]. In two large comparative trials [2, 16], anastrozole has been shown to produce comparable response rates to megestrol acetate in postmenopausal breast cancer patients. Furthermore, combined analysis of the data from these two trials has shown a significant improvement in survival in women treated with anastrozole, 1 mg, compared with those treated with megestrol acetate [3].

Most studies with anastrozole (and other aromatase inhibitors) have been conducted in Western populations; there are few data on the endocrinological and clinical effects of aromatase inhibitors in Japanese women. Anastrozole is currently being evaluated in a trial of Japanese women with advanced breast cancer. Fadrozole is the only aromatase inhibitor approved for use in Japan. This agent, however, is not completely selective for aromatase and suppression of aldosterone secretion is seen at therapeutic doses [5, 9].

Marked differences in circulating oestrogen concentrations have been reported between Japanese women and Caucasian women [14, 23] and have been considered as a possible factor in the difference in breast cancer incidence between the two populations. Hence, it is important to determine whether the endocrine effects of aromatase inhibitors are comparable in the two populations. This report describes the first comparative study of the pharmacokinetics and pharmacodynamic effects of anastrozole on serum oestrogens in Japanese and Caucasian women.

Materials and methods

Subjects

A total of 48 healthy female volunteers (24 Caucasian, 24 Japanese), recruited in the UK and Japan, took part in this study. Calculation of the number of volunteers for this trial was based on serum oestradiol data obtained from previous anastrozole pharmacology trials carried out in the West [12] and Japan (unpublished data). Anastrozole, 1 mg daily, in Western patients causes mean plasma oestradiol concentrations to fall to approximately 3 pmol/l [12]. A mean serum oestradiol concentration of 4.5 pmol/l or higher in Japanese volunteers was considered to be a clinically significant difference from the Caucasian volunteers. Sample size calculations using an estimate of standard deviation of 0.42 on the log-scale showed that 24 Japanese and 24 Caucasian volunteers were required to complete the trial to have a 90% chance of observing mean serum oestradiol concentrations in Japanese volunteers of 4.5 pmol/l or higher, compared with the predicted value of 3 pmol/l in Western volunteers and a 5% chance of falsely concluding that there was a difference, if no difference existed.

All the women were aged 50–75 years and had been postmenopausal for at least 2 years, as judged by follicle-stimulating hormone (FSH) concentrations within the postmenopausal range and the absence of menstruation. All subjects were required to be within 20% of normal weight for their height. Exclusion criteria included: regular use of medication other than occasional use of analgesics and hypnotics; acute illness within 2 weeks prior to the start of the study; clinically significant abnormalities in clinical chemistry, haematology or urinalysis; definite or suspected history of hypersensitivity to triazole drugs; and any medical condition likely to interfere with drug pharmacokinetics.

Written informed consent was obtained from all subjects before entry to the study, which was approved by the local Ethics Committee (in the UK) and the Institutional Review Board (in Japan), and conducted according to the principles of the Good Clinical Practice for Trials on Drugs.

Study design

An open parallel-group comparative trial design was used and considered appropriate because serum oestrogen concentrations are known to be stable in women who have been postmenopausal for more than 2 years [21]. The effects of anastrozole on serum oestrogens were assessed by measuring plasma oestradiol and oestrone sulphate levels on three consecutive days at the beginning and end of treatment. In addition, the pharmacokinetics of anastrozole were assessed by measuring trough drug concentrations (24 h after dosing) during the same periods.

All subjects received anastrozole 1 mg orally daily for 16 days. Treatment was given at approximately 0900 hours, 2 h after a light breakfast. Subjects were requested to refrain from eating for 2 h after dosing, to maintain constant levels of caffeine intake and constant smoking habits throughout the study, and to avoid grapefruit, grapefruit juice and alcohol from 72 h before the first dose.

In the UK, subjects attended the clinic for blood sampling on three consecutive days beginning 2 days before the first dose of anastrozole, and on a further 3 days beginning on day 15 of treatment. In Japan, subjects were hospitalized during the same periods. Venous blood samples (10 ml) were obtained at approximately 0900 hours on the 2 days before the first dose, before dosing with anastrozole on days 1, 15 and 16, and approximately 24 h after the last dose (day 17). Samples were collected into glass tubes and allowed to clot for 60 min. Serum was separated by centrifugation at approximately 1000 *g* for 10 min and stored frozen at −20 °C prior to dispatch to the Royal Marsden Hospital for analysis. Further blood samples (7 ml) for measurement of trough plasma levels of anastrozole were obtained before dosing with anastrozole on days 1, 15 and 16, and 24 h after the last dose. These samples were collected in heparinized tubes and the plasma was separated by centrifugation at 1000 *g* for 10 min at 4 °C. Plasma was stored frozen at −20 °C prior to dispatch to AstraZeneca Pharmaceuticals (Wilmington, Del.) for analysis. Additional blood and urine samples were obtained for clinical chemistry, haematology and urinalysis at different times during the study.

Serum concentrations of oestradiol and oestrone sulphate were determined by radioimmunoassay methods reported elsewhere [8, 10]. All analyses on the same patient were included in the same assay batch. To reduce the potential impact of batch variability on the degree of change with treatment, equal numbers of patients from Japan and the UK were included in each assay batch. Plasma anastrozole concentrations were determined using a gas chromatographic method with electron capture detection. The method was validated from 3 to 100 ng/ml [1]. Information on adverse events was recorded throughout the trial.

Statistical methods

The primary measure for statistical analysis was serum oestradiol concentration. Secondary measures were serum oestrone sulphate concentration and minimum plasma anastrozole concentration at steady-state (anastrozole C_{min}). For both the primary and secondary measures, the median of the three values obtained at the end of treatment was log-transformed and comparisons between

Caucasian and Japanese subjects performed by an analysis of variance (ANOVA) model. Where individual values fell below the sensitivity limit of the respective assay, they were assigned a value equal to that sensitivity limit. The results of the analyses are presented as geometric least squares (gls) means (gls-means) and 95% confidence intervals. Gls-means were used because it was found that fitting the baseline data as a covariate significantly improved the fit of the model.

Results

The mean ages (± standard deviation) of the Caucasian and Japanese subjects were 61.5 ± 6.7 years and 60.9 ± 6.5 years, respectively. The Caucasian subjects tended to be heavier than the Japanese subjects (mean weight 66.0 ± 9.4 kg versus 54.2 ± 8.0 kg, respectively).

Of the 48 women recruited to the study, 45 were included in the analysis. Three women were excluded as subsequent analysis of study samples showed that their oestradiol concentrations were in the premenopausal range. For these patients, pretrial FSH levels were 60.9, 50.5 and 46.5 IU/l, and pretrial oestradiol levels on study days −2, −1 and 1 were 356.9, 219.4 and 183.6 pmol/l, 370.6, 333.2 and 396.0 pmol/l, and 157.1, 198.0 and 196.0 pmol/l, respectively. The ages of the patients were 50, 53 and 52 years, and this relatively young age suggests some residual ovarian function at the time of the study in these women despite their high FSH levels. Although the exclusion of the three women meant that there were fewer than had been required for the trial to have 90% power, the standard deviation from the analysis of oestradiol was found to be 0.27, much less than the estimate of 0.42 used in the calculation of volunteer numbers, which more than compensated for the reduction in the number of volunteers.

Serum concentrations of oestradiol and oestrone sulphate before and during treatment are summarized in Table 1. The geometric means for serum oestradiol (end of treatment) were 3.3 pmol/l and 2.7 pmol/l in the Caucasian and Japanese groups, respectively, while the geometric means for serum oestrone sulphate (end of treatment) were 32.0 pmol/l and 39.5 pmol/l, respectively.

Anastrozole reduced serum oestradiol and oestrone sulphate by approximately 87% and 93%, respectively, compared with baseline values in both the Caucasian and Japanese groups. Plasma anastrozole C_{min} values at the end of treatment were also similar in the two groups. The geometric means of the median concentration of anastrozole C_{min} were 25.7 ng/ml in the Caucasian group and 30.4 ng/ml in the Japanese group.

The statistical analyses of the serum oestradiol and oestrone sulphate concentrations and plasma anastrozole C_{min} are summarized in Table 2. The 95% confidence limits of the ratio of the gls-means in the two groups overlapped unity, which indicates that there was no significant difference in serum oestrogen concentrations or plasma anastrozole C_{min} between the groups.

No serious adverse events were observed in this study, and no subject withdrew from the trial because of adverse events. In 8 of the Japanese and 17 of the Caucasian volunteers, the investigator considered at least one of their reported adverse events as an adverse drug reaction, but all of these were mild-to-moderate and were consistent with those reported in the anastrozole Summary of Product Characteristics.

Discussion

Substantially higher concentrations of oestradiol and oestrone sulphate have previously been found in postmenopausal white American women than in postmenopausal Japanese women [23], and significant differences in the binding of oestradiol by sex hormone binding globulin between Japanese and British women have also been reported [20]. In the present study, there was no indication of higher levels of oestradiol in the Caucasian group. It is notable that in the study by Shimizu et al. [23], the Japanese women were selected from a rural background to reflect a traditional Japanese environment. The Japanese women in this study were mainly from an urban background.

Serum oestradiol and oestrone sulphate levels were suppressed by approximately 87% and 93%, respectively, in both Caucasian and Japanese women during treatment with anastrozole. These figures are consistent

Table 1 Serum concentrations of oestradiol and oestrone sulphate in Japanese and Caucasian subjects before and during treatment with anastrozole. Results are presented as the geometric means of the medians of three consecutive measurements, with ranges

	Japanese (n = 22)				Caucasian (n = 23)			
	Oestradiol (pmol/l)		Oestrone sulphate (pmol/l)		Oestradiol (pmol/l)		Oestrone sulphate (pmol/l)	
	Baseline	End of treatment	Baseline	End of treatment	Baseline	End of treatment	Baseline	End of treatment
Mean	21.4	2.7	561	40	25.9	3.3	476	32
Range	8.7–44.8	1.5–4.9	124–3693	10–227	11.0–67.5	1.5–5.3	98–1751	10–85
Coefficient of variation (%)	57.8	30.7	102.2	96.8	50.9	34.2	75.7	60.7
Suppression from baseline (%)		87.3		93.0		87.2		93.3

Table 2 Comparison of serum oestradiol and oestrone sulphate, and plasma anastrozole C_{min} in Japanese and Caucasian subjects during treatment with anastrozole 1 mg/day. Results are presented as geometric least squares means and as a consequence they differ from the means shown in Table 1

	Japanese (n = 22)	Caucasian (n = 23)	Ratio Japanese/ Caucasian	95% confidence limits	P-value
Oestradiol	2.8 pmol/l	3.2 pmol/l	0.87	0.74 to 1.03	0.102
Oestrone sulphate	37.2 pmol/l	33.9 pmol/l	1.10	0.84 to 1.43	0.475
Plasma anastrozole C_{min}	30.4 ng/ml	25.7 ng/ml	1.18	0.97 to 1.44	0.093

with the results of studies in Western populations [12, 22, 24], which have shown that maximal suppression of oestrogen levels is achieved with anastrozole doses of 1 mg/day and above. The comparison of oestradiol suppression may not always yield meaningful data as on-treatment levels are frequently near the sensitivity limit of the assay. However, the much higher plasma levels of oestrone sulphate allow greater sensitivity in comparisons between agents. The very similar suppression of both oestrogens adds confidence to the data. Although oestrone is the principle product of aromatization in postmenopausal women, the sensitivity of available assays in relation to the prevailing plasma levels of oestrone provides no more sensitivity for the comparison of effects on oestrogen suppression than oestradiol assays. Oestrone was therefore not included in the present study. Given that the effects on oestrogen suppression of anastrozole were similar in Japanese and Caucasian subjects, it was considered that measurement of the androgenic substrates of aromatization would not add significant value to the study.

In a comparative study in postmenopausal women with breast cancer, anastrozole, 1 mg/day, has been shown to suppress circulating oestradiol concentrations to a greater extent than the steroidal aromatase inhibitor formestane [17], and indirect comparisons suggest that anastrozole, 1 mg/day, has a greater effect on aromatase than aminoglutethimide, fadrozole or rogletimide at their therapeutic dosages [7]. The finding that trough anastrozole concentrations were similar in the two groups studied here suggests that the pharmacokinetics of anastrozole are not significantly different in Japanese women. Together with the similarity of oestrogen suppression, the same sensitivity of oestrogen synthesis to anastrozole is suggested in Japanese and Caucasians.

In conclusion, this study showed that anastrozole has similar effects on serum oestrogen levels in postmenopausal Japanese and Caucasian women. Studies in Western populations have shown that aromatase inhibitors have an important place in the treatment of breast cancer and that oestrogen suppression is widely accepted as a good surrogate for the clinical potential of aromatase inhibitors. The results of this study therefore suggest that the clinical activity of anastrozole in Japanese patients with breast cancer should be similar to that seen in Western patients and support the use of anastrozole in Japanese patients with breast cancer.

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