In Vivo Inhibition of Aromatization by Exemestane, a Novel Irreversible Aromatase Inhibitor, in Postmenopausal Breast Cancer Patients

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ABSTRACT

The effect of exemestane (6-methylenandrosta-1,4-diene-3,17-dione) 25 mg p.o. once daily on in vivo aromatization was studied in 10 postmenopausal women with advanced breast cancer. Aromatization was determined before treatment and after 6–8 weeks on therapy by administering a bolus injection of [3H]androstenedione (500 μCi) and [14C]estrone (5 μCi) followed by measurement of the isotope ratio of urinary estrogens after high-performance liquid chromatography purification. In addition, plasma endogenous estrogens were measured with highly sensitive radioimmunoassays after separation with high-performance liquid chromatography. Treatment with exemestane suppressed whole body aromatization from a mean pretreatment value of 2.059% to 0.042% (mean suppression of 97.9%). Plasma levels of estrone, estradiol, and estrone sulfate were found to be suppressed by 94.5%, 92.2%, and 93.2%, respectively. This is the first study revealing near total aromatase inhibition in vivo with the use of a steroidal aromatase inhibitor. The observation that exemestane is a highly potent aromatase inhibitor, together with the fact that the drug is administered p.o. and causes limited side effects, suggests that exemestane is a promising new drug for the treatment of hormone sensitive breast cancer.

INTRODUCTION

Aromatase inhibition is a well-established therapeutic option in postmenopausal breast cancer patients. The first generation aromatase inhibitor AG3 has been used for breast cancer treatment for more than two decades (1). Because of substantial side effects associated with AG treatment, several new aromatase inhibitors have been introduced in clinical trials.

Aromatase inhibitors can be divided into two major classes of compounds, steroidal and nonsteroidal drugs. Nonsteroidal aromatase inhibitors include AG and the imidazole/triazole compounds. With the exception of testolactone, a testosterone derivative (2), steroidal aromatase inhibitors are all derivatives of A, the natural substrate for the aromatase enzyme (3).

The second generation steroidal aromatase inhibitor, 4-hydroxyandrostenedione (4-OHA, formestane), was found to inhibit peripheral aromatization by ~85% when administered by the i.m. route at a dosage of 250 mg every 2 weeks as recommended (4) but only by 50–70% (5) when administered p.o. Thus, a major disadvantage with this drug is that it has to be administered by the i.m. route to maximize its pharmacological effect (6).

Exemestane (6-methylenandrosta-1,4-diene-3,17-dione) is a third generation steroidal aromatase inhibitor developed for oral administration. Treatment with exemestane given p.o. has been shown to be well tolerated, and chronic treatment was found to suppress plasma estrogen levels by about 85–95% when the drug was administered at a dose of ≥10 mg o.d. (7). Although this suggests exemestane to be a potent aromatase inhibitor, direct measurement of in vivo aromatase inhibition during treatment with exemestane has not been conducted thus far.

In the present study, we measured in vivo aromatization before and during treatment with exemestane (25 mg o.d. by the oral route) by administering radiolabeled bolus injections of [3H]androstenedione and [14C]estrone followed by determination of the isotope ratio in urinary estrogens. In addition, plasma levels of E1, E2, and E1S were determined before and during treatment with exemestane.

PATIENTS AND METHODS

Patients. Postmenopausal women suffering from locally advanced or metastatic breast cancer failing on tamoxifen were eligible and were treated as part of a larger multicenter, Phase II study evaluating the clinical effects of exemestane 25 mg o.d. The associated biochemical study presented here was approved by the regional ethical committee, and the patients gave their written informed consent in addition to their consent for enroll-

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2 To whom requests for reprints should be addressed, at Department of Oncology, Haukeland University Hospital, N-5021 Bergen, Norway. Phone: 47-55-97-20-10; Fax: 47-55-97-20-46.
Aromatase Inhibition with Exemestane

The study was a biochemical study. A total number of 11 patients was enrolled in the clinical study. Previous treatment was terminated at least 4 weeks before commencing treatment with exemestane, and no other anticancer treatment was allowed during the study period. Urine was collected for a period of 96 h, pooled, and kept frozen (−20°C) until processing. A major estrogen metabolite in the urine is 2-hydroxyestrone (14). Enhanced 2-hydroxylation, that the reason for the low amount of radioactivity in the crude urine samples in both test situations, little radioactivity was recovered in one patient (patient 11). This patient received her proper tracer dose, but although radioactivity was recovered from the crude urine samples in both test situations, little radioactivity (14°C and 3H) was recovered from the purified estrogen fractions in the second test situation. Repeated measurements suggested that the reason for the low amount of radioactivity in the urinary E1 and E2 fractions in this particular patient could not be due to technical flaws. A major estrogen metabolite in the urine is 2-hydroxyestrone (14). Enhanced 2-hydroxylation,

### Table 1: Influence of treatment with exemestane on percentage of peripheral aromatization

<table>
<thead>
<tr>
<th>Patient</th>
<th>Pretreatment</th>
<th>Exemestane</th>
<th>% suppression</th>
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<td>0.174</td>
<td>91.7</td>
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<td>2</td>
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<td>0.063</td>
<td>97.6</td>
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<td>3</td>
<td>1.399</td>
<td>0.028</td>
<td>98.0</td>
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<td>1.174</td>
<td>0.042</td>
<td>96.4</td>
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<tr>
<td>5</td>
<td>1.531</td>
<td>0.051</td>
<td>96.7</td>
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<tr>
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<td>4.462</td>
<td>0.032</td>
<td>&gt;99.1</td>
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<tr>
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<td>0.015</td>
<td>99.0</td>
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<tr>
<td>8</td>
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<td>&gt;99.1</td>
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<td>9</td>
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<td>0.091</td>
<td>95.2</td>
</tr>
<tr>
<td>10</td>
<td>2.052</td>
<td>0.020</td>
<td>99.0</td>
</tr>
<tr>
<td>Geo. mean</td>
<td>2.059</td>
<td>0.042</td>
<td>97.9</td>
</tr>
</tbody>
</table>

CI (1.498–2.829) (0.025–0.071) (96.3–98.8)

* Percentage of aromatization.

### Table 2: Influence of treatment with exemestane on plasma estrogen levels (pmol/liter)

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<td>24.2</td>
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<td>&gt;89.4</td>
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<td>90.4</td>
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<td>&gt;96.3</td>
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<tr>
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<td>&gt;95.3</td>
<td>17.6</td>
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<tr>
<td>CI</td>
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<td>88.7–94.7</td>
<td>798–2181</td>
<td>57–141</td>
<td>91.2–94.8</td>
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</table>

* Pretreat., pretreatment value; % sup., percentage of suppression from baseline; Geo. mean, geometric mean value.

### Study Protocol
Whole body aromatization was measured before treatment with exemestane and after 6 to 8 weeks on treatment (8, 9). Exemestane was administered by the oral route at a dose of 25 mg o.d. The patients received their first dose of exemestane after the completion of the first urine collection period.

### Measurement of Whole Body Aromatization
Aromatization of A to E1 can be measured in vivo by either administration of a steady-state infusion or a bolus injection of A and E1 labeled with different isotopes followed by determination of the isotope ratio in plasma estrogens or urinary estrogens, respectively. In this study, we used a HPLC technique to measure the isotope ratio in urinary estrogens after a bolus injection of [3H]androstenedione (500 μCi) and [14C]estrone (5 μCi) dissolved in 50 ml of saline containing 8% ethanol (w/w; Ref. 8). Aliquots of the isotopes in the injection mixture were taken to calculate the 3H:14C ratio. Urine was collected for a period of 96 h, pooled, and kept frozen (−20°C) until processing. A recent assessment of the sensitivity of this method revealed that inhibition of whole body aromatization up to 99.1% is detectable (10).

### Hormone Measurements
Blood samples for hormone measurements were obtained immediately before the radiotracer injections administered on day −4 and after 6–8 weeks on treatment with exemestane. After an overnight fast, the blood samples were collected between 8 and 10 a.m. before intake of the drug. Plasma was separated by centrifugation and stored at −20°C until processing. Each plasma estrogen (E1, E2, and E3) was measured by RIAs after HPLC purification according to the technical system used for the purification of the steroids. The intra-assay coefficient of variation was <10% for each estrogen.

### Statistical Methods
Previous studies by our group have shown plasma estrogen levels in postmenopausal women to be log-normal distributed (13). Thus, plasma estrogen levels as well as aromatization levels obtained before and during treatment with exemestane are given as their geometric mean value with 95% CI of the mean. For estrogen levels below the sensitivity limit of the assays, the sensitivity limit was used for statistical analysis.

### RESULTS
The percentage of aromatase inhibition could not be evaluated in one patient (patient 11). This patient received her proper tracer dose, but although radioactivity was recovered from the crude urine samples in both test situations, little radioactivity (14C and 3H) was recovered from the purified estrogen fractions in the second test situation. Repeated measurements suggested that the reason for the low amount of radioactivity in the urinary E1 and E2 fractions in this particular patient could not be due to technical flaws. A major estrogen metabolite in the urine is 2-hydroxyestrone (14). Enhanced 2-hydroxylation,
Mechanisms of action of steroidal (Type-I) and nonsteroidal (Type-II) aromatase inhibitors at the aromatase complex. 

A. A occupies the SBS. B, the steroidal aromatase inhibitor exemestane occupies the SBS and excludes A. Heme activity is required for the suicide inhibition (inactivation) of the enzyme. The 6-methylene group of exemestane probably sticks into one of the hydrophobic pockets (HP). C, the triazole group of the nonsteroidal aromatase inhibitor letrozole coordinates the iron atom of the heme, occupies part of the SBS, and reversibly excludes A.

Fig. 1

which may be caused by, among other factors, dietary compounds (15), will increase the synthesis of this metabolite (and other catechol estrogens also) with a similar drop in the excretion of other estrogen metabolites. Because no antioxidant was added to our solvent systems, all catechol estrogens would be destroyed during the purification procedures (16). Thus, one possible explanation for the finding of little E1 and E3 could be enhanced production of catechol estrogens in this patient in her second test situation, which left 10 patients evaluable for the determination of in vivo aromatase inhibition (Table 1). One of these patients (patient 5) had plasma E2 levels below the detection limit and an E1 level close to the detection limit in the pretreatment situation, which left nine patients for the assessment of plasma E1, E2, and E1S suppression (Table 2).

The percentage of total body aromatization was 2.059% before the initiation of treatment (geometric mean value; 95% CI, 1.498–2.829) and fell to a mean value of 0.042% (95% CI, 0.025–0.071) during treatment with exemestane. This corresponds to an aromatase inhibition of 97.9% (geometric mean value; 95% CI, 96.3–98.8). Thus, all but 1 of the patients experienced a suppression of total body aromatization by >95%, and 5 of 10 patients experienced suppression by at least 98%.

The influence of exemestane therapy on plasma estrogen levels is shown in Table 2. Plasma E2 was suppressed from a mean level of 35.6 pmol/liter (95% CI, 24.1–52.7 pmol/liter) before treatment to a mean of 2.8 pmol/liter (95% CI, 2.4–3.2 pmol/liter) during exemestane therapy, whereas plasma levels of E1 fell from a mean pretreatment value of 162.4 pmol/liter (95% CI, 114.5–230.5 pmol/liter) to 8.9 pmol/liter (95% CI, 7.3–10.9 pmol/liter) during treatment with exemestane. In addition, plasma levels of E1S fell from a mean level of 1320 pmol/liter (95% CI, 798–2181 pmol/liter) before treatment to 89 pmol/liter (95% CI, 57–141 pmol/liter). Accordingly, treatment with exemestane decreased plasma levels of E1, E2, and E1S by mean values of 94.5% (95% CI, 92.8–95.8%), 92.2% (95% CI, 88.7–94.7%), and 93.2% (95% CI, 91.2–94.8%), respectively. Comparing the percentage of plasma estrogen suppression with the percentage of aromatase inhibition in individual patients revealed 0.403 ≤ R ≤ 0.477 for all of the three plasma estrogens.

DISCUSSION

The present trial reveals for the first time near total in vivo aromatase inhibition by an aromatase inhibitor belonging to the steroidal class. Administered at a dose of 25 mg o.d. by the oral route (the dose currently recommended for clinical use), we found exemestane to inhibit aromatization by a mean of 97.9% in breast cancer patients. This aromatase inhibition is significantly better than that reported for the second generation steroidal aromatase inhibitor, formestane (4), and testolactone (2), the only other steroidal aromatase inhibitors for which in vivo aromatase inhibition has been determined. The findings also reveal exemestane to be pharmacologically more effective than first and second generation nonsteroidal inhibitors like AG (1), rogletimide (17), and fadrozole (18) at their clinically used doses. The extent of whole body aromatase inhibition found during treatment with exemestane is comparable to what has recently been found for anastrozole and close to that found for letrozole, two highly potent aromatase inhibitors belonging to the triazole class (10, 19). Although the degree of aromatase inhibition achieved with these drugs has not been compared in randomized trials, all of these results were obtained in the same laboratory within a limited time frame, which suggests that these results may be reliably compared.

Plasma estrogen measurements in patients on exemestane treatment require HPLC purification before RIA analysis.
The invalidity of conventional RIA methods in patients on exemestane treatment may be caused by nonspecific cross-reactions to exemestane metabolites in the RIAs (20). Although the exact mechanism remains unknown, it is notable that the only modification of the steroidal structure with exemestane is the incorporation of a 6-methylene group. The estrogen antibodies used in most assays are derived from 6-conjugated hapten, which would tend to cross-react with modified steroids. Whether similar analytical problems may explain sustained plasma estrogens in patients on treatment with other steroidal aromatase inhibitors, such as formestane, is presently not known because plasma estrogen analysis, with and without HPLC purification, has not been performed on samples obtained from such patients. In any case, the given degree of uncertainty with estrogen measurements in patients treated with aromatase inhibitors in general and steroid drugs in particular underlines the necessity of tracer studies to estimate the pharmacological effectiveness. Using HPLC purification before estrogen measurements, we found exemestane to suppress plasma levels of E1, E2, and E3S by 94.5, 92.2, and 93.2%, respectively. These findings confirm our results obtained previously in a Phase I study (7) and reveal exemestane also to suppress plasma estrogens by a percentage comparable to that demonstrated for the triazole drugs vorozole (21), letrozole (22), and anastrozole (19). Interestingly, for all these drugs, the degree of estrogen suppression approaches the level of aromatase inhibition, which reveals an internal consistency between these observations.

The encouraging endocrine and clinical results obtained recently with nonsteroidal aromatase inhibitors such as letrozole (10) and anastrozole (19, 23) may challenge the role of new aromatase inhibitors such as exemestane for breast cancer treatment. However, steroidal aromatase inhibitors differ substantially in their biochemical action when compared with the nonsteroidal drugs. Whereas steroidal drugs bind to the substrate binding site of the enzyme ("type-I aromatase inhibitors"), nonsteroidal drugs bind to the heme part of the aromatase complex ("type-II aromatase inhibitors"; Fig. 1; Ref. 24). Furthermore, steroidal aromatase inhibitors have been shown to inactivate the aromatase enzyme irreversibly ("suicide inhibition"; Ref. 25). Thus, the lack of cross-resistance between steroidal and nonsteroidal aromatase inhibitors has been demonstrated in clinical trials in which patients who were resistant to AG subsequently responded to formestane (26, 27) and also to exemestane therapy (28), which suggests that these drugs may act partly by different mechanisms of action. There is evidence from in vitro studies (29) that intratumoral aromatase may respond differentially to aromatase inhibitors, which suggests that steroidal and nonsteroidal aromatase inhibitors may be used sequentially or in concert to improve anticancer therapy. Our finding that exemestane is a most potent aromatase inhibitor in vivo suggests that this drug is an appropriate steroidal inhibitor for such clinical trials.

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REFERENCES


In vivo inhibition of aromatization by exemestane, a novel irreversible aromatase inhibitor, in postmenopausal breast cancer patients.

J Geisler, N King, G Anker, et al.