Endocrine and Clinical Effects of Exemestane (PNU 155971), a Novel Steroidal Aromatase Inhibitor, in Postmenopausal Breast Cancer Patients: A Phase I Study

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ABSTRACT

Clinical and endocrinological effects of exemestane (6-methylandrosta-1,4-diene-3,17-dione; PNU 155971) were evaluated in an open Phase I study. Thirteen postmenopausal women suffering from advanced breast cancer received exemestane in escalating doses over a 12-week period. Starting on 5 mg once daily (o.d.), exemestane was subsequently escalated at 2-week intervals to 10, 25, 50, 100, and 200 mg o.d. Each patient subsequently continued treatment on the highest tolerated dose until time of progression.

One patient terminated treatment after 6 days due to diarrhea that was probably not related to drug therapy, although a relationship could not be excluded. Apart from this, no serious side effects were seen during the dose escalation period.

Exemestane (10 mg o.d.) caused maximal suppression of plasma estradiol (E2) and estrone (E1) to a mean of 14.6 and 5.8% of pretreatment levels, respectively, whereas 25 mg of exemestane o.d. suppressed estrone sulfate (E1S) to 8.9% of pretreatment levels. No fall in adrenal steroid levels was recorded. Exemestane (5 mg o.d.) suppressed urinary E2 and E1 to a mean of 11.9 and 12.2% of pretreatment levels, respectively. Administering exemestane at doses of 50–200 mg o.d. caused no further suppression of urinary E1, whereas urinary E2 fell to 6–7% of pretreatment levels. Median time to progression was 63 weeks.

We conclude that exemestane is a well-tolerated aromatase inhibitor that effectively suppresses plasma and urinary estrogens in postmenopausal patients with breast cancer.

INTRODUCTION

Aromatase inhibition has become a well-established treatment of postmenopausal breast cancer patients. Although the first-generation aromatase inhibitor AG4 effectively blocks the aromatase enzyme and suppresses plasma estrogens, the drug causes several side effects (1).

Aromatase inhibitors belong to one of two classes: nonsteroidal aromatase inhibitors, which are AG derivatives or belong to the imidazole or triazole classes, and steroidal aromatase inhibitors, which are all derivatives of the natural substrate for the aromatase enzyme, androstenedione (2).

Although several nonsteroidal aromatase inhibitors are in clinical trials (3–6), thus far, only one steroidal aromatase inhibitor, formestane, has been implemented for clinical use (7).

There are several reasons to develop steroidal aromatase inhibitors in addition to the nonsteroidal ones. Steroidal and nonsteroidal aromatase inhibitors differ in their biochemical action in that steroidal inhibitors bind to the substrate site on the aromatase enzyme whereas nonsteroidal inhibitors bind to the heme part of the molecule (10). Unlike nonsteroidal inhibitors, steroidal aromatase inhibitors seem to inactivate the aromatase enzyme irreversibly, in so-called “suicide” inhibition (11).

These biochemical differences may be of clinical relevance because patient studies have shown lack of cross-resistance between formestane and AG (12, 13).

Exemestane is a steroidal aromatase inhibitor developed for oral administration. In vitro studies have shown the drug to be a very potent aromatase inhibitor, inhibiting placental aromatase with a Ki of 4.3 nmol/liter, compared with a Ki of 671 nmol/liter for AG (14). Exemestane also causes irreversible inactivation of the aromatase enzyme, with an efficacy similar to

4 The abbreviations used are: AG, aminoglutethimide; formestane, 4-hydroxyandrostenedione; exemestane, 6-methylandrosta-1,4-diene-3,17-dione (PNU 155971, previously known as FCE 24304); o.d., once daily; FSH, follicle-stimulating hormone; LH, luteinizing hormone; E1, estrone; E2, estradiol; E,S, estrone sulfate; HPLC, high-performance liquid chromatography; SHBG, sex hormone binding globulin; DHEAS, dehydroepiandrosterone sulfate; CI, confidence interval; CR, complete response; PR, partial response; NC, no change; PD, progressive disease; RIA, radioimmunoassay; IRMA, immunoradiometric assay; CV, coefficient of variation.
that of formestane ($K_{i,u}$ of 26 and 29 nmol/liter, respectively; Ref. 15). Exemestane effectively reduced DMBA-induced mammary tumors in ovariec- tomized, testosterone-supplemented rats after oral as well as s.c. administration (16), and a single dose study in human volunteers has shown the drug to be an effective suppressor of plasma estrogens (17). This study was developed to evaluate the safety and endocrine effects of exemestane in escalating doses in breast cancer patients.

**PATIENTS AND METHODS**

**Patients.** Postmenopausal women suffering from locally advanced or metastatic, estrogen receptor-positive or receptor-negative breast cancer were eligible. All patients had amenorrhea for ≥1 year. Except for one patient (patient 13) who was subsequently found to have FSH/LH levels in the perimenopausal range (see below), all patients had FSH and LH levels in the postmenopausal range. All forms of previous endocrine therapy were allowed. Demographic data are summarized in Table 1. The patients had a predicted survival of not less than 6 months and a performance status ≤2 according to the Eastern Cooperative Oncology Group performance scale.

All previous treatment for breast cancer was terminated at least 4 weeks before commencement of treatment with exemestane. No other form of anticancer treatment or endocrine therapy was allowed during the study period.

Of a total of 13 patients enrolled, 1 patient (patient 7) withdrew from the study after 6 days due to diarrhea (probably not drug-related, although a possible relationship may not be excluded). One patient (patient 11) did not reveal use of estriol vagitories for senile colpitis at time of inclusion in the protocol. In a third patient (patient 13), subsequent hormone analyses revealed cyclic values of estrogens, FSH, and LH in the perimenopausal range despite a history of amenorrhea for more than 4 years. This left 13 patients for analysis of clinical effects on an intention-to-treat basis and 10 patients for evaluation of endocrine effects of treatment.

**Study Protocol.** The protocol was approved by the regional ethical committee. All patients gave their written informed consent. Exemestane was administered p.o. in escalating doses over a 12-week period starting with 5 mg o.d. [one-fifth of the lowest dose to cause plasma estrogen suppression as determined in a single-dose study (17)]. The dose was escalated at 2-week intervals to 10, 25, 50, 100, and 200 mg o.d. provided that no serious side effects occurred (National Cancer Institute common toxicity criteria grade ≥2). Adverse events were recorded weekly, and drug compliance was accounted for every second week during the dose escalation period and at 4-week intervals thereafter.

All patients were staged at baseline, and their response to treatment was assessed at 12-week intervals. In case of tumor control, the treatment was continued at the highest tolerable dose of exemestane until disease progression.

**Plasma and Urinary Hormone Measurements.** Blood samples for hormone measurements were obtained on day 1 and at the end of each 2-week dose interval during the 12-week dose escalation period. In addition, blood samples were collected after 1 week of treatment with each drug dose for plasma estrogen measurements. Each sample was obtained between 8:00 and 10:00 a.m. after an overnight fast. Plasma was obtained by centrifugation and stored at −20°C until analysis.

Plasma estrogen levels (E$_2$, E$_3$, and E$_{1S}$) were first measured by methods described elsewhere with some modifications: E$_2$ and E$_{1S}$ were purified on a Celite (Chromatolite A; BioMérieux SA, Lyon, France) column using isooctane/ethylacetate (7 ml of 94%/6%, 5 ml of 78%/22%, and 5 ml of 60%/40%) as solvent. The sensitivity limits for plasma E$_2$, E$_3$, and E$_{1S}$ were 2.1, 6.3, and 25.6 pmol/liter, respectively (18).

In addition, plasma estrogens were reanalyzed following HPLC purification in samples obtained by the end of each 2-week interval in eight patients from whom plasma samples were still available. In this more specific procedure, the various estrogens were measured by specific RIAs after solid-phase extraction and HPLC purification. A 2-ml plasma sample, acidified with 1 ml of 2 mol/liter acetic acid, was loaded onto a preconditioned Amrep C18 cartridge (500 mg; Amersham Corp.) and then washed with 4 ml of water. The fraction containing E$_{1S}$ was eluted with 4 ml of 24% acetonitrile in water (fraction a). The free steroids were then eluted with 4 ml of acetonitrile (fraction b). Fraction a was concentrated up to about 2 ml and hydrolyzed with arylsulfatase (Helix Pomatia; Merck) and 0.5 ml of 1 mol/liter acetic buffer, pH 5, at 45°C for 18 h. The sample was further loaded onto a C18 cartridge, and deconjugated E$_2$ was eluted with 4 ml of 100% acetonitrile (fraction c). Fractions b and c were evaporated, reconstituted with 250 µl of mobile phase, and subjected to HPLC. The HPLC conditions were as follows: Nova Pack C18 column.
The procedure described above for HPLC of plasma free estrogens was used. Fraction b was evaporated and subjected to HPLC, and individual fractions containing testosterone and androstenedione were collected. The hormones were assayed by kits from BioMérieux. The overall recovery was 80% for testosterone and 85% for androstenedione. The detection limit was 105 and 140 pmol/liter for testosterone and androstenedione, respectively, and the intra-assay CV was <10% for both assays. Due to the limited amount of plasma available, plasma testosterone, androstenedione, and DHEAS were determined in 8 patients only.

**Table 2**: Plasma estrogen values (pmol/liter) before and during treatment with exemestane determined after purification by HPLC

<table>
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<tr>
<th>Exemestane dose o.d. (mg)</th>
<th>E₁</th>
<th>CI</th>
<th>E₂</th>
<th>CI</th>
<th>E₃</th>
<th>CI</th>
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<td>19.3</td>
<td>15.4–24.2</td>
<td>1194</td>
<td>814–1751</td>
</tr>
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<td>8.8–24.0</td>
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<td>2.5–6.5</td>
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<td>100–394</td>
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<tr>
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<td>8.5</td>
<td>6.4–11.4</td>
<td>2.8</td>
<td>2.5–3.2</td>
<td>133</td>
<td>73–244</td>
</tr>
<tr>
<td>25</td>
<td>8.6</td>
<td>6.7–11.2</td>
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<td>2.3–3.6</td>
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<tr>
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<td>6.5–11.5</td>
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<td>2.3–3.5</td>
<td>102</td>
<td>61–171</td>
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<td>6.8–11.4</td>
<td>2.7</td>
<td>2.4–3.0</td>
<td>106</td>
<td>66–167</td>
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<tr>
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<td>8.0–14.4</td>
<td>2.6</td>
<td>2.6–2.6</td>
<td>120</td>
<td>66–218</td>
</tr>
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</table>

*Geometric mean values with 95% CI of the mean.*

(Waters); 300 × 3.9 mm; particle size, 3 μm; temperature, 40°C; mobile phase, acetonitrile-water (35:65); flow rate, 1 ml/min; and injection volume, 200 μl. The fractions containing individual estrogens (2 ml) were collected (Fraction Collector 202, Gilson) and evaporated in a centrifugal concentrator. The dried samples were used for the specific 125I-labeled E₁ and 125I-labeled E₂ RIA kits from Diagnostic System Laboratories (Webster, TX) and Sorin Biomedica (Saluggia, Italy). The overall recoveries, monitored in each run of the assay by adding the labeled tritiated hormones in a set of appropriate plasma samples (to avoid the mass interference of the tracer in the very sensitive iodinated RIAs), were ≈60, 70, and 55% for E₂, E₁, and E₃, respectively, and were used for the final calculations. Thus, measurement of plasma E₁, E₂, and E₃ levels in plasma pools made from untreated subjects by similarRIA methods with or without HPLC purification revealed no significant differences in the concentration of any of the estrogens. The detection limits for plasma E₂, E₁, and E₃ with use of this method were 2.6, 6.7, and 22.2 pmol/liter, respectively. The intra-assay CV was <10% for all of the assays.

To determine total (free and conjugated) urinary E₁ and E₂, urine samples (1 ml) were hydrolyzed with β-glucuronidase/arylsulfatase (Helix Pomatia, Merck) and 0.5 ml of 1 mol/liter acetate buffer, pH 5, in a 45°C water bath for 18 h. Deconjugated estrogens were isolated with solid-phase C₁₈ extraction columns and further purified and separated by HPLC with a C₁₈ column, using the procedure for plasma estrogens described above. The fractions containing E₁ and E₂ were evaporated, and the dried samples were used for RIA (same kits as for plasma estrogen measurements). Final results were corrected for mean overall recovery of the procedure (60%), monitored with [³H]E₁, E₂. The intra-assay CV was between 8 and 9% for both estrogens.

IRMA kits for LH, FSH, and SHBG and RIA kits for cortisol were obtained from Orion Corporation (Turku, Finland). Aldosterone and 17-hydroxyprogesterone were measured by IRMA and RIA kits, respectively, obtained from Diagnostic Products Corporation (Los Angeles, CA). DHEAS was measured by a direct RIA kit supplied by Sorin Biomedica.

Because preliminary results indicated that exemestane and its metabolite 17-hydroxexemestane markedly cross-react with the androstenedione and testosterone antibodies, these two hormones were measured by RIA after purification with HPLC. The procedure described above for HPLC of plasma free estrogens was used. Fraction b was evaporated and subjected to HPLC, and individual fractions containing testosterone and androstenedione were collected. The hormones were assayed by kits from BioMérieux. The overall recovery was 80% for testosterone and 85% for androstenedione. The detection limit was 105 and 140 pmol/liter for testosterone and androstenedione, respectively, and the intra-assay CV was <10% for both assays. Due to the limited amount of plasma available, plasma testosterone, androstenedione, and DHEAS were determined in 8 patients only.

**RESULTS**

Thirteen patients were evaluable for clinical response based on an intention-to-treat analysis, whereas endocrine effects could be determined in 10 patients (see “Materials and Methods”).

**Endocrine Effects.** Plasma levels of E₂, E₁, and E₃ before and during exemestane treatment are shown in Table 2. In addition, plasma estrogens expressed as percentage of pretreatment levels are shown in Figs. 1–3. Each figure depicts estrogen levels measured with conventional methods (Celite chromatography; Figs. 1–3, top) and following HPLC purification (bottom). As plasma estrogens were determined with the HPLC method in samples obtained by the end of each 2-week interval, only values from these samples are presented in all of the figures. It is noteworthy that there was no difference between plasma estrogen levels determined after 1 or 2 weeks of treatment with any of the drug doses as determined by the Celite chromatography/RIA method (data not shown).

The correlation coefficients of plasma estrogen values obtained before treatment with the different RIA methods used in this investigation were 0.85, 0.54, and 0.20 for E₁, E₂, and E₃, respectively. However, purification by HPLC significantly improved the percentage of suppression of all of the three plasma estrogens. This was particularly evident during treatment with high doses of exemestane, suggesting nonspecific drug interactions occurring in samples obtained during exemestane treatment not subjected to HPLC purification.

Following purification by HPLC, plasma E₁ was suppressed from a mean value of 146.0 pmol/liter (95% CI, 99.5–
214.2 pmol/liter) at baseline to a mean of 8.5 pmol/liter (95% CI, 6.4–11.4 pmol/liter) during treatment with 10 mg of exemestane o.d. This corresponds to a suppression to 5.8% of pretreatment level. No further suppression was achieved by escalating the doses. Plasma E\textsubscript{1} was suppressed from a mean value of 19.3 pmol/liter (95% CI, 15.4–24.2 pmol/liter) at baseline to a mean of 2.8 pmol/liter (95% CI, 2.5–3.2 pmol/liter) during treatment with 10 mg of exemestane o.d. This corresponded to a mean suppression to 14.6% of baseline values. Again, no further suppression was achieved by escalating the doses. Notably, 4 of 8 and 5 of 8 patients, respectively, had plasma E\textsubscript{1} and E\textsubscript{2} levels suppressed to the detection limit of the assays following HPLC purification. In these cases, the detection limit value was used for statistical analysis.

Plasma levels of E\textsubscript{1}S were suppressed from a mean value of 1194 pmol/liter (95% CI, 814–1751 pmol/liter) at baseline to a mean of 133 pmol/liter (95% CI, 73–244 pmol/liter) during treatment with 10 mg of exemestane o.d. This corresponds to a suppression to 11.1% of pretreatment values. It is noteworthy that plasma E\textsubscript{1}S was suppressed somewhat further, to 107 pmol/liter (95% CI, 59–193) corresponding to a suppression to 8.9% of pretreatment levels, by escalating the dose of exemestane to 25 mg o.d. Further dose escalation caused a mean suppression to a level varying between 8.5% and 10.8%.

Urinary estrogens (E\textsubscript{1} and E\textsubscript{2}) were measured at baseline and during treatment with 5 mg of exemestane o.d. in all patients and in subgroups during treatment with 50 mg o.d. (n = 4), 100 mg o.d. (n = 3), or 200 mg o.d. (n = 3) (Fig. 4). Treatment with 5 mg of exemestane o.d. suppressed urinary E\textsubscript{1} from a mean value of 8.05 nmol/24 h (95% CI, 5.18–12.51 nmol/24 h) to 0.95 nmol/24 h (95% CI, 0.56–1.77 nmol/24 h), while E\textsubscript{2} was suppressed from a mean value of 1.34 nmol/24 h (95% CI, 0.86–2.10 nmol/24 h) to 0.16 (95% CI, 0.08–0.32 nmol/24 h). This corresponds to a suppression of urinary E\textsubscript{1} and E\textsubscript{2} to a mean of 12.2 and 11.9% of control levels, respectively. Increasing the drug doses caused no further suppression of urinary E\textsubscript{1} and E\textsubscript{2}. However, urinary E\textsubscript{1} was suppressed to a mean value of 6–7% of control levels during treatment with exemestane in doses of 50–200 mg o.d.

During the twelve weeks of dose escalation, no significant alteration in plasma FSH, LH, cortisol or aldosterone was seen (Figs. 5 and 6). There was a trend toward an increase in plasma 17-hydroxyprogesterone, DHEAS, and testosterone when the dose of exemestane was escalated to ≥50 mg o.d. and for androstenedione with doses ≥100 mg, but the 95% CI spanned the 100% value, except for 17-hydroxyprogesterone and DHEAS during treatment with exemestane of 200 mg o.d. (Figs. 6 and 7). Interestingly, both plasma LH and FSH increased nonsignificantly during treatment with exemestane in low doses but fell below pretreatment levels during treatment with exemestane in high doses.

SHBG showed a reduction from a mean pretreatment level of 48.6 nmol/liter (95% CI, 36.9–64.0 nmol/liter) to a mean of 22.5 nmol/liter (95% CI, 15.7–28.2 nmol/liter) during treatment with 200 mg of exemestane o.d. This corresponds to a suppression...
sion to 43% (95% CI, 33–57%) of pretreatment values (Fig. 5). The fall in SHBG was dose dependent and became significant at treatment with 25 mg of exemestane o.d.

Drug Tolerability. One patient (patient 7) decided to withdraw from the study after 6 days due to moderate diarrhea (which was probably not drug-related); all of the remaining 12 patients completed 12 weeks of treatment with dose escalation to 200 mg o.d. without any major side effects. One patient terminated treatment with exemestane after 12 weeks due to progressive disease; the other patients continued treatment with exemestane at the highest tolerable dose (200 mg o.d.).

Seven patients experienced drug-related mild adverse events during treatment with exemestane in doses from 50 to 200 mg o.d. during the dose escalation period or during follow-up-treatment (after 12 weeks of treatment with exemestane). Side effects included hot flashes, hypertrichosis, myalgia, abdominal pain, diarrhea, flatulence, and dyspepsia.

Due to nerve compression after 3 weeks on treatment with exemestane, one patient (patient 1) received methylprednisolone in high doses and radiation therapy to the sacral bone for 2 weeks. The radiated area was subsequently deleted for response evaluation to exemestane therapy. Because the glucocorticoid was administered for a short time period, it was considered unlikely that it would influence the response to systemic treatment. However, the patient was deleted for evaluation of endocrine response during the period in which she received concomitant glucocorticoid treatment. None of the other patients received concomitant medication known to influence breast cancer cell growth or plasma steroid hormone levels during the 12 weeks of dose escalation.

The dose was subsequently reduced in five patients during the follow-up period due to possible drug-related side effects. In one patient, this was due to development of minor hirsutism (recognized after 52 weeks on treatment with 200 mg o.d.) followed by a temporary dose reduction to 100 mg o.d. for 1 month when 200 mg o.d. was reintroduced. Two patients complained of abdominal discomfort after 24 weeks on treatment, and two patients complained about muscular pain after 28 and 51 weeks on treatment. Three patients experienced a decrease in symptoms (abdominal disorder and muscular pain) following a dose reduction of exemestane from 200 to 100 mg o.d. No decrease in hirsutism was seen, and one case of myalgia progressed shortly after dose reduction. In addition, another patient showed signs of mild hirsutism in the follow-up period, but in this case it was not considered necessary to reduce the drug dose.

Clinical Response. Median time to failure (13 patients) was 50 weeks (range, 1–174), whereas median time to disease progression (12 patients) was 63 weeks (Table 1). It is noteworthy that two patients (patients 5 and 6) obtained disease control lasting for more than two years. Patients 11 and 13 (the protocol violators) had a time to progression of 36 and 77 weeks; maximal tumor response was classified as CR and NC, respectively. Of the 12 patients who were evaluable for clinical response, 4 obtained a CR, 3 had a PR, and 4 patients had NC, whereas 1 patient had PD diagnosed following 12 weeks on treatment. One patient (patient 9) developed pancreatic cancer while her breast cancer was still responding to treatment with exemestane. She subsequently died of her pancreatic carcinoma.
Blood Chemistry. Dose escalation of exemestane to 200 mg o.d. caused a nonsignificant increase in plasma creatinine values by 7% (95% CI, −1 to 15%) by the end of the 12-week period. Whereas six patients had creatinine values above the normal range recorded on some occasions during long-term treatment (in one of these patients, the value was elevated before commencement of treatment), in two patients, sustained elevated levels only slightly above the normal range were recorded. In no case did elevation in plasma creatinine values cause therapeutic interventions. Treatment with exemestane had no influence on other biochemical parameters.

DISCUSSION

This study revealed that exemestane in doses of up to 200 mg o.d. is well tolerated in postmenopausal breast cancer patients. The side effects were mild and appeared during treatment with exemestane doses of ≥50 mg o.d. only. It is noteworthy that some of the side effects recorded during long-term treatment responded to a reduction in dose from 200 to 100 mg o.d.

The effect of exemestane single doses on plasma estrogen levels has been studied in healthy volunteers (17) and, recently, in cohorts of breast cancer patients on chronic treatment with exemestane in doses varying from 2.5 to 25 mg o.d. (19). These studies revealed that exemestane suppresses plasma E₁ and E₂ levels but only to about 30% of pretreatment levels. Similar results were obtained in this study when plasma estrogen levels were determined with RIA following conventional column chromatographic separation. The metabolism of exemestane in humans is complex, with several identified and unidentified metabolites (20). To test for the possibility of nonspecific cross-reactions in the RIAs, we reanalyzed plasma estrogen levels following purification by HPLC. When plasma estrogens were

Fig. 5 Plasma gonadotropin and SHBG levels during treatment with exemestane, expressed as a percentage of pretreatment levels. Data points, geometrical mean; bars, 95% CI of the mean.

Fig. 6 Plasma levels of cortisol, 17-hydroxypregesterone, and aldosterone during treatment with exemestane, expressed as a percentage of pretreatment levels. Data points, geometrical mean; bars, 95% CI of the mean.
The finding that exemestane is a very potent suppressor of estrogen synthesis in postmenopausal women was further documented by its influence on urinary estrogen excretion. Treatment with exemestane at the lowest dose (5 mg o.d.) suppressed urinary excretion of E1 and E2 to 11.9 and 12.2% of pretreatment levels, respectively. Urinary samples were not collected during treatment with drug doses of 10 and 25 mg o.d. However, among the subgroups having urinary estrogens determined during treatment with 50–200 mg of exemestane o.d., urinary E2 excretion was suppressed to about 6% of pretreatment values. It is difficult to explain why urinary E1 was not suppressed to a similar extent; however, the fact that exemestane is metabolized into several unidentified metabolites raises the possibility of nonspecific interactions in the RIA despite HPLC purification. Exemestane caused no suppression in plasma levels of testosterone, androstenedione, DHEAS, 17-hydroxyprogesterone, cortisol, or aldosterone. This observation suggests that the drug is a selective aromatase inhibitor that does not inhibit adrenal steroid synthesizing enzymes. Although a trend toward an increase in plasma levels of 17-hydroxyprogesterone, androstenedione, and DHEAS during treatment with exemestane in high doses was recorded, it is difficult to see how exemestane may enhance the production or hamper the metabolism of these steroids. Instead, these observations may be due to nonspecific cross-reactions in the RIAs by exemestane metabolites.

Similar to what was reported from the single dose study in healthy females (17), we found that chronic treatment with exemestane has no significant influence on plasma levels of LH and FSH. Interestingly, however, a nonsignificant increase in plasma levels of both gonadotropins was observed during treatment with exemestane in low doses, probably precipitated by the fall in plasma estrogen levels. On the other hand, the fall in plasma FSH and LH that was observed when the dose of exemestane was increased further may be secondary to an androgenic effect of exemestane metabolites on the pituitary. A modest suppression in plasma LH levels has also been recorded in rats treated with exemestane by the parenteral but not by the oral route (24).

Treatment with exemestane provoked a dose-dependent fall in plasma SHBG, which reached statistical significance when the drug dose reached 25 mg o.d. A similar effect on SHBG is seen in patients treated with formestane p.o., whereas the effect seem to be smaller when the drug is given parenterally (8, 9). Androgens are known to inhibit and estrogens to enhance the secretion of SHBG from hepatocytes in vitro (25); however, relative high concentrations are required (26). In a recent study (18), we found that plasma levels of SHBG are negatively correlated not only to plasma levels of androstenedione and DHEA but also to plasma levels of E,S, suggesting that differences in plasma estrogen concentrations in postmenopausal women do not influence plasma SHBG levels. The finding of a dose-dependent suppression of plasma SHBG when exemestane was administered in doses ≥25 mg but no increase in plasma androgen levels (except for a slight increase in plasma DHEAS at treatment with 200 mg) does not suggest that an increase in any of these androgens is the mechanism behind the suppression of SHBG. A more likely explanation could be exposure of the liver to exemestane metabolites with intrinsic androgenic activity. Exemestane itself has minimal intrinsic androgenic activity (27), whereas its metabolite 17-hydroexemestane has significant

![Fig. 7](https://example.com/fig7.png) Plasma levels of DHEAS, androstenedione, and testosterone during treatment with exemestane, expressed as a percentage of pretreatment levels. Data points, geometrical mean; bars, 95% CI of the mean.

determined by RIA following HPLC purification, this revealed suppression of the different plasma estrogens down to 6–11% of pretreatment levels. This suppression is comparable to what has recently been observed with novel, potent aromatase inhibitors of the triazole class, such as letrozole (21) and anastrozole (22), and significantly better than what has been achieved with another steroidal aromatase inhibitor on the market, formestane, given by the oral (8) but also by the parenteral (9, 23) route. It is noteworthy that many patients had plasma E1 and E2 levels suppressed to the detection limit of the assay, suggesting that our findings may represent an underestimate of the effect. Maximal suppression was achieved with a drug dose as low as 10 mg o.d. with the possible exception of plasma E,S, for which a dose of 25 mg o.d. probably aggravated the suppression somewhat. These findings support the hypothesis that previous observations on sustained estrogen levels in patients on treatment with exemestane could be due to nonspecific cross-reactions in the assays.
intrinsic androgenic activity, and previous studies in rats revealed that exemestane high-dose treatment causes androgenic effects (28). However, despite long-term treatment with 200 mg of exemestane o.d., clinical signs of hirsutism were mild and appeared in only a few patients.

The patients investigated in this study responded well to treatment with 11 of 12 evaluable patients achieving either an objective response or stable disease for ≥24 weeks. An interesting observation was the long period of time to disease progression, with a median interval of 63 weeks; two patients achieved disease control for ≥2 years.

In conclusion, exemestane seems to be an effective nontoxic oral aromatase inhibitor suitable for treatment of postmenopausal breast cancer patients with estrogen receptor-positive tumors.

ACKNOWLEDGMENTS

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Endocrine and clinical effects of exemestane (PNU 155971), a novel steroidal aromatase inhibitor, in postmenopausal breast cancer patients: a phase I study.
