Letrozole (CGS 20267)

A Phase I Study of a New Potent Oral Aromatase Inhibitor of Breast Cancer

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Background. **Letrozole (CGS 20267), a triazole derivative, is a new, once-daily, oral nonsteroidal inhibitor of aromatase activity.**

Methods. **In this Phase I trial, 23 heavily pretreated postmenopausal patients with metastatic breast cancer received letrozole at doses ranging from 0.1 to 5.0 mg once daily.**

Results. **No hematologic, biochemical, or significant clinical toxicity was encountered. Serial steroid measurements were determined in 19 of these patients. Letrozole at all doses tested produced a marked suppression of plasma estrone, estradiol, estrone sulfate, and urine estrone and estradiol. This was observed within 24 hours of the initial dose of letrozole and resulted in a greater than 90% suppression of plasma and urinary estrogen levels within 2 weeks. Letrozole appears to be highly selective in its action and does not compromise glucocorticoid or mineralocorticoid production or thyroid function. Of the 21 evaluable patients, there were 2 with partial responses and 7 with stable disease.**

Conclusions. **Letrozole is a well tolerated, potent, and specific inhibitor of estrogen biosynthesis in postmenopausal patients with metastatic breast cancer. *Cancer* 1995;75:2132–8.**

Key words: letrozole, CGS 20267, aromatase inhibitor, breast cancer.

Approximately one-third of human breast cancers are hormone dependent or rely on estrogen to stimulate their growth. Two clinical strategies are available to block estrogen action. The first uses antiestrogens and the second uses enzyme inhibitors to block estrogen biosynthesis.

Nonovarian estrogen production in postmenopausal or castrate women is caused by the metabolic conversion of androstenedione (D^4-A) to estrone (E_1).[1] The adrenal gland is the principal source of androstenedione, a weak androgen, which circulates to peripheral tissues, where aromatization to estrone occurs, followed by enzymatic reduction to estradiol. Aromatase is the rate limiting enzyme that catalyzes the aromatization of androstenedione. Fat and muscle are the richest source of extraglandular aromatase activity.[2–6] More recently, several reports have shown that two-thirds of human breast cancers contain measurable aromatase activity.[7–11]

Aromatase inhibitors are effective second-line treatment for hormone dependent (estrogen receptor [ER] positive) metastatic breast cancer in postmenopausal women. Two classes of aromatase inhibitors are available: (1) competitive, nonsteroidal inhibitors, which inhibit aromatase activity by binding to the heme group, and (2) substrate analogues, which compete with the normal substrate (androstenedione) at the binding site of the aromatase enzyme.

The first clinically useful aromatase inhibitor was aminoglutethimide. This drug initially was developed as an anticonvulsant but was abandoned for that indication when marked adrenal suppression was noted. To treat breast cancer, it was used at high doses to inhibit adrenal steroid biosynthesis (medical adrenalectomy).[12–15] Results of aminoglutethimide treatment of metastatic breast cancer are equal to those achieved by surgical ablation.[16] Studies have demonstrated that aminoglutethimide is a competitive, nonsteroidal inhibitor of aromatase activity at lower concentrations.[12–15,17–19] However, the primary problems with aminoglutethimide are bothersome central

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nervous system and dermatologic toxicity. In addition, the glucocorticoid inhibitory properties of aminoglutethimide require glucocorticoid replacement therapy or, during administration of low doses alone, careful patient observation for signs of adrenal insufficiency.[20]

A second generation nonsteroidal competitive aromatase inhibitor, fadrozole hydrochloride (CGS 16949A), recently has been studied. This drug is well tolerated and produces clinical responses in patients with metastatic breast cancer. Two small studies have demonstrated that treatment with fadrozole hydrochloride results in a statistically significant decrease in serum aldosterone levels.[21,22] However, in most patients, this decrease in serum aldosterone is not associated with clinical symptoms or electrolyte changes. One investigator has reported alterations in electrolyte balance in patients receiving fadrozole hydrochloride therapy.[23]

Lentaron (4-hydroxyandrostenedione) is a substrate analogue of androstenedione that has undergone extensive clinical testing. It binds irreversibly to the enzyme aromatase (suicide inhibitor), and also is an active drug in patients with metastatic breast cancer. Lentaron is administered as an intramuscular injection every 2 weeks. It generally is well tolerated; rare instances of injection site reactions and sterile skin abscesses have been reported. Although a weak androgenic effect has been observed in animals receiving this agent,[24–29] no androgenic effect has been reported in the limited published clinical trials of this drug.

Letrozole is a new, nonsteroidal, competitive aromatase inhibitor. It is 10–30 times more potent than fadrozole hydrochloride in inhibiting androstenedione induced uterine hypertrophy in a uterine weight assay for aromatase. In addition, in female rats, it is highly effective in eradicating estrogen dependent mammary tumors.[30]

We recently reported preliminary results of eight patients with metastatic breast cancer treated with letrozole at doses of 0.1 and 0.25 mg once a day in ascending doses during a 12-week period.[31] This report summarizes our experience from the recently completed Phase I study of letrozole.

Patients and Methods

Patient Selection

Twenty-three postmenopausal women with measurable or evaluable metastatic breast cancer were entered in this study. Patients were required to be ambulatory and to have a Karnofsky performance of 50% or greater or an Eastern Cooperative Oncology Group (ECOG) rating of 0–2. All patients had breast cancer that had been shown to be ER positive (19 patients), ER negative/progesterone receptor positive (1 patient), or ER unknown (3 patients). All patients had metastatic carcinoma of the breast and were treated previously with single or multiple hormonal agents (Table 1). In addition, 10 patients had received adjuvant chemotherapy and 12 had received chemotherapy for metastatic disease. There was no systemic treatment administered for at least 28 days before initiation of therapy with CGS 20267. The postmenopausal state was defined as the following: at least 5 years after spontaneous cessation of menses; if less than 5 years since spontaneous menopause or chemotherapy induced amenorrhea, follicle stimulating hormone and luteinizing hormone levels in the postmenopausal range (>40 mIU/ml); or bilateral oophorectomy or radiation castration. Other eligibility criteria included normal bone marrow reserve (leukocyte count, >3000/μl; granulocytes, >1500/μl; and platelets, >100,000/μl), adequate renal function (serum creatinine, <2 mg/dl), and normal hepatic function (bilirubin, <1.5 mg/dl; serum glutamic oxaloacetic acetate, <2 × normal; alkaline phosphatase, <2 × normal; prothrombin time, <1.5 × normal). Patients requiring therapy with corticosteroids or another investigational drug were excluded from the study. Informed consent was obtained from all patients according to institutional policy.

Table 1. Pretreatment Characteristics of Patients Treated With Letrozole

No. of patients entered	23
Age (yr) (range)	41–77
ER status	
Positive	19
Unknown	3
ER − PR$^+$	1
Pretreatment sites of disease	
Local disease	6
Skin other than chest wall	2
Bone	20
Liver	4
Lung	2
Previous endocrine therapies	
1	4
2	8
3	5
4+	6

ER: estrogen receptor; PR: progesterone receptor.

Study Design

Patients were seen at the M. S. Hershey Medical Center (Hershey, PA) on the day before and on the day of treatment initiation and at weeks 2, 4, 6, 8, 10, and 12 of

drug administration. Letrozole was administered by mouth on the morning of day 1 after basal blood collections were obtained. Thereafter, it was administered each morning before 9:00 a.m.

Patients were assigned to groups sequentially in order of entry into the trial. The groups and dosages are as follows:

Group I: 0.1 mg letrozole orally once daily for 6 weeks and then 0.25 mg/day (8 patients);
Group II: 0.5 mg letrozole/day for 6 weeks and then 1.0 mg/day (7 patients); and
Group III: 2.5 mg letrozole/day for 6 weeks and then 5.0 mg/day (8 patients).

Patients were given a physical examination and had blood work performed for routine hematology, chemistry profile, and urinalysis at each biweekly clinic visit. An assessment was completed at each visit to determine drug side effects. Blood and 24-hour urine samples for measurement of hormone levels and creatinine were collected every 2 weeks for 12 weeks. On each visit to the clinic, the patients' vital signs were taken after the patient had been recumbent for 15 minutes and again after 3 minutes of standing. A cortrosyn stimulation test with assessment of cortisol and aldosterone was performed at baseline and at 6-week intervals for 12 weeks. Patients completing the 12-week evaluation period continued into an extension study on their last dose of letrozole if they experienced no progression of their breast cancer.

Drug Preparation and Patient Compliance

Letrozole was supplied by CIBA-GEIGY Corp. (Summit, NJ) as 0.1-, 0.25-, 1.0-, and 2.5-mg tablets. Patients were questioned regarding the number of tablets missed during each 2-week visit to the outpatient clinic, and tablet counts were performed. One patient mistakenly received 0.25 mg, instead of 0.5 mg, during weeks 3 and 4, but all other patients received their drug dose as required by the protocol. Tablet counts coincided with protocol dose requirements, except in fewer than 2% of cases in which patients had spilled or discarded tablets. In these instances, fewer than the appropriate number of tablets were returned.

Radioimmunoassays

Measurements of plasma estrone and estrone sulfate and urinary estrone and estradiol were performed by radioimmunoassays as previously described.[32,33] In this study, we used a highly specific antiserum to estrone that was obtained from Buhlmann Labs (Basel, Switzerland) through CIBA-GEIGY. The high specific activity estrone trace used in the assay (E1[2,4,6,7-3H(N)] was purchased from DuPont (Billerica, MA). The estrone assay had a sensitivity of 1.0 pg/ml in a direct diethyl ether extraction assay. Between-run precision at a mean concentration of 42 pg/ml was 12%.

The plasma estradiol assay was modified to include extraction of 1.8 ml of plasma with ether, followed by direct radioimmunoassay with a highly sensitive and specific antiserum. The estradiol antiserum was obtained from CIBA-GEIGY (Basel, Switzerland). The assay used a high specific activity trace (Estradiol-6-(0-carboxymethyl)oximino-2-[125I]iodohistamine) obtained from DuPont. The estradiol assay had a sensitivity of 0.1 pg/ml in a direct diethyl ether extraction assay. Between-run precision at a concentration of 7 pg/ml was 14%.

Plasma androstenedione, testosterone, 17-hydroxyprogesterone, adrenocorticotropic hormone, cortisol, aldosterone, and DHEA-sulfate were assayed according to established methods from our laboratory.[33] All samples from the same patient were routinely run in the same radioimmunoassay to eliminate interassay variability.

Treatment Evaluation

Pretreatment evaluation included a complete history and physical examination, superficial lesion measurements, chest radiograph, electrocardiogram, abdominal computed axial tomography scan, and bone scan plus radiographs of areas indicative of disease. Physical examination was repeated every 2 weeks. Superficial lesion measurements were repeated at 6 and 12 weeks and every 3 months thereafter. The electrocardiogram and radiograph studies were repeated every 3 months.

Response Criteria

The ECOG response and toxicity criteria were used. Complete response required complete disappearance of all evidence of disease. A partial response was defined as a 50% or greater decrease in the sum of the products of the two greatest dimensions of measurable lesions for at least 3 months. Stable disease was defined as a decrease in tumor size of less than 50%, no change, or an increase of less than or equal to 25%. Progressive disease was defined as a greater than 25% increase in the size of measurable lesions or the appearance of new lesions. Tumor evaluation was performed every 3 months.

Statistical Analysis

For each outcome, a one-sample t test was used to detect a significant change from baseline. Results obtained

Table 2. Toxicity in 23 Patients Treated With Letrozole

Toxicity	No. of patients
Hot flashes	5
Hair thinning	2
Nausea	5
Diarrhea	2
Dyspepsia	2
Increased sweating	2
Leg cramps	1
Peripheral edema	1
Dizziness	1
Bone pain	1

at baseline were compared with results obtained at 2, 4, 6, 8, 10, and 12 weeks of therapy. Results in the figures are expressed as the mean ± SEM. The detection limits used in these calculations for the individual estrogen results are as follows: serum E2, 0.1 pg/ml; serum E1, 0.5 pg/ml; serum E1-S, 0.5 pg/ml; urine E2, 0.01 μg/24 hours; and urine E1, 0.01 μg/24 hours.

Results

Patient Characteristics

Twenty-three patients were treated with letrozole (Fig. 1). The median age of these patients was 61 years (range, 41–77 years). Table 1 gives the pretreatment characteristics of all of the patients entered. Nineteen patients previously had a positive ER tumor or metastatic focus (1 was ER negative PR positive, and 3 were ER unknown). Bone, soft tissue, and lymph node were the most common sites of disease involvement. Most patients were heavily pretreated, with 19 (83%) having received at least two previous endocrine therapies.

Toxicity

No hematologic or biochemical toxicity was observed at any dose level. One patient experienced bone pain that was interpreted as a possible tumor flare. Two weeks after the start of the 0.5-mg dose, this patient was noted to have Grade 4 bone pain. The patient had extensive bone metastases and was evaluated to have Grade 3 bone pain at baseline. The patient was treated with sulindac with improvement and continued in the study for 12 weeks, at which time the progression of disease in bones was documented. All other toxicities encountered were mild to moderate (Grades 1 or 2). Five patients reported hot flashes, two noted increased sweating, and two noted hair thinning. Gastrointestinal side effects manifested as nausea, dyspepsia, and diarrhea were reported by five, two, and two patients, respectively (Table 2). In no patient did symptoms prevent dose escalation. Toxicity could not be directly correlated with dose administered.

No skin or allergic reactions were encountered in any patients. Orthostatic hypotension is defined as a greater than 15 mmHg drop in systolic blood pressure from the supine to the upright position, with a concomitant increase in heart rate of greater than 15 beats per minute, with both sustained for at least 3 minutes. These criteria were not met in any patient receiving treatment with letrozole.

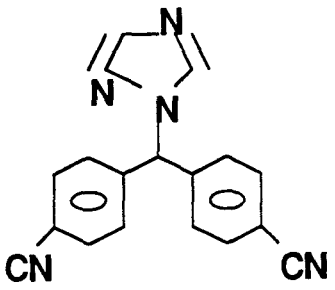
Endocrine Effects

The endocrine results presented are from 19 patients from whom complete endocrine samples/analyses are available. Treatment with letrozole produced a marked suppression in serum estrone, estradiol, and estrone sulfate, which reached more than 95% suppression within 2 weeks of therapy (Fig. 2, top left, top middle, top right). A similar pattern was observed for urinary estrone and estradiol (Fig. 2, bottom left and bottom right). Similar suppression of estrogens was observed for patients in Groups I, II, and III (Fig. 2, top left–bottom right). This degree of suppression in the urine was supported by results obtained by gas chromatography-mass spectrometry (data not shown). The 24-hour data after the first dose (first dose, 0.1, 0.5, and 2.5 mg) were available only for serum estrone and estradiol. At 24 hours, suppression from baseline for the 0.1-mg dose was 55% for E1 and 62% for E2.

There were no other changes in any other endocrine parameters. The adrenal gland was unaffected by CGS 20267 therapy. Both cortisol and aldosterone responsiveness to cortrosyn stimulation were unaffected throughout 12 weeks of drug therapy. Urine electrolytes also were not altered. There was no evidence of sodium loss or change in potassium excretion during the 12-week period of observation. In similar fashion, thyroid function was maintained. No changes in the levels of thyroid stimulating hormone, T4 and thyroid hormone binding were noted.

Tumor Response

Twenty-one patients were evaluable for antitumor response (Table 3). Two patients voluntarily withdrew from the study before completion of the 12 weeks of treatment. Response was measured from the date of initiation of letrozole therapy. At 12 weeks, one patient had a partial response for 18 months. Seven patients had stable disease for 6, 7, 7, 7, 14, 16 and 17 months, respectively. The other 13 patients had progression of their malignancy. Patients who might benefit from treatment were allowed to continue therapy beyond 12



LETROZOLE

Figure 1. Structure of letrozole (CGS 20267).

weeks on an extension protocol. A second partial response was seen during the extension.

Discussion

Aromatase inhibition is an established strategy in the palliative treatment of postmenopausal women with metastatic breast carcinoma. The first clinically useful aromatase inhibitor, aminoglutethimide, requires glucocorticoid supplementation and has substantial central nervous system and dermatologic side effects. The next aromatase inhibitor to enter clinical trials was Lentaron, a potent competitive inhibitor of aromatase.[24–26] Lentaron has shown good activity but requires a biweekly intramuscular injection for administration. Fadrozole HCL is an imidazole derivative that showed higher potency and much greater specificity in its inhibition of aromatase than did aminoglutethimide. This nonsteroidal competitive aromatase inhibitor was well tolerated and was clinically effective in postmenopausal women with advanced breast cancer.[21,23,32,34,35] However, even with these new agents there is a failure of plasma and urinary estrogens to be completely suppressed at all doses tested. For example, fadrozole HCL treatment resulted in suppression of urinary estrone to 73% ± 3% (±SE) of basal, followed in order by plasma estrone sulfate, 70%; plasma estrone, 68%; urine estradiol, 55%; and plasma estradiol, 35% from basal levels.[32] In addition, fadrozole hydrochloride in doses several times greater than the recommended therapeutic dose caused a decrease in serum aldosterone levels and a blunting

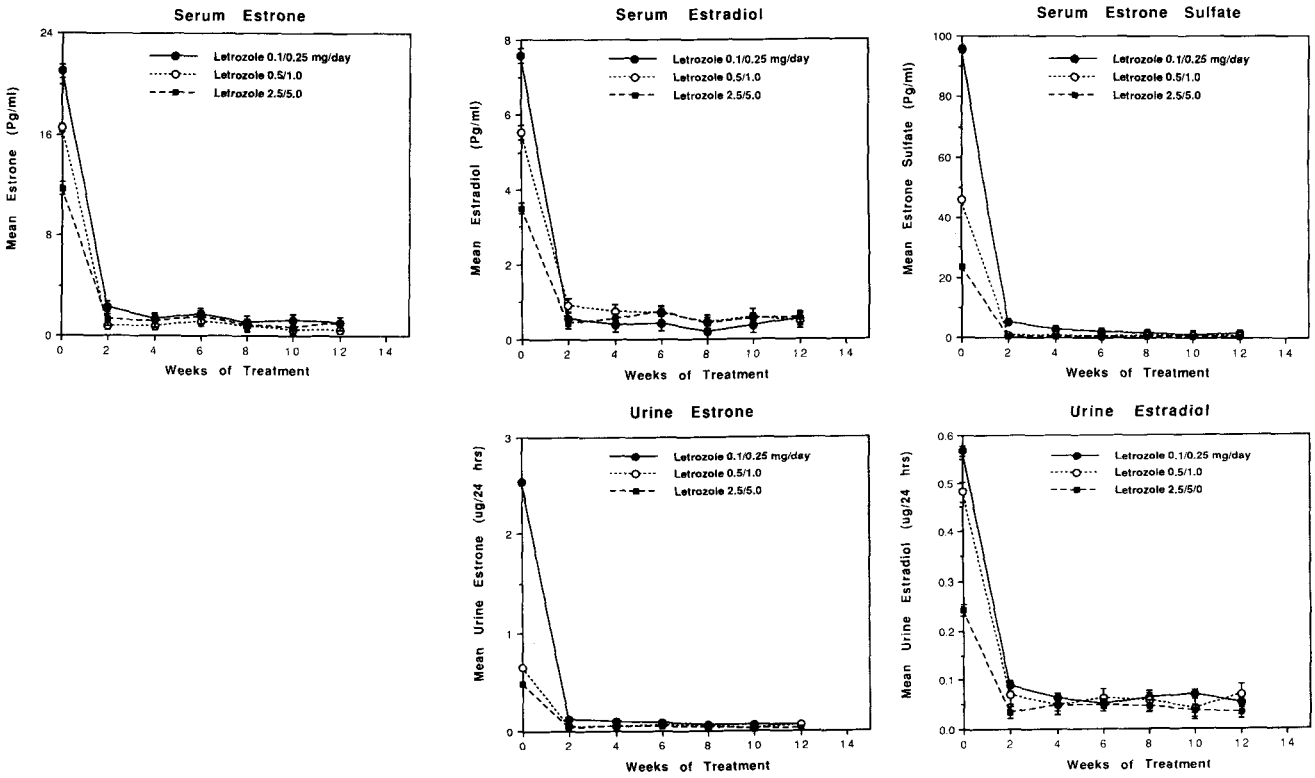


Figure 2. (Top left) The effects of letrozole (CGS 20267) on serum estrone. (Top middle) The effects of letrozole (CGS 20267) on serum estradiol. (Top right) The effects of letrozole (CGS 20267) on serum estrone sulfate. (Bottom left) The effects of letrozole (CGS 20267) on urine estrone. (Bottom right) The effects of letrozole (CGS 20267) on urine estradiol. Patients received 0.1, 0.5, or 2.5 mg/day letrozole during the first 6 weeks, followed by 0.25, 1.0, or 2.5 mg during the second 6 weeks. Results expressed are the mean ± SEM. Results were significantly suppressed ($P < 0.05$) from baseline values at weeks 2–12 of treatment for all serum and some urine estrogens.

Table 3. Results of Therapy With Letrozole

Response	No. of patients	Duration (mo)
Partial response	1	18
Stable	7	6, 7, 7, 7, 14, 16, 17
Progressive disease	13	—

of the plasma cortisol response to adrenocorticotropic hormone.[22] Thus, there remains a need for a more potent and specific aromatase inhibitor.

Letrozole, a triazole derivative is the newest nonsteroidal aromatase inhibitor tested in clinical trials. In vitro studies using human placental microsomal preparations showed that letrozole is more potent than are aminoglutethimide and lentaron.[36] In vivo letrozole is approximately 10–30 times more potent than is fadrozole hydrochloride in inhibiting androstenedione induced uterine hypertrophy in the rat uterine assay for aromatase.[37,38]

In this Phase I study, 23 heavily pretreated postmenopausal women with metastatic breast cancer were treated with letrozole. The drug was extremely well tolerated. Minor side effects included hot flashes, increased sweating, hair thinning, nausea, diarrhea, and dyspepsia. These side effects usually were mild and transient. No skin or allergic problems were encountered. A Phase I study of letrozole from the United Kingdom was published recently. Iveson et al.[39] reported the drug to be well tolerated by 21 patients.

Letrozole is a potent inhibitor of estrogen biosynthesis in postmenopausal women. Using a highly sensitive radioimmunoassay for estrone and estradiol, letrozole at the lowest dose studied (0.1 mg/day) produced more than 90% suppression of plasma and urinary estrogens. In addition, the inhibitory effects of letrozole on aromatase activity were observed within 24 hours of the initial dose of medication. This degree of estrogen suppression is greater than that previously reported with aminoglutethimide, lentaron, or fadrozole HCL.[32] Confirmatory data are not available from the study by Iveson et al.[39] because of differences in assay sensitivity.

Letrozole appears to be a specific aromatase inhibitor. In this study and in that of Iveson et al., there was no alteration of basal or cortrosyn stimulated cortisol or aldosterone. There was no evidence of urinary sodium loss or significant change in potassium excretion. Thyroid function was unaltered.

Finally, letrozole appears to have an antitumor effect in 20 evaluable patients with metastatic breast carcinoma. We observed one patient with partial response and seven with stable disease, with an additional patient in the companion extension study having partial response. Iveson et al.[39] reported 1 patient with complete response, 6 with partial response, and 6 with stable disease of the 21 patients who received therapy. The difference in response rate may be attributable to patient selection because only 4 of our patients, compared with 11 of those in the study by Iveson et al., had only one prior endocrine treatment. Phase II–III studies to document efficacy are under way.

In summary, letrozole is a well tolerated, potent, and specific inhibitor of estrogen biosynthesis in postmenopausal patients with metastatic breast cancer. A Phase III study comparing two doses of letrozole (0.5 mg and 2.5 mg) with megestrol acetate is in progress.

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