Pilot Study Evaluating the Pharmacokinetics, Pharmacodynamics, and Safety of the Combination of Exemestane and Tamoxifen

Edgardo Rivera,1 Vicente Valero,1 Deborah Francis,1 Aviva G. Asnis,2 Larry J. Schaaf,2 Barbara Duncan,2 and Gabriel N. Hortobagyi1
1Department of Breast Medical Oncology, The University of Texas M. D. Anderson Cancer Center, Houston, Texas, and 2Pharmacia Corporation, Peapack, New Jersey

ABSTRACT

Purpose: We conducted a pilot study assessing the effects of the selective estrogen receptor modulator, tamoxifen, on the pharmacokinetics, pharmacodynamics, and safety of the steroidal, irreversible aromatase inhibitor (AI), exemestane, when the two were coadministered in postmenopausal women with metastatic breast cancer.

Experimental Design: Patients with documented or unknown hormone receptor sensitivity were eligible. Patients received oral exemestane at 25 mg once daily. Starting day 15, oral tamoxifen at 20 mg once daily, was added. We measured plasma concentrations of exemestane, estrone, estradiol, estrone sulfate, and estradiol after 14 days of exemestane monotherapy and after ~4 weeks of combination therapy. The incidence and severity of adverse events were assessed by physical examination and patient reporting.

Results: We treated 18 patients. All had received prior chemotherapy and/or hormonal therapy, eight and six, respectively, with single-agent selective estrogen receptor modulators or irreversible aromatase inhibitors; no hormonal therapy was given within 30 days of study entry. Plasma exemestane concentrations and estrone, estrone sulfate, and estradiol suppression were unchanged after ~4 weeks of exemestane coadministration. All drug-related adverse events were grades 1 or 2; none was unexpected. Although not a formal study end point, antitumor activity was noted, with single-agent tamoxifen activity of two partial responses and four cases of stable disease among the 17 evaluable patients after a 9-month median follow-up (range, 2.5–19 months).

Conclusions: This pilot study provides evidence that coadministration of tamoxifen does not affect exemestane pharmacokinetics or pharmacodynamics and that the combination is well-tolerated and active. Further clinical investigation is warranted.

INTRODUCTION

Breast cancer growth frequently is promoted by estrogen, and approximately one-third of tumors respond to estrogen deprivation therapy (1). In current clinical practice, two main single-agent strategies are used to deprive breast cancer cells of estrogen. A selective estrogen receptor modulator (SERM), such as tamoxifen, is given to block binding of the hormone to the cancer cells. Alternately, in postmenopausal women, an inhibitor of the enzyme, aromatase, is administered to suppress their main source of estrogen synthesis, conversion of androgens by this enzyme.

One aromatase inhibitor (AI) that has been studied extensively and shown activity as a single agent in postmenopausal women with breast cancer is exemestane (Aromasin, Pharmacia Corp., Peapack, NJ; Ref. 2–4). In contrast to other currently approved AIs, which are nonsteroidal (type II) agents that temporarily inhibit aromatase activity, exemestane is a steroidal (type I) agent that permanently inactivates the enzyme (5–7). As an androstenedione derivative, exemestane serves as a false substrate for aromatase. The drug is processed through the normal catalytic mechanism to a transformed product, which binds covalently and irreversibly to the catalytic site of the enzyme (“suicide inhibition”). Resumption of estrogen production depends on the synthesis of new aromatase molecules.

The separate and potentially complementary mechanisms of action of SERMs and AIs have prompted study of combination therapy of breast cancer with tamoxifen plus individual AIs. Initial investigation in a nude mouse model of breast carcinoma suggested that coadministration of tamoxifen and either of the nonsteroidal AIs, letrozole or anastrozole, provided no added activity compared with single-agent use of these AIs (8). A pair of early pharmacokinetic and pharmacodynamic clinical studies showed that the combination of tamoxifen plus a nonsteroidal AI at clinically recommended doses decreased mean plasma concentrations of the AI relative to levels attained with single-agent therapy; for example, there was a decrease by 37.6% in the case of letrozole (9) and 27% in the case of anastrozole (10). However, both studies showed no effect of the addition of tamoxifen on the reduction in plasma estrogens effected by the nonsteroidal AIs.

In contrast to the preclinical experience with tamoxifen plus a nonsteroidal AI, an early study in rats with 7,12-dimethylbenzanthracene-induced mammary tumors showed that the combination of exemestane plus tamoxifen had greater activity than did either agent alone (11). On the basis of these results and on the demonstrated clinical efficacy of single-agent exemestane (2–4) and tamoxifen (12) in this setting, we conducted a pilot study in postmenopausal women with metastatic breast...
cancer to evaluate the pharmacokinetics, pharmacodynamics, and safety of exemestane when administered in combination with tamoxifen. We now report the results of this trial.

PATIENTS AND METHODS

Study End Points. To assess the pharmacokinetics of exemestane when administered in combination with tamoxifen, we measured plasma concentrations of exemestane after an initial period of single-agent administration and a subsequent period of combination therapy. To assess the pharmacodynamics of exemestane in this setting, we performed similar measurements for plasma estrone (E1), estrone sulfate (E1S), and estradiol (E2). Additional endpoints were the incidence rate and grade of observed toxicities during the study, assessed by physical examination and patient reporting.

We retrospectively plotted plasma exemestane concentration-time profiles after both the exemestane monotherapy and exemestane plus tamoxifen combination therapy periods. In addition, we performed a retrospective statistical comparison of mean plasma estrogen values between these periods, with the study population serving as its own control. Compliance with clinical response to therapy were not formal study end points but were recorded.

Eligibility Criteria and Ethical Considerations. Patients were eligible for the study if they had stage IV breast cancer with documented or unknown hormone receptor sensitivity, were postmenopausal, and were candidates for hormonal therapy. Patients who had received single-agent tamoxifen or AIs in the adjuvant or metastatic setting were eligible, as long as these drugs had not been given within 21 days of study entry. Patients were required to have measurable or evaluable disease, a life expectancy of 12 weeks and a Zubrod performance status (13) ≤ 1. Other eligibility criteria included adequate bone marrow, liver and kidney function, respectively, defined as an absolute granulocyte count of ≥ 1,500/µl and platelet count of ≥ 100,000/µl, a bilirubin concentration of < 1.5 mg/dl and creatinine < 2.5 mg/dl. Exclusion criteria included a history of deep venous thromboembolism, pulmonary embolism, or stroke. The use of hormone replacement therapy or of over-the-counter products or supplements considered to have estrogenic effects, such as ginseng, ginkgo biloba, and black cohosh, was not allowed during the study. Also prohibited during the trial was the use of raloxifene for osteoporosis.

The study was approved by the Institutional Review Board of the University of Texas M. D. Anderson Cancer Center, and all patients provided written informed consent to participate.

Treatment Plan and Evaluation. Patients were given single-agent exemestane (provided by Pharmacia Corp.), 25-mg once daily, starting on day 1. On day 15, tamoxifen, 20-mg once daily, was added. Both drugs were taken orally with a light meal.

Before study entry, patients underwent a complete history and physical examination, including evaluation of performance status and weight and documentation of all prior anticancer treatments and their residual side effects, if any. Baseline imaging studies were obtained to define the extent of disease. Baseline laboratory tests included a complete blood cell count with differential and platelet counts and blood chemistry including tumor marker studies (cancer antigens 27–29 and carcino-embryonic antigen). Physical examination and all laboratory assessments were repeated every 8–12 weeks. Patients were told to report toxicities as any arose and were queried about adverse events during all follow-up visits. Toxicities were graded using the National Cancer Institute Common Toxicity Criteria, version 2.0. Follow-up radiological evaluations were performed as requested by the primary physician. To verify compliance, patients were asked to bring their bottles of exemestane and tamoxifen on each follow-up visit and to document any missed doses.

Patients continued on treatment until they had disease progression, became unable to tolerate or were noncompliant with therapy, or withdrew consent. Patients were evaluated 4 weeks after their last dose of the combination and assessed for any toxicities.

Pharmacokinetic and Pharmacodynamic Measurements. Collection, storage, and processing of blood specimens for pharmacokinetic and pharmacodynamic (i.e., estrogen) measurements were carried out according to specified procedures. Briefly, blood samples were collected via venipuncture or an indwelling i.v. cannula on two occasions: at the end (day 14) of the 2-week single-agent exemestane treatment period, and after 4 weeks of combination treatment (i.e., 6 weeks after the first dose of exemestane). These collection times were chosen in light of the published times to attain steady-state serum concentrations of 7 days for exemestane and 3–4 weeks for tamoxifen given as single agents at the doses used in our study (7, 14). For exemestane pharmacokinetic measurements, ~5 ml of venous whole blood was obtained before (0 h) and 1, 2, 4, 6, 8, and 24 h after dosing. For pharmacodynamic measurements, a single 16-ml venous whole blood specimen was obtained on the mornings of the two collection occasions (postexemestane monotherapy period and postcombination treatment period).

All blood samples were drawn into chilled blood collection tubes containing sodium heparin as anticoagulant and immediately placed in ice-water before centrifugation (1000–1200 × g for 10–15 min at 4°C) within 30 min of collection. The harvested plasma was transferred to screw-cap polypropylene storage tubes and stored frozen at approximately −20°C until analysis.

 Plasma concentrations of exemestane were measured using a validated, sensitive, and specific high-performance liquid chromatography method with tandem mass spectrometric detection (15). The lower limit of quantitation (LLOQ) of this assay was 0.1 ng/ml using 0.50 ml of aliquots.

Pharmacodynamic samples were assayed for plasma concentrations of E1, E1S, and E2 using solid-phase extraction and high-performance liquid chromatography purification followed by highly sensitive radioimmunoassay, according to a procedure described previously (16). The LLOQ of the assay were 1.8 pg/ml for E1, 6 pg/ml for E1S, and 0.7 pg/ml for E2. Estrogen concentrations below the LLOQ were recorded as the value of that limit.

Statistical Analyses. Because this was a pilot study originally planned to generate only descriptive statistics, no formal sample-size calculations were performed. Retrospectively, mean plasma concentrations of E1, E1S, and E2 following the exemest-
tane monotherapy and the tamoxifen plus exemestane combination therapy periods were compared using the nonparametric Mann-Whitney U test. Ps < 0.05 were considered to be statistically significant.

RESULTS

Patient Characteristics. Between September 2001 and April 2002, 18 eligible patients were recruited; Table 1 lists their characteristics. Most patients were in late middle age, had bone as their dominant disease site, and were treated previously. Six patients had experienced progressive disease on single-agent tamoxifen, and two patients on single-agent exemestane.

All patients received therapy for at least the planned 8 weeks, except one individual who withdrew from the study for personal reasons after 2 weeks of combination treatment. Thirteen of the 17 remaining patients took all study medication prescribed per protocol, and an additional three missed only 2 days of dosing each because of surgeries.

Pharmacokinetic Measurements. For exemestane pharmacokinetic measurements, blood samples were available from at least one collection occasion (i.e., the end of the exemestane monotherapy period and/or after ~4 weeks of combination treatment) for all 18 patients. Samples were available for all time points during both collection occasions for seven patients. Two of the 18 patients did not have any specimens collected during the combination treatment period and nine patients had a specimen for at least one time point missing or with labels that became detached during shipping to the laboratory. Four patients had concentrations at 24 h after dosing that were much higher than at earlier time points, and these findings were attributed to specimen collection after the patients had taken their next morning dose. These four 24-h specimens were not included in subsequent analysis.

Mean plasma concentration-time profiles of exemestane when exemestane was administered alone were similar to those observed when exemestane was combined with tamoxifen (Fig. 1). This suggests that tamoxifen does not alter the pharmacokinetics of exemestane.

Estrogen Measurements. Estrogen specimens were received at the laboratory for 16 patients during the monotherapy period and for 15 patients during the combination treatment period. E1 concentrations could not be determined for one patient, and E1S concentrations could not be quantified for three patients because of insufficient volume available for reanalysis of the samples; no reportable result had been obtained during initial analysis. E2 concentrations were determined for all patients.

In this study, baseline estrogen levels were not measured. However, in a similar population of breast cancer patients, and using the same assay methodology, mean pretreatment levels of 40.8, 349.8, and 10.3 pg/ml were reported for E1, E1S, and E2, respectively (2). A profound suppression of all plasma estrogens after 2 weeks of exemestane monotherapy was confirmed in the present study, with mean concentrations of 2.3, 25.4, and 0.7 pg/ml for E1, E1S, and E2, respectively (Table 2). The E1 and E1S suppression that was achieved after 2 weeks of exemestane monotherapy was maintained after ~4 additional weeks of combination therapy (Table 2). Mean E2 levels were suppressed below the LLOQ in all but one evaluable patient after 2 weeks of exemestane monotherapy and were suppressed below the LLOQ in all evaluable patients after an additional ~4 weeks of combination therapy (Table 2). In a retrospective analysis, no statistically significant differences were noted between plasma concentrations of any measured hormone after the monotherapy period versus after ~4 weeks of combination therapy (Table 2).

Toxicity. All 18 patients were evaluable for toxicity. After a median follow-up of 9 months (range, 2.5 to 19 months), both single-agent exemestane and combination therapy with exemestane and tamoxifen were very well tolerated. All drug-related adverse events were classified as mild or moderate (Table 3), and no patient withdrew from the study because of toxicity. No unexpected toxicities were reported. Three of six

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. of patients/value</th>
</tr>
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<tbody>
<tr>
<td>Total patients</td>
<td>18</td>
</tr>
<tr>
<td>Age / Median (range)</td>
<td>62 yrs (46–84 yrs)</td>
</tr>
<tr>
<td>Performance status</td>
<td>1</td>
</tr>
<tr>
<td>Dominant metastatic site</td>
<td>Viscera: 2, Bone: 15, Soft tissue: 6</td>
</tr>
<tr>
<td>Prior therapy</td>
<td>Chemotherapy: 15, Hormones: 16</td>
</tr>
<tr>
<td>Prior hormonal therapy</td>
<td>SERM: 8, AI: 6, Progestins: 2, Androgens: 2</td>
</tr>
</tbody>
</table>

AI, aromatase inhibitor; SERM, selective estrogen receptor modulator.
patients with bone pain and one of nine with hot flashes during the study had reported these symptoms at baseline. Although a comparison of toxicity during exemestane monotherapy versus during combination therapy was not a formal study objective, our clinical impression was that toxicity was similar during both periods.

Efficacy. Response-related data were not end points of this study. However, response was evaluable in all 17 patients who received the planned duration of therapy. After a median follow-up of 9 months (range, 2.5 to 19 months) from study entry, we observed antitumor activity of the combination of exemestane and tamoxifen, with two partial responses and four cases of stable disease in the 17 patients. The two partial responders previously had disease progression despite adjuvant therapy with single-agent tamoxifen. Neither one of these two patients had previously received AIs. In the current trial, their time to response was 3 and 2 months, and their time-to-tumor progression was 7 and 19+ months. In the patients with stable disease, time-to-tumor-progression was 8, 19+, 15+, and 5 months. Three of the four patients with stable disease, including two of the three patients with stable disease for >6 months, had received a prior single-agent AI.

DISCUSSION

This pilot study provides preliminary evidence suggesting that coadministration of tamoxifen does not affect the pharmacokinetics or pharmacodynamics of exemestane in postmenopausal women with metastatic breast cancer. Mean plasma concentration-time profiles of exemestane were virtually identical after 2 weeks of exemestane monotherapy and ~4 weeks of coadministration with tamoxifen. Plasma E1, E2S, and E2 concentrations were rapidly and markedly suppressed by exemestane without such therapy during a 3-week “washout period” before study entry, and in practice, none was received within 30 days of study entry. Therefore, our pharmacokinetic and pharmacodynamic results are unlikely to have been influenced by prior hormonal therapy.

This pilot study also provides preliminary evidence of the safety of exemestane and tamoxifen coadministration. The combination was very well tolerated, with only mild or moderate drug-related toxicity and no unexpected adverse events observed during a median follow-up of 9 months. We did not measure markers of the effects of combination therapy on the bone or on hepatic metabolism of lipids or lipoproteins; it will be of interest to do so in future studies.

Although efficacy was not an end point of this pilot study, over a 9-month median follow-up, we noted encouraging anti-tumor activity of the combination. Clinical benefit (i.e., partial response or stable disease lasting >6 months) was seen in 5 of 17 patients evaluable for efficacy. It is noteworthy that both partial responders in our trial previously had disease progression despite adjuvant therapy with single-agent tamoxifen and that two of four patients with stable disease, including two of three with stable disease for >6 months, had received prior treatment with a single-agent AI.

Our findings of no effect of SERM coadministration on pharmacokinetics or pharmacodynamics of a steroidal AI agree with preliminary results from two clinical studies reported because we conducted our trial. A German pharmacokinetic study noted no effect of the SERM, toremifene, on the mean area under the curve or Cmax of the steroidal AI, atamestane, in 12 evaluable patients (17). In an initial 14-patient cohort, a University of Wisconsin pharmacokinetic and pharmacodynamic study (18) in postmenopausal women receiving adjuvant treatment for early-stage breast cancer observed a mean area under the curve of exemestane after 8 weeks of coadministration with tamoxifen that was similar to the published mean area under the curve of single-agent exemestane in healthy postmenopausal women. Also in the Wisconsin study, after 8 weeks of combination therapy, plasma E1 and E2 were suppressed to undetectability in 8 of 12 and in all 14 patients, respectively. The Wisconsin study design differed from ours in preceding combination therapy with tamoxifen rather than exemestane monotherapy. Also, the Wisconsin trial took measurements after 8 rather than 4 weeks of combination therapy and 12 rather than ~6 weeks of therapy in general. However, both studies used the same doses for tamoxifen and exemestane, 20- and 25-mg once daily, respectively.

Table 3  Drug-related adverse eventsa (N = 18)

<table>
<thead>
<tr>
<th>Toxicity</th>
<th>No. of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Grade 1b</td>
</tr>
<tr>
<td>Nausea</td>
<td>6</td>
</tr>
<tr>
<td>Alopecia</td>
<td>1</td>
</tr>
<tr>
<td>Bone pain</td>
<td>0</td>
</tr>
<tr>
<td>Hot flashes</td>
<td>5</td>
</tr>
<tr>
<td>Myalgias</td>
<td>2</td>
</tr>
<tr>
<td>Nail changes</td>
<td>1</td>
</tr>
<tr>
<td>Vaginal bleeding</td>
<td>2</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>2</td>
</tr>
<tr>
<td>Constipation</td>
<td>2</td>
</tr>
<tr>
<td>Edema</td>
<td>1</td>
</tr>
<tr>
<td>Fatigue</td>
<td>5</td>
</tr>
</tbody>
</table>

a Median follow-up of 9 months (range, 2.5–19 months).

b Toxicities were graded according to the National Cancer Institute Common Toxicity Criteria; no grade 3 or 4 events were observed.
the German and Wisconsin studies observed no effects of the steroidal AI on SERM pharmacokinetics, including, in the case of the Wisconsin study, those of the tamoxifen metabolites, 4-hydroxy-tamoxifen and N-desmethyl tamoxifen. A similar lack of altered SERM pharmacokinetics has been observed in studies of tamoxifen plus letrozole (9) or anastrozole (10).

The body of the evidence thus far, then, would suggest that when a SERM and an AI are coadministered, the type of AI that is given (i.e., steroidal versus nonsteroidal) affects the pharmacokinetics of the AI but not the SERM. It is possible that the observation of reduced plasma levels of nonsteroidal but not steroidal AIs in combination with SERMs is an artifact of the duration of follow-up of the studies in question, which tended to be longer (12 weeks to 8 months) in the nonsteroidal AI than in the steroidal AI trials (≤12 weeks; 9, 10, 17, 18). However, the consistency of this observation across several studies suggests that an artifactual explanation is unlikely. An alternative hypothesis might be that SERMs induce enzymes that metabolize nonsteroidal but not steroidal AIs. This mechanism has been speculated to occur with tamoxifen plus letrozole (9) or anastrozole (10). In separate single-agent studies, these nonsteroidal AIs have been shown to be metabolized differently than is the steroidal AI, exemestane, for example. Anastrozole is metabolized by N-dealkylation, hydroxylation and glucuronidation and letrozole, by the isoenzymes, cytochrome P-450 3A4 and 2A6, whereas exemestane is broken down by cytochrome P-450 3A4 and by aldoketoreductases (7, 19, 20).

The clinical relevance of the difference in SERM-AI pharmacokinetic interactions is unclear. Studies of tamoxifen plus letrozole (9) or anastrozole (10) found no apparent diminution of estrogen suppression with combination therapy, although in the latter study, the pharmacokinetic and pharmacodynamic observations were made in different cohorts. Recently, an initial report of the entire “Arimidex and Tamoxifen Alone or in Combination” trial (21), which involves over 9000 postmenopausal women receiving adjuvant therapy of early breast cancer, found that anastrozole plus tamoxifen was equivalent to tamoxifen and inferior to anastrozole monotherapy with respect to disease-free survival. The Arimidex and Tamoxifen Alone or in Combination investigators speculated that their findings might be explained by the inability of the estrogen inhibition attained by anastrozole to counteract the mild estrogenic effects of tamoxifen, which as an estrogen receptor agonist mediates some estrogen-like signaling. No data were provided on estrogen suppression in the three subgroups of the entire Arimidex and Tamoxifen Alone or in Combination study. However, the investigators noted that the reduced anastrozole concentrations during combination therapy that had been documented in a subprotocol of their study (10), also might account for the lack of efficacy benefits of the combination in the larger population.

A recent animal study (22) has confirmed earlier preclinical findings (11) of superior antibreast cancer efficacy with tamoxifen plus exemestane than with either agent alone. Together with these results, our observations of activity of this combination in patients who had failed prior single-agent hormonal therapy suggest that incremental clinical benefit might be seen when a SERM is coadministered with a steroidal AI. It is possible that any such benefits would stem not from the unaltered pharmacokinetics but from other properties of the steroidal AI. These properties might include the mild androgenic effects of this class of drug on breast tumor tissue (4); indeed, a recent animal study (23) suggests that androgens inhibit mammary epithelial proliferation.

Whether clinical results differ when a steroidal instead of a nonsteroidal AI is given in combination with a SERM will be determined by a recently developed multicenter phase III North Central Cancer Treatment Group study. This trial will compare the efficacy and safety of exemestane and tamoxifen as single agents and in combination, in postmenopausal women with metastatic breast cancer.

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