

# Influence of Letrozole and Anastrozole on Total Body Aromatization and Plasma Estrogen Levels in Postmenopausal Breast Cancer Patients Evaluated in a Randomized, Cross-Over Study

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**Purpose:** To compare the effects of the two novel, potent, nonsteroidal aromatase inhibitors anastrozole and letrozole on total-body aromatization and plasma estrogen levels.

**Patients and Methods:** Twelve postmenopausal women with estrogen receptor-positive, metastatic breast cancer were treated with anastrozole 1 mg orally (PO) and letrozole 2.5 mg PO once daily, each given for a time interval of 6 weeks in a randomized sequence. Total-body aromatization was determined before treatment and at the end of each treatment period using a dual-label isotopic technique involving isolation of the metabolites with high-performance liquid chromatography. Plasma levels of estrone (E<sub>1</sub>), estradiol (E<sub>2</sub>), and estrone sulfate (E<sub>1</sub>S) were determined in samples obtained before each injection using highly sensitive radioimmunoassays.

**Results:** Pretreatment aromatase levels ranged from 1.68% to 4.27%. On-treatment levels of aromatase were detectable in 11 of 12 patients during

treatment with anastrozole (mean percentage inhibition in the whole group, 97.3%) but in none of the 12 patients during treatment with letrozole (> 99.1% suppression in all patients; Wilcoxon,  $P = .0022$ , comparing the two drug regimens). Treatment with anastrozole suppressed plasma levels of E<sub>1</sub>, E<sub>2</sub>, and E<sub>1</sub>S by a mean of 81.0%, 84.9%, and 93.5%, respectively, whereas treatment with letrozole caused a corresponding decrease of 84.3%, 87.8% and 98.0%, respectively. The suppression of E<sub>1</sub> and E<sub>1</sub>S was found to be significantly better during treatment with letrozole compared with anastrozole ( $P = .019$  and  $.0037$ , respectively).

**Conclusion:** This study revealed letrozole (2.5 mg once daily) to be a more potent suppressor of total-body aromatization and plasma estrogen levels compared with anastrozole (1 mg once daily) in postmenopausal women with metastatic breast cancer.

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AROMATASE INHIBITION is a well-established treatment modality for postmenopausal women with hormone-sensitive metastatic breast cancer.<sup>1</sup> Recently, several new aromatase inhibitors shown to be more potent and less toxic compared with the first and second generation aromatase inhibitors have become available.<sup>2</sup>

Anastrozole (Arimidex; Zeneca Pharmaceuticals [Astra-Zeneca], Macclesfield, United Kingdom) and letrozole (Femara; Novartis Pharmaceuticals, Basel, Switzerland) are two third-generation, highly potent, nonsteroidal aromatase inhibitors used for the treatment of advanced breast cancer in postmenopausal patients. Anastrozole and letrozole, as well as the steroidal aromatase inactivator exemestane, have revealed clinical superiority compared with megestrol acetate or aminoglutethimide as second-line therapy for metastatic breast cancer in postmenopausal women.<sup>3-6</sup> Currently, all these drugs are under evaluation as first-line therapy for metastatic disease and in the adjuvant setting.

Clinical studies have compared each of these novel aromatase inhibitors to conventional therapy, but so far, no study has compared the in vivo biochemical efficacy or clinical effects of third-generation aromatase inhibitors head to head. The finding that these novel drugs show clinical superiority compared with conventional treatment, together with recent in vitro studies revealing estrogens in low

concentrations to stimulate tumor cell growth,<sup>7</sup> indirectly supports the hypothesis that the degree of estrogen suppression may be of importance for clinical outcome. Clinical support for such clinical-pharmacologic relationships is provided by the greater efficacy of letrozole 2.5 mg daily compared with aminoglutethimide 500 mg daily<sup>3</sup> together with previous findings that letrozole<sup>8</sup> inhibits whole-body aromatization by approximately 99% compared with an inhibition of approximately 90% recorded with aminoglutethimide (1,000 mg/d).<sup>9</sup>

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Table 1. Patient Characteristics

Patient No.	Age (years)	BMI	ER	PGR	Metastasis	Pretreatment Therapy
1	78	19.2	Pos	Pos	N, L	T, E, M
2	79	29.2	Pos	Pos	L	None
3	75	29.8	91.2	252.0	L	T
4	55	27.1	79.3	161.0	B, S, N	T
5	79	23.3	Pos	Pos	N, S	T
6	69	34.0	Pos	Pos	B, S	None
7	54	26.9	Pos	Unk	N, L	T
8	70	18.4	Pos	Pos	S	T, A
9	76	18.3	Pos	Pos	N, S	T
10	69	32.5	51.5	14.7	S	D, T
11	77	23.6	932.0	79.1	L	None
12	64	25.1	112.4	87.5	B	T

Abbreviations: BMI, body mass index; ER, estrogen receptor (either as fmol/mg or staining by immunohistochemistry); Pos, positive; PGR, progesterone receptor; Unk, unknown; N, lymph nodes; L, lung; B, bone; S, skin; T, tamoxifen; D, droloxifene; A, aminoglutethimide; E, exemestane; M, megestrol.

Although different studies have revealed anastrozole<sup>10</sup> as well as letrozole<sup>11</sup> to be highly potent aromatase inhibitors, in vitro potency may not be directly correlated to in vivo efficacy because the latter will depend on drug disposition. However, studies in nude mice model systems have suggested a more potent antitumor efficacy of letrozole compared with anastrozole.<sup>12</sup> In addition, previous studies conducted by our groups<sup>8,13</sup> revealed both drugs to inhibit in vivo aromatization by 97% to 99% and suggested a somewhat better efficacy of letrozole 2.5 mg compared with anastrozole 1 mg daily (the drug doses recommended for clinical use). Because of interindividual variation in drug response, firm conclusions could not be drawn from such an indirect comparison. The aim of the present study was to compare the biochemical efficacy of anastrozole and letrozole in breast cancer patients by evaluating their influence on total-body aromatization and plasma estrogen levels in the same patients using a randomized cross-over design.

## PATIENTS AND METHODS

### Patients

Twelve postmenopausal women (median age, 72 years; range, 54 to 79 years) with estrogen receptor–positive, metastatic breast cancer suitable for treatment with an aromatase inhibitor were enrolled (Table 1). Postmenopausal status was defined as amenorrhoea for the duration of 1 year or more, with luteinizing hormone and follicle-stimulating hormone levels in the postmenopausal range. Previous anticancer treatment was terminated at least 4 weeks before commencing treatment, and no other anticancer treatment was allowed during the study period. Patients treated with any other drugs known to influence plasma estrogen levels (like anti-epileptics)<sup>14,15</sup> were ineligible.

### Treatment

All patients were treated at the Department of Oncology, Haukeland University Hospital, Bergen, Norway. Each patient received anastro-

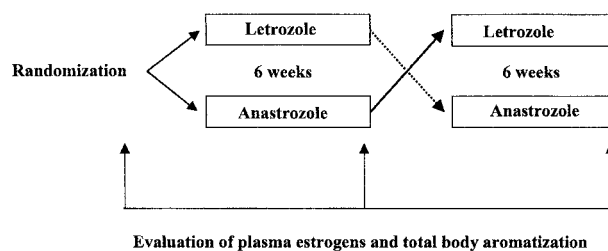


Fig 1. Study design.

zole 1 mg orally (PO) or 2.5 mg letrozole PO once daily in a double-blind cross-over study (Fig 1). Each regimen was administered for a period of 6 weeks. Six patients were randomly allocated to start treatment with anastrozole followed by letrozole, whereas the other six received letrozole upfront. The protocol was approved by the local ethical committee, and every patient gave her written informed consent. After completion of the study protocol period, treatment was continued with either anastrozole or letrozole until disease progression.

### Tracer Administration

In vivo aromatization was determined before therapy and at the end of each treatment period. On each occasion, the patient received a bolus injection of [<sup>3</sup>H]androstenedione (500  $\mu$ Ci) and [<sup>14</sup>C]estrone (5  $\mu$ Ci) dissolved in 50 mL of saline containing 8% ethanol (w/w) as described elsewhere.<sup>16</sup> All injections were administered between 08:00 and 09:00 AM after an overnight fast and before the daily intake of anastrozole or letrozole.

### Blood Samples and Estrogen Measurements

Blood samples for hormone measurements were obtained into heparinized vials (two vials containing 10 mL each) immediately before each tracer injection after an overnight fast. Plasma was separated by centrifugation and stored at  $-20^{\circ}\text{C}$  until analyzed. Estradiol ( $\text{E}_2$ ) and estrone ( $\text{E}_1$ ) were determined by radioimmunoassay, as reported elsewhere.<sup>17,18</sup> Plasma levels of estrone sulfate ( $\text{E}_1\text{S}$ ) were determined by a novel highly sensitive assay involving purification and derivatization into  $\text{E}_2$  and radioimmunoassay analysis using  $\text{E}_2$ -6-carboxy-methylloximine-[2-<sup>125</sup>I]iodohistamine as tracer ligand.<sup>19</sup> The sensitivity limits for plasma levels of  $\text{E}_2$ ,  $\text{E}_1$ , and  $\text{E}_1\text{S}$  were 2.1, 6.3, and 2.7 pmol/L, respectively.<sup>17-19</sup>

### Urine Collection for the Measurement of Total-Body Aromatization

Urine was collected for a period of 96 hours after each tracer injection, pooled, and kept frozen ( $-20^{\circ}\text{C}$ ) until analyzed. The estrogen fraction was isolated and purified through multiple chromatographic steps involving high-performance liquid chromatography, and the percentage aromatization was calculated from the  $^3\text{H}/^{14}\text{C}$  isotope ratio in the intravenous vehicle and urine estrogen fractions, as described elsewhere.<sup>8,16</sup> On formal assessment, this method was shown to allow detection of up to 99.1% inhibition of aromatization.<sup>8</sup> The 96-hour urine collection was completed before any change of therapy.

### Statistical Methods

Previous work by our group has shown plasma estrogen levels in postmenopausal women to be well fitted to a log-normal distribution.<sup>18</sup>

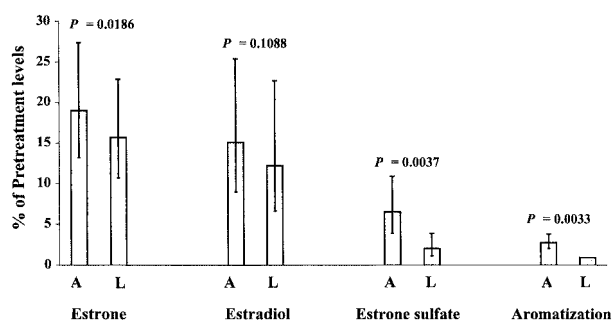


Fig 2. Percentage of pretreatment levels of  $E_1$ ,  $E_2$ , and  $E_{1S}$ , and aromatase (A: during treatment with anastrozole; L: during treatment with letrozole; geometric mean values with 95% CI of the mean). The  $P$  values refer to the differences between the two treatment regimens.

Thus, all plasma estrogen levels and the percentage of aromatization obtained before therapy and during treatment with anastrozole or letrozole are given as their geometric mean values with 95% confidence intervals (CI) of the mean. Whenever estrogen levels below the sensitivity limits of the assays were found, the corresponding sensitivity limit was used for statistical analysis. Considering measurement of in vivo aromatization, a previous study revealed an inhibition of 99.1%<sup>8</sup> as the limit of detection, and this value was used for statistical comparisons whenever a value above this threshold was recorded. The Friedman test was used to compare three data groups, whereas data obtained during treatment with anastrozole or letrozole were compared using the Wilcoxon matched-pair signed rank test. In addition, we performed a two-way analysis of variance to test for interaction between effects of sequence of administration on estrogen levels and degree of aromatization. The mean percentage change was calculated as  $100 - x$ , where  $x$  is the geometric mean value of the individual parameters in the on-treatment situation expressed as percentage of pretreatment values.<sup>13</sup>

## RESULTS

The geometric mean pretreatment plasma levels of  $E_1$ ,  $E_2$ , and  $E_{1S}$  were 78.1 pmol/L (95% CI, 56.1 to 108.7 pmol/L),

17.2 pmol/L (95% CI, 9.2 to 32.1 pmol/L), and 422.8 pmol/L (95% CI, 182.4 to 980.4 pmol/L), respectively. Treatment with anastrozole suppressed plasma levels of  $E_1$ ,  $E_2$ , and  $E_{1S}$  to a mean of 14.8 pmol/L (95% CI, 12.4 to 17.7 pmol/L), 2.6 pmol/L (95% CI, 1.9 to 3.5 pmol/L), and 27.6 pmol/L (95% CI, 14.0 to 54.3 pmol/L), respectively, corresponding to a mean suppression among individuals of 81.0%, 84.9%, and 93.5%, respectively. Letrozole treatment suppressed plasma levels of  $E_1$ ,  $E_2$ , and  $E_{1S}$  to a mean of 12.3 pmol/L (95% CI, 11.2 to 13.5 pmol/L), 2.1 pmol/L (95% CI, 2.1 to 2.1 pmol/L), and 8.9 pmol/L (95% CI, 4.9 to 16.0 pmol/L), respectively, corresponding to a mean suppression of 84.3%, 87.8%, and 98.0%, respectively (Fig 2). Notably, nine and 12 patients had their plasma levels of  $E_2$  suppressed below the sensitivity limit of the assay during treatment with anastrozole and letrozole, respectively, making any comparison between on-treatment values of  $E_2$  impossible. None of the patients had plasma  $E_1$  levels below the sensitivity limit, whereas three patients had plasma  $E_{1S}$  suppressed below the sensitivity limit during treatment with letrozole. The suppression of plasma  $E_1$  and  $E_{1S}$  levels was significantly greater during treatment with letrozole compared with treatment with anastrozole ( $P = .019$  and  $P = .0037$ , respectively), with lower levels of plasma  $E_1$  and  $E_{1S}$  in nine and 11 patients during treatment with letrozole compared with anastrozole, respectively.

Pretreatment aromatization levels ranged from 1.68% to 4.27%. Although on-treatment levels of aromatase were detectable in 11 of 12 patients during treatment with anastrozole, we found whole-body aromatization to be suppressed below the detection limit ( $> 99.1\%$  suppression) of the method in all 12 patients during treatment with letrozole (Table 2 and Fig 3). Thus, inhibition of aromati-

Table 2. Influence of Letrozole and Anastrozole on Total-Body Aromatization

Patient No.	Pretreatment Aromatization (%)	Letrozole		Anastrozole	
		Aromatization (%)	Suppression (%)	Aromatization (%)	Suppression (%)
1	1.942	0.013	> 99.1	0.092	95.3
2	3.025	0.004	> 99.1	0.075	97.5
3	1.767	0.013	> 99.1	0.047	97.2
4	1.675	0.007	> 99.1	0.038	97.8
5	2.366	0.009	> 99.1	0.062	97.4
6	3.854	0.006	> 99.1	0.091	97.6
7	1.687	0.002	> 99.1	0.031	98.2
8	2.029	0.001	> 99.1	0.144	92.9
9	2.952	0.012	> 99.1	0.100	96.6
10	4.270	0.005	> 99.1	0.011	> 99.1
11	2.238	0.005	> 99.1	0.058	97.4
12	2.384	0.010	> 99.1	0.096	96.0
Geometric mean	2.399	0.006	> 99.1	0.059	97.3
95% CI	1.966-2.927	0.004-0.009		0.038-0.092	96.2-98.0

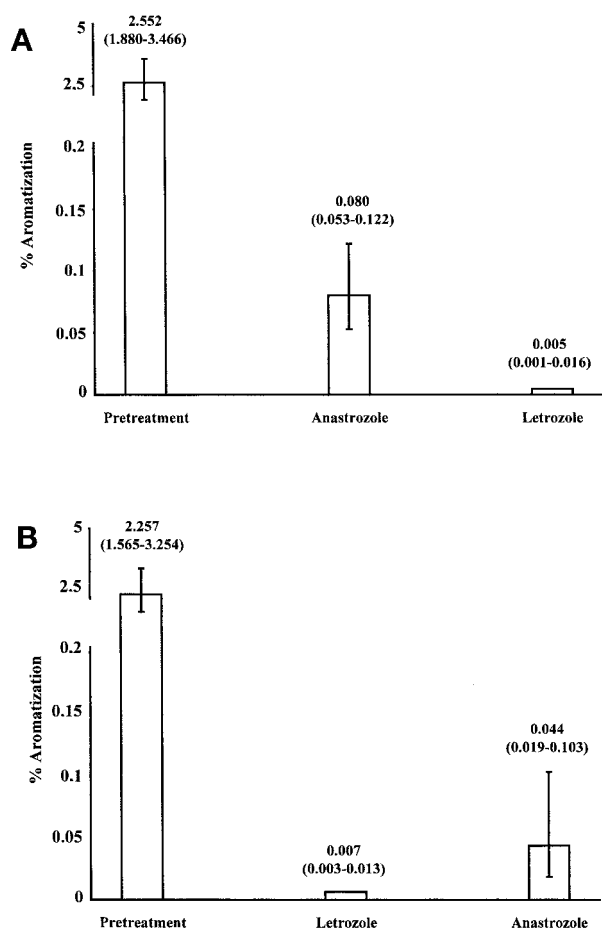


Fig 3. Influence of the treatment with (A) anastrozole followed by letrozole ( $n = 6$ ) and (B) letrozole followed by anastrozole on the percentage of aromatization ( $n = 6$ ). Geometric mean values with 95% CI of the mean.

zation was demonstrably greater in 11 of 12 patients treated with letrozole. The geometric mean suppression of aromatization was 97.3% during treatment with anastrozole and more than 99.1% during letrozole treatment (Wilcoxon  $P = .0022$ ).

A two-way analysis of variance, taking account of the cross-over design of the study, confirmed a significant difference in the degree of aromatase inhibition ( $P < .001$ ) as well as plasma levels of  $E_1$  ( $P = .05$ ) and  $E_1S$  ( $P = .012$ ) between treatment with letrozole and anastrozole but no interaction between sequence of treatment and the two drug regimens for any of these parameters ( $P > .15$  for each; Figs 3 and 4).

## DISCUSSION

Anastrozole and letrozole are two third-generation aromatase inhibitors belonging to the triazole class. Both drugs

have previously been shown to be highly potent aromatase inhibitors causing profound suppression of plasma estrogen levels in postmenopausal women.<sup>8,13</sup>

Indirect evidence suggests a dose-response relationship between the degree of estrogen suppression and clinical effects in breast cancer. Drugs acting on adrenal steroid synthesis as well as glucocorticoids have been reported to cause modest suppression of plasma estrogen levels and low response rates in breast cancer patients.<sup>20</sup> Several pilot studies have confirmed the benefits of stepwise estrogen suppression in patients progressing after treatment with castration, adrenalectomy, or an aromatase inhibitor.<sup>21-24</sup> Although the mechanism of action of progestins in high doses in breast cancer is not fully understood, megestrol acetate given as 160 mg daily causes plasma estrogen suppression comparable with what has been recorded with the first generation aromatase inhibitor aminoglutethimide.<sup>6,25,26</sup> The degree of plasma estrogen suppression achieved with aminoglutethimide as well as megestrol acetate is of a smaller magnitude compared with the degree of suppression achieved with novel drugs like anastrozole, letrozole, and exemestane, which are all found to be superior with respect to clinical effects.<sup>3,5,6,27</sup> In particular, the large randomized trial demonstrating greater efficacy of 2.5 mg letrozole in comparison with 500 mg aminoglutethimide<sup>3</sup> suggests a dose relationship within the limits of aromatase inhibition achieved with these two drugs, ie, more than 99% and approximately 85%, respectively.

With several novel aromatase inhibitors at hand, an important question is whether one drug may be superior to the others with respect to biochemical and clinical efficacy. Although several studies have revealed lack of complete cross-resistance between different nonsteroidal aromatase inhibitors and steroidal so-called aromatase inactivators,<sup>24,28-31</sup> there may be several explanations to this observation.<sup>32</sup> Comparison of the biochemical and clinical efficacy between different drugs of the same class, like anastrozole and letrozole, is interesting for several reasons. First, any difference in clinical efficacy would select the appropriate drug for patient treatment. Second, any correlation (or lack of such) between the degree of estrogen suppression and clinical outcome with the different drugs would provide us with information about whether the degree of estrogen deprivation is of clinical importance or there may be thresholds for estrogen levels with respect to clinical response. Data regarding a potential dose-response effect with anastrozole<sup>33</sup> or letrozole<sup>3,5</sup> are conflicting. Interestingly, data from our previous investigations suggested that letrozole, at its lower dose (0.5 mg once daily), achieved a greater aromatase inhibition compared with anastrozole at its higher dose (10 mg once daily).<sup>8,13</sup> A

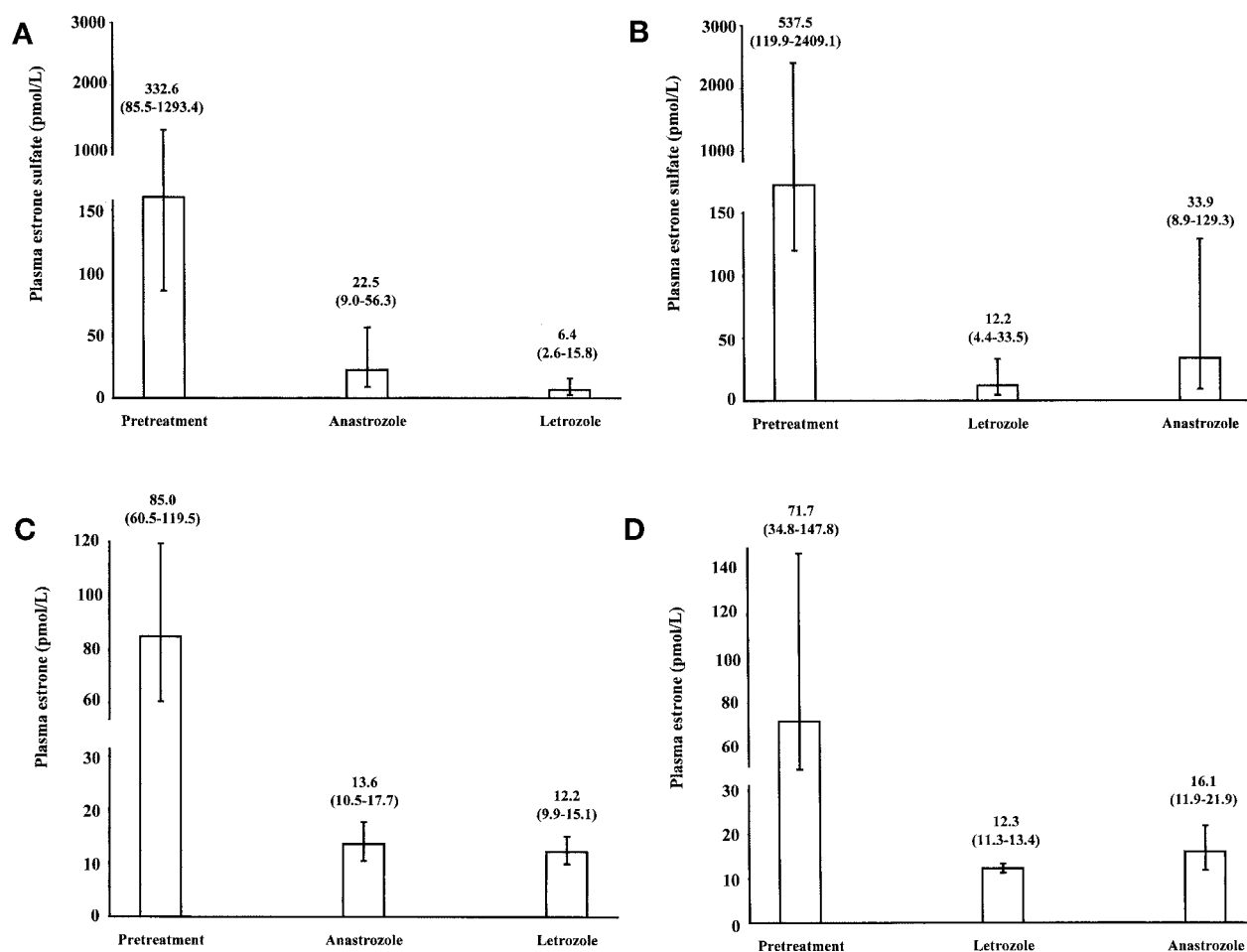


Fig 4. A. Influence of the cross-over (A) from anastrozole to letrozole ( $n = 6$ ) and (B) from letrozole to anastrozole on plasma  $E_1S$  levels ( $n = 6$ ). Influence of the cross-over (C) from anastrozole to letrozole ( $n = 6$ ) and (D) from letrozole to anastrozole on plasma  $E_1$  levels ( $n = 6$ ). Geometric mean values with 95% CI of the mean.

study comparing the clinical efficacy of letrozole (2.5 mg daily) to anastrozole (1 mg daily) administered as second-line endocrine therapy for metastatic breast cancer is ongoing. The results of that trial will be instrumental in assessing whether suppression of aromatase to below 3% residual activity is clinically important in that context.

This study revealed a significant difference in the biochemical efficacy of letrozole and anastrozole with regard to *in vivo* aromatase inhibition as well as plasma estrogen suppression in postmenopausal breast cancer patients. The results on inhibition of aromatase are closely similar to those which we have reported previously from separate studies of these inhibitors.<sup>8,13</sup> A significant difference in suppression of plasma  $E_1$  and  $E_1S$  in addition to a more effective inhibition of aromatization was recorded during therapy with letrozole compared with anastrozole. Any

potential difference in the effect on plasma  $E_2$  could not be evaluated for technical reasons because of the fact that all patients obtained plasma levels below the sensitivity limit of the assay during treatment with letrozole and nine patients had values below the limit on treatment with anastrozole.

Similar to other studies on aromatase inhibitors,<sup>13,34</sup> we found a better suppression of plasma  $E_1S$  compared with the other estrogen fractions and that this effect on  $E_1S$  more closely reflected differences in aromatase inhibition. It should also be noted that the degree of plasma  $E_1S$  suppression has been found to correspond more closely to the percentage *in vivo* aromatization than suppression of  $E_1$  and  $E_2$  also in other studies using the same methods as here.<sup>13</sup> This is probably a result of technical difficulties measuring plasma  $E_1$  and  $E_2$  levels in the low range obtained during therapy with these drugs, indicating the importance of

simultaneous measurements of several estrogen fractions in the same sample. The ratio between mean plasma hormone levels in untreated postmenopausal patients and the sensitivity limit of the assay is about 1:10 for  $E_2$  and  $E_1$  but below 1:100 for  $E_1S$ . Use of alterations in plasma  $E_1S$  levels as a surrogate marker for alterations in plasma estrogens in general is further supported by knowledge that plasma levels of  $E_1$ ,  $E_2$ , and  $E_1S$  are at equilibrium provided no interaction with interconverting enzymes occurs.<sup>35</sup> Overall, plasma  $E_1S$  seems to be the most reliable indicator of estrogen suppression in patients treated with potent aromatase inhibitors.<sup>36</sup>

The cross-over design of the present study was chosen to avoid interindividual variation concerning aromatase inhibition and plasma estrogen suppression in our comparison. The mean half-life of both letrozole and anastrozole is approximately 40 to 50 hours. Thus, in theory, more than 99.99% of the drugs will be eliminated over a time period of 4 weeks after the final dose. We used a time period of 6 weeks on each regimen to avoid any possible hang-over effects. A wash-out period without any treatment could have detrimental effects on the therapeutic outcome in this group of patients with metastatic breast cancer. The finding of a greater aromatase inhibition and estrogen suppression of letrozole when given either first- or second-line together with a negative test for interaction demonstrates that any hang-over effects are unlikely in this study. Notably, the design of this study required a short-term course with each drug for valid comparison. Although we may not exclude the possibility of alterations in drug disposition over time during treatment with these compounds, the two studies addressing long-term endocrine effects of an aromatase

inhibitor (aminoglutethimide) both reported an increase in plasma androgens<sup>37,38</sup> and an increase in plasma estrone at time of progression.<sup>37</sup> These results are consistent with a nonspecific stress reaction causing elevated adrenal hormone secretion and do not suggest any escape from the aromatase inhibitory effect. In a recently published study, patients progressing on different nonsteroidal aromatase inhibitors were treated with exemestane. Interestingly, patients failing on novel nonsteroidal aromatase inhibitors were found to have consistently suppressed plasma estrogen levels.<sup>31</sup> Thus, although our short-term data may not be directly extrapolated to long-term treatment, currently there is little evidence suggesting alterations in drug pharmacology during chronic treatment.

In conclusion, this study is the first head-to-head comparison evaluating potential endocrine differences between third-generation aromatase inhibitors *in vivo*. We found letrozole to cause a significantly better inhibition of *in vivo* aromatization and a better plasma estrogen suppression compared with anastrozole. Together with clinical data from ongoing trials, we believe the results from this study may provide important information not only to select between two drugs for clinical use but to also understand the important relationship between degree of estrogen suppression and clinical efficacy of estrogen suppressors in breast cancer patients.

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