Phase I Study of the Oral Nonsteroidal Aromatase Inhibitor CGS 20267 in Postmenopausal Patients with Advanced Breast Cancer

T. J. Iveson, I. E. Smith, J. Ahern, D. A. Smithers, P. F. Trunet, and M. Dowsett

ABSTRACT

A phase I study was performed of CGS 20267, an oral nonsteroidal, highly potent, and selective aromatase inhibitor, in 21 postmenopausal patients with advanced breast cancer. The patients were recruited in 3 successive groups of 7, receiving 0.1, 0.5, and 2.5 mg p.o./day, respectively. All patients had received at least one prior endocrine treatment (range, 1–4), and six patients had received prior chemotherapy. The treatment was very well tolerated, and no toxicity was seen at any of the three doses. There was a statistically significant suppression of estradiol (E2) and estrone (E1) levels by 74% and 79% from baseline levels, respectively (P < 0.0001). Suppression occurred in all three patient groups, with many patients having serum concentrations of estradiol and estrone, which were below the limit of detection of the assays (3 and 10 pM, respectively), which are responsive to CGS 20267 (duration of response; 4+, 6+, 6+, 9+, 9, 12+, and 12+ months). Five had stable disease for more than 3 months, and 9 had progressive disease.

These results suggest that CGS 20267 is a very potent and specific aromatase inhibitor, and phase II studies are now required to confirm its clinical efficacy.

INTRODUCTION

Approximately 30% of human breast cancers are sensitive to estrogen deprivation. Aminogluthethimide has been shown to be clinically effective in postmenopausal women with breast cancer, and its main action is inhibition of aromatase (the enzyme which catalyzes the conversion of androgens to estrogens), leading to a reduction in estrogen synthesis. This is achieved by competition with the normal substrate (androstenedione) at the binding site of the aromatase enzyme and, in some cases (e.g., 4-hydroxyandrostenedione), bind irreversibly to the enzyme (suicide substrates).

4-Hydroxyandrostenedione was the first of the new aromatase inhibitors to be used in clinical trials. 4-Hydroxyandrostenedione is effective clinically by both the i.m. and oral routes, but it is first-pass hepatic metabolism means that much higher doses are required for effective oral treatment (10, 11). The i.m. route is therefore the preferred route, although this is complicated by local side effects, including sterile abscesses (8). The incidence of these local adverse events is less with i.m. injection of medroxyprogesterone acetate. Use of 4-hydroxyandrostenedione 250 mg i.m. once every 2 weeks provides effective aromatase inhibition and minimizes the incidence of these local adverse events.

Fadrozole hydrochloride, a nonsteroidal competitive aromatase inhibitor, has been shown to suppress effectively estrogen levels in postmenopausal women (12, 13). It is well tolerated (14) and has been shown to be clinically effective in postmenopausal women with advanced breast cancer (15). However, at doses required to suppress estrogen synthesis (2 and 4 mg/day), fadrozole hydrochloride has been shown to cause a significant fall in serum aldosterone levels (16), which in one study was found to be associated with changes in electrolyte balance (13). In a different study fadrozole hydrochloride was found to cause a significant fall in serum aldosterone levels only at doses of 8 and 16 mg/day, and at these doses no change in electrolyte balance was detected (17). Nonetheless, it is clear there is still a need to develop a potent and specific aromatase inhibitor for use in postmenopausal women with breast cancer.

CGS 20267 is a novel nonsteroidal agent, which has been shown in vitro and in animal studies to be a potent, selective competitive aromatase inhibitor. In addition, in female rats bearing estrogen-dependent dimethylbenzanthracene-induced mammary tumors, CGS 20267 causes almost complete regression of tumors present at the start of treatment (18).

We report here on the results of a phase I study of CGS 20267 in postmenopausal women with advanced breast cancer.

PATIENTS AND METHODS

Patient Selection. Twenty-one postmenopausal patients with histologically or cytologically proven, locally advanced, or metastatic breast cancer at the Royal Marsden Hospital Breast Unit, Fullham Road, London, were entered into the study during March 1991 and December 1991. Demographic details are listed in Table 1. All patients had received at least one previous endocrine treatment, and their performance status was 0, 1, or 2, according to WHO criteria (19). Postmenopausal status was defined as either (a) 5 years or more since a spontaneous menopause or (b) FSH and LH levels > 20 IU/liter if less than 5 years since spontaneous menopause or less than 5 years of age for

The abbreviations used are: Fadrozole hydrochloride, 4-[5,6,7,8-tetrahydroimidazo-[1,5a]pyridin-5-yl]benzonitrile mono-hydrochloride (CGS 16949A); CGS 20267, 4-[4-[1H,1,2,4-triazol-1-ylmethene]-bis-benzonitrile; FSH, follicle-stimulating hormone; LH, luteinizing hormone; TSH, thyroid-stimulating hormone; CV, coefficient of variation; IC50, concentration of inhibitor required to inhibit enzyme activity by 50%; ED50, median effective dose.
patients who had a hysterectomy, chemotherapy-induced amenorrhea, bilateral oophorectomy, or radiation castration.

Patients were ineligible if they had endocrine disorders such as diabetes mellitus, hypothyroidism, hyperthyroidism, Addison’s disease, or Cushing’s syndrome or if they had other concurrent malignant disease or were taking concomitant anticancer treatments. Patients were also ineligible if there was significant renal or hepatic dysfunction (creatinine >1.5 times the upper limit of normal, bilirubin >1.5 times the upper limit of normal, and/or transaminases >3 times the upper limit of normal) or calcium >2.75 mmol/liter. Likewise, patients were ineligible if hemoglobin <9.5 g/dl, WBC <3000/mm³, platelets <100,000/mm³, or prothrombin time was 1.5 times greater than control.

There was no selection of patients for this phase I study on the basis of estrogen receptor status of primary tumors. All previous systemic anticancer therapy was stopped at least 4 weeks prior to entry into the study. All patients gave written informed consent, and the study was approved by the Royal Marsden Hospital Ethical Committee.

Study Design. CGS 20267 was supplied by Ciba-Geigy in 0.025-, 0.25-, and 2.5-mg tablets and was taken p.o. at 9 a.m. daily. The first 7 patients entered into the study received 0.1 mg CGS 20267/day, the next 7 patients received 0.5 mg CGS 20267/day, and the final 7 patients received 2.5 mg CGS 20267/day.

On entry into the study a full clinical examination was made. Staging consisted of full blood count, urea, creatinine and electrolytes, liver function tests, electrocardiogram, chest X-ray, bone scan, and liver ultrasound.

Patients were seen at the Royal Marsden Hospital the day before treatment started, when they were examined and staging investigations were performed. On the day of starting treatment blood samples were taken during the day for endocrine and pharmacokinetic studies. Patients were then seen on days 1, 7, 14, and 28, when toxicity was assessed and blood samples were taken at 9 a.m. for hematological, biochemical, endocrine, and pharmacokinetic studies (data reported separately). All blood samples were taken after the patient had been lying supine for 15 min to exclude a postural effect on aldosterone. Blood samples for the pharmacokinetic study were taken into lithium-heparin tubes, and those for endocrine samples were taken into plain tubes. The samples were centrifuged, and the plasma/serum was removed and stored at −20°C until analysis. If their disease had not progressed after 1 month’s treatment patients were continued on the same dose of CGS 20267 and were assessed for clinical response (according to criteria of the Union Internationale Contre Cancer) every 3 months. Treatment was stopped at the time of documented disease progression.

Radioimmunoassays. Serum levels of estrone (21), estradiol (22), LH, FSH (23), and testosterone (24) were all measured according to previously described methodology. Serum TSH and aldosterone were measured by immunoradiometric assay kits obtained from Serono Diagnostics. The performance data for these assays were as follows: TSH, sensitivity 0.04 mU/ml, intraassay CV 3.1%, and interassay CV 3.9%; aldosterone, sensitivity 23 pmol/liter, intraassay CV 7.7%, and interassay CV 7.9%; 17α-Hydroxyprogesterone, cortisol, and androstenedione were measured by radioimmunoassay kits obtained from Biogenesis. The performance data for these assays were as follows: 17α-hydroxyprogesterone, sensitivity 120 pmol/liter, intraassay CV 8.9%, and interassay CV 10.4%; androstenedione, sensitivity 360 pmol/liter, intraassay CV 9.1%, and interassay CV 9.1%; androstenedione, sensitivity 360 pmol/liter, intraassay CV 8.9%, and interassay CV 10.3%. For each parameter all samples on the same patient were analyzed in the same assay batch.

Statistics. Because CGS 20267 was so effective at suppressing estrogen synthesis many of the serum samples had estradiol and estrone levels below the detection limit of the assays (3 and 10 pm, respectively), and therefore for calculation of the mean estradiol and estrone levels those samples with undetectable estradiol and estrone have been given nominal values of 2.9 and 9.9 pm, respectively. For each dose level of CGS 20267 tested at each time point the mean estradiol and estrone concentration ± SEM has been calculated. Estrone concentration during treatment was compared to the baseline estrone concentration using Student’s paired t test. Since the patients were sequentially allocated the drug rather than being randomized, no statistical comparison of estrogen suppression between the different doses has been attempted.

For the other endocrine parameters, which were measured before treatment and after 28 days of treatment, the difference between these two values has been calculated, and the mean change is shown together with the 95% confidence limits.

**RESULTS**

**Patient Characteristics.** Twenty-one patients were treated with CGS 20267. Their median age was 60 years (range, 39–81 years). The three groups receiving the different doses of CGS 20267 were similar, with median ages of 61, 63, and 62 years.

<table>
<thead>
<tr>
<th>Dose Level</th>
<th>No. of Patients</th>
<th>Median Age</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.025 mg</td>
<td>3</td>
<td>61</td>
<td>59–64</td>
</tr>
<tr>
<td>0.25 mg</td>
<td>7</td>
<td>62</td>
<td>59–68</td>
</tr>
<tr>
<td>2.5 mg</td>
<td>11</td>
<td>63</td>
<td>59–69</td>
</tr>
</tbody>
</table>

**Endocrine Results.** The mean serum estradiol and estrone levels before and after treatment are shown in Table 2 and Fig. 1. Serum estradiol and estrone levels were significantly suppressed with treatment (P < 0.001) at all three dose levels of CGS 20267 (Fig. 1), with the greater part of the suppression occurring within 24 h of starting treatment. CGS 20267 proved so effective at suppressing estradiol and estrone levels that many of the serum samples had estradiol and estrone levels below the detection limit of the assays (3 and 10 pm, respectively). The mean on-treatment values are therefore overestimates because of the assignment of 2.9 pm and 9.9 pm, respectively, for those samples with undetectable levels.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before Treatment</th>
<th>After Treatment</th>
<th>Mean Change (pm)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estradiol</td>
<td>159</td>
<td>&lt;3</td>
<td>-156</td>
<td>-161</td>
</tr>
<tr>
<td>Estrone</td>
<td>108</td>
<td>&lt;3</td>
<td>-105</td>
<td>-110</td>
</tr>
</tbody>
</table>

**Toxicity Data.** CGS 20267 was very well tolerated. The main symptoms observed were headache in 6 patients and gastrointestinal symptoms in 5 patients. All symptoms were mild and are shown in Table 4. There were no serious side effects which could be attributed to the drug. One patient on CGS 20267 who received concomitant treatment with cimetidine, ibuprofen, and dexamethasone developed an urticarial rash which resolved on stopping all treatment. It reappeared when she was rechallenged with dexamethasone; therefore, it was considered unlikely to be caused by CGS 20267. Two patients reported vaginal discharge, but this was not thought to be related to...
responses occurred at all three doses (1 at 0.1 mg, 2 at 0.5 mg, and 4 at 2.5 mg). Five patients had stable disease for 5, 6+, 7, 9, and 12+ months (2 at 0.1 mg and 3 at 0.5 mg). The other 9 patients had progressive disease.

Six patients had previously received aromatase inhibitors. Two patients had stable disease on CGS 20267, having previously had stable disease on a different aromatase inhibitor. One patient responded to CGS 20267, having previously had stable disease on aminoglutethimide.

Nine patients had received tamoxifen as adjuvant therapy (of which 4 responded to CGS 20267), 4 patients had previously responded to tamoxifen (of which one responded to CGS 20267), and 6 had previously had stable disease on tamoxifen (2 of these subsequently responded to CGS 20267).

DISCUSSION

In experimental systems CGS 20267, a triazole derivative, has been shown to be a highly potent and selective aromatase inhibitor. Potency has been assessed by both in vitro and in vivo methods. When microsomal preparations of aromatase obtained from human placenta are used (25), the potency of an aromatase inhibitor may be expressed in vitro as an IC50. With this assay CGS 20267 has been shown to be a potent aromatase inhibitor with an IC50 of 11.5 nm (18). It is 2 orders of magnitude more potent than aminoglutethimide, 5 times more potent than 4-hydroxyandrostenedione, and one-half as potent as fadrozole hydrochloride (26) (see Table 5). In an in vivo assay of

| Table 2 Serum estrone and serum estradiol concentrations (pmol/L) ± SEM |
|---------------------------------|-----------------|-----------------|-----------------|
|                                 | CGS 20267 (0.1 mg/day) | CGS 20267 (0.5 mg/day) | CGS 20267 (2.5 mg/day) |
| Estrone Serum                  |                  |                  |                  |
| Pretreatment                   | 62.7 ± 8.7       | 72.3 ± 10.5      | 70.0 ± 16.2      |
| Day 1                          | 26.5 ± 4.8 (42.2 ± 7.6%) | 26.1 ± 4.5 (37.2 ± 4.6%) | 14.4 ± 2.1 (27.5 ± 8.2%) |
| Day 7                          | 17.3 ± 2.3 (30.2 ± 5.1%) | 15.8 ± 2.8 (26.7 ± 7.3%) | 12.8 ± 2.9 (27.2 ± 8.4%) |
| Day 14                         | 17.4 ± 3.3 (29.1 ± 4.9%) | 15.2 ± 2.8 (22.7 ± 4.1%) | 11.3 ± 1.4 (20.7 ± 1.1%) |
| Day 28                         | 14.2 ± 2.3 (23.8 ± 3.3%) | 11.2 ± 0.7 (18.2 ± 3.6%) | 11.8 ± 1.6 (21.3 ± 1.1%) |
| Estradiol Serum                |                  |                  |                  |
| Pretreatment                   | 21.2 ± 2.8       | 23.4 ± 4.8       | 21.0 ± 3.8       |
| Day 1                          | 11.6 ± 3.9 (52.5 ± 16.5%) | 7.6 ± 0.5 (41.0 ± 8.5%) | 6.3 ± 0.8 (31.5 ± 2.0%) |
| Day 7                          | 4.8 ± 0.8 (22.7 ± 2.8%) | 6.0 ± 0.7 (30.4 ± 3.9%) | 3.9 ± 0.4 (20.6 ± 2.4%) |
| Day 14                         | 5.5 ± 1.7 (26.3 ± 7.7%) | 4.8 ± 0.4 (26.2 ± 5.7%) | 4.9 ± 1.1 (23.4 ± 2.4%) |
| Day 28                         | 4.8 ± 0.7 (22.8 ± 1.9%) | 4.8 ± 0.3 (26.7 ± 6.2%) | 6.7 ± 3.3 (29.1 ± 8.5%) |

" Concentration as a percentage of baseline level in parentheses.

CNS responses to CGS 20267. No hematological or biochemical toxicity was observed at any dose level.

Tumor Response. All 21 patients were evaluable for tumor response. One patient had a complete response (nodal disease), and 6 patients had a partial response: 2 had local, 2 nodal and bone disease; one had local, nodal, and skin disease; and one nodal disease alone. The patient who achieved a complete response received 2.5 mg CGS 20267/day, and the duration of this complete response (calculated from the time the complete response was achieved) is 4+ months. The duration of response for those patients achieving a partial response (calculated from the time that they started treatment with CGS 20267) has been 6+, 6+, 7, 9, 12+, and 12+ months. These responses occurred at all three doses (1 at 0.1 mg, 2 at 0.5 mg, and 4 at 2.5 mg). Five patients had stable disease for 5, 6+, 7, 9, and 12+ months (2 at 0.1 mg and 3 at 0.5 mg). The other 9 patients had progressive disease.

Six patients had previously received aromatase inhibitors. Two patients had stable disease on CGS 20267, having previously had stable disease on a different aromatase inhibitor. One patient responded to CGS 20267, having previously had stable disease on aminoglutethimide.

Nine patients had received tamoxifen as adjuvant therapy (of which 4 responded to CGS 20267), 4 patients had previously responded to tamoxifen (of which one responded to CGS 20267), and 6 had previously had stable disease on tamoxifen (2 of these subsequently responded to CGS 20267).
androstenedione-induced uterine hypertrophy CGS 20267 has an ED$_{50}$ of 1–3 µg/kg for aromatase inhibition (18). In this assay CGS 20267 is 4 orders of magnitude more potent than aminoglutethimide (27) (see Table 5). The apparent difference between the potency of fadrozole hydrochloride and that of CGS 20267 in these two assays is probably due to the much longer plasma half-life of CGS 20267.

In vitro studies on the selectivity of aromatase inhibition with CGS 20267 have shown no inhibition of progesterone and corticosterone production (18). Inhibition of aldosterone synthesis occurred only at concentrations 10,000 times greater than that required to inhibit estrone production. Selectivity of an aromatase inhibitor may therefore be expressed as a ratio of the IC$_{50}$ for aromatase inhibition compared to the IC$_{50}$ for other hormones. For CGS 20267 these are $>$17,500 and 10,500 for corticosterone and aldosterone, respectively (see Table 6). Thus CGS 20267 is a much more selective aromatase inhibitor than aminoglutethimide and fadrozole hydrochloride (18) (see Table 6).

CGS 20267 has been shown to cause marked dose-dependent regression of estrogen-dependent dimethylbenzanthracene-induced mammary tumors in adult female rats (26). The ED$_{50}$ for this antitumor effect for CGS 20267 was estimated to be 30 µg/kg/day. This compares with an ED$_{50}$ for fadrozole hydrochloride of between 0.1 and 0.5 µg/kg/day in the same assay and aminoglutethimide, which was only marginally effective at the maximally tolerated dose of 100 mg/kg/day (28).

Over 3 months in rats 3 mg/kg/day CGS 20267 caused no toxicity, and 30 mg/kg/day caused minor clinical signs but no toxicity. The median lethal dose for mice was estimated to be above 2000 mg/kg p.o., and that for rats was estimated to be above 500 mg/kg i.p. (Ciba-Geigy data on file).

In male volunteers treatment doses of CGS 20267 up to 30 mg p.o./day have been tolerated without serious side effects and resulted in the suppression of plasma estrogen levels to 60–85% of baseline levels. We have performed a study investigating aromatase inhibition with CGS 20267 in normal postmenopausal women volunteers. In these subjects CGS 20267 (0.1, 0.5, and 2.5 mg p.o. stat) was well tolerated with no serious side effects. All three doses suppressed estrone and estradiol concentrations to 75–80% from baseline levels, with statistically significant suppression occurring within 24 h of taking CGS 20267.4

In this phase I clinical study we investigated the effect of estrogen suppression with CGS 20267 on postmenopausal women with advanced breast cancer, since previously tested aromatase inhibitors have been shown to be ineffective in premenopausal women (11, 29, 30). CGS 20267 suppressed both serum estrone and estradiol levels by 79% and 74% from baseline levels, respectively. These estimates of estrogen suppression are underestimates, since after starting treatment many serum samples had levels of estradiol and estrone below the detection limit of the assays (<3 and <10 µM, respectively). It is inappropriate to compare statistically the effectiveness of aromatase inhibition at the three doses tested because the patients were prescribed the CGS 20267 dose in a sequential rather than randomized way. In any case, given that the lowest dose of 0.1 mg/day suppressed estradiol levels to below 3 µM it is unlikely that a randomized study of this size (or possibly even a much larger size) could distinguish the effectiveness of the doses on the basis of serum estrogen levels.

This degree of suppression of plasma estrogen levels seen with CGS 20267 appears to be greater than that which we have previously seen with aminoglutethimide, 4-hydroxyandrostenedione, and fadrozole hydrochloride (2, 16, 22). It is important, however, to be cautious with such data since the assay performance may vary with time, and direct comparison of the estrone data is not possible since the assay used is different from that used in trials of older aromatase inhibitors. In vivo measurement of aromatase inhibition (31) is probably a more sensitive technique to compare such potent aromatase inhibitors, and we are now carrying out these studies.

A possible alternative source of estrogen in breast cancer patients is estrone sulfate. Unfortunately, estrone sulfate was not measured in this study, but it would be interesting to measure in future studies.

This study has also confirmed the selectivity of CGS 20267, by the lack of effect on other endocrine parameters measured. In particular, CGS 20267 had no effect on the circulating levels of androstenedione, 17α-hydroxyprogesterone, and cortisol, which gives it an advantage over other nonsteroidal aromatase inhibitors on which there are published clinical data. Measurement of cortisol concentration following an adrenocorticotropin hormone challenge would provide further evidence of the specificity of CGS 20267. This was not measured in this study but is to be measured in future studies. The mean value of aldosterone fell at the 0.5 mg dose, but this was not statistically significant. Likewise, the circulating levels of FSH, LH, and TSH were unaffected by CGS 20267.

In this phase I study CGS 20267 was very well tolerated, with no major side effects. The response rate of 33% (95% confidence interval, 15–57%) with another 24% achieving disease stabilization is very encouraging, since these were a group of patients with advanced breast cancer who had all previously received at least one endocrine treatment. In addition, patients were recruited to this study regardless of their estrogen receptor status (2 were negative) or prior response to endocrine therapy. Indeed, 6 patients (29%) had failed to respond to prior aromatase inhibitors (although 4 had stable disease), and one of these patients subsequently responded to CGS 20267. Also, 2 patients who obtained stable disease only on tamoxifen subsequently responded to CGS 20267, and one patient with an estrogen receptor-negative tumor responded to CGS 20267. It was particularly encouraging that one patient achieved a response with the 0.1 mg dose, only 1/25 of a dose (2.5 mg/day), which lacked significant clinical and endocrine side effects. This suggests that CGS 20267 may be the first

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3 P. F. Trunet, manuscript in preparation.

4 T. J. Iveson, manuscript in preparation.
clinically useful aromatase inhibitor to approach the target of maximal aromatase inhibition and estrogen suppression at a dose which lacks significant clinical or endocrine side effects.

With previous aromatase inhibitors (aminoglutethimide, 4-hydroxyandrostenedione, fadrozole hydrochloride, and rogeleitimide) aromatase inhibition and estrogen suppression have been incomplete (2, 16, 22, 32). In each case dose-related side effects have prevented the escalation of dose to allow complete pharmacological effectiveness. Therefore, it has not been possible so far to test clinically whether complete suppression of peripheral aromatization might lead to higher response rates, although a recent study gives indirect support that this might be true: the addition of aminoglutethimide to the treatment of patients already receiving 4-hydroxyandrostenedione improved estrogen suppression and resulted in additional clinical responses (33). In light of these results it is interesting that in this study more responses occurred at higher doses of CGS 20267. In addition, breast cancer cell lines which have been conditioned by estrogen deprivation can respond mitogenically to estradiol stimuli at concentrations as low as 10^-13 M, suggesting that more complete suppression of peripheral aromatization might result in higher response rates.

Our clinical findings in this phase I study reinforce preclinical data suggesting that CGS 20267 may allow maximal or at least significantly greater aromatase inhibition than with previous agents, without significant toxicity. All 3 doses tested effectively suppressed estrogen biosynthesis. J. Clin. Endocrinol. Metab., 68: 99–106, 1989.


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