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Sex Hormone Levels in Postmenopausal Women With Advanced Metastatic Breast Cancer Treated with CGS 169 49A

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30 postmenopausal patients with metastatic breast cancer were treated with three different doses of fadrozole hydrochloride (CGS 169 49A), a non-steroidal competitive aromatase inhibitor. The effect of 0.5, 1 and 2 mg given twice daily upon the levels of oestrogens, their androgen precursors and upon the concentration of sex hormone binding globulin (SHBG) was investigated after 1 and 3 months and then every 3 months until progression of disease. A significant reduction in the serum concentration of oestrone ($P < 0.0001$) was obtained at all doses. Also, the serum concentration of oestrone sulphate was significantly reduced ($P < 0.001$). However, after 1 month, the concentration was significantly different from pretreatment levels ($P < 0.01$) only at the 4 mg daily dose. A decline was also observed in the concentration of SHBG ($P < 0.05$), with a concomitant elevation of the percentage non-SHBG-bound oestradiol. The androgens, testosterone and dehydroepiandrosterone sulphate, were unaltered during treatment, while androstendione was significantly elevated at the 2 mg daily dose ($P < 0.001$).
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INTRODUCTION

HORMONES ARE often implicated in various aspects of genesis and growth of cancer. Malignant cells tend to lose their ability to differentiate, but some malignant tumours retain their responsiveness to the specific hormones that are necessary for growth and maintenance of function in the normal tissue from which the tumour originated. Oestrogens are considered to be important in the development of breast cancer, although they are not directly carcinogenic. The growth of a proportion of human breast carcinomas is dependent on oestrogens, and this oestrogen dependency has led to the use of several therapeutic regimens aimed at reducing oestrogen production. In postmenopausal women, extra glandular tissues such as fat, muscle and skin are considered to be principal sites of oestrogen production from adrenal androgen precursors [1–3]. No consistent differences in serum levels of oestrogens in women with or without breast

tumours have been observed. However, in breast tissue, the concentration of oestrogens has been reported to be higher than in serum, and in cancerous breast tissue higher than in normal breast tissue. Furthermore, the concentration of oestrogens in breast tissue is found to be not related to serum levels, and independent of the menopausal status of the women [4–6]. These observations indicate that local factors are involved in accumulation and synthesis of oestrogens in breast tissue, particularly in postmenopausal women [7, 8].

A major goal of endocrine treatment of breast cancer is oestrogen deprivation, and one approach is inhibition of the aromatase enzyme system which convert androgen precursors to oestrogens. Aminogluthetimide was the first clinically used aromatase inhibitor, but as it showed dermatological and central nervous system toxicity and required glucocorticoid supplementation [9, 10], additional inhibitors were developed to enhance aromatase inhibitory potency while improving specificity and reducing side-effects [11]. One of these novel aromatase inhibitors is CGS 169 49A, 4-(5,6,7,8-tetrahydroimidazo-[1,5-α] pyridin-5-yl)-benzonitrile-monohydrochloride, which has been shown to be very potent *in vitro*, effectively blocking the conversion of androgenic precursors to oestrogens in human placental microsomes [12]. The drug was also found to be 300–800 times more potent than aminogluthetimide *in vivo*, reducing the growth of hormone-dependent DMBA (dimethylbenzylthracene)-induced rat mammary tumours [13]. In spite of the higher potency *in vitro* and in the animal model system, the suppression of oestrogens in postmenopausal breast cancer patients treated with the drug, in doses giving maximal suppression, is similar to that seen in patients receiving aminoglutethimide [14]. CGS 169 49A is heavily metabolised, but with a plasma half-life of approximately 10 h [15]. Furthermore, increases in oestrogen precursor levels at increasing doses of this drug have been described [16]. This study was designed to evaluate effects on serum levels of oestrogens, androgens, and their binding protein sex hormone binding globulin (SHBG) of different doses of CGS 169 49A, given twice a day, to postmenopausal women with advanced metastatic breast cancer.

MATERIAL AND METHODS

Patients

30 postmenopausal women with metastatic breast carcinoma and with a performance status of 0–2 (WHO) gave their written informed consent to participate in the study. The study was approved by the regional scientific ethical committee. All patients had either oestrogen receptor-positive or oestrogen receptor unknown breast cancer. The postmenopausal status was defined as cessation of menstruation more than 5 years ago, gonadotrophins in the postmenopausal range, or bilateral oophorectomy or castration by radiation. No treatment with tamoxifen or any other endocrine therapy was permitted for at least 4 weeks before entering the study. Patients previously treated with adjuvant tamoxifen were only included if progressive disease was diagnosed later than 12 months after cessation of the treatment.

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Table 1. *Patients' characteristics*

	No. of patients
Patients registered/evaluable	30/28
Median age (years)	64
Range	(45–78)
Oestrogen receptor positive/unknown	17/13
Prior therapy	
Adjuvant tamoxifen	6
Adjuvant chemotherapy	2
Tamoxifen for advanced disease	29
Chemotherapy for advanced disease	13
Sites of metastases	
Lung	7
Liver	3
Bone	25
Soft tissue	15
Number of metastatic sites	
1	16
2	8
≥ 3	6

Patients were excluded if serum creatinine was > 165 μmol/l, bilirubin > 25 μmol/l or serum glutamic oxalacetic transaminase (SGOT) > 120 U/l. The patients were included in a clinical multicentre, multinational study with the aim of defining the optimal dose level of CGS 169 49A. Patients' characteristics are given in Table 1.

Methods

Patients were randomised and allocated to one of the following three treatments with CGS 169 49A: 0.5 mg twice daily, 1 mg twice daily or 2 mg twice daily. CGS 169 49A was supplied by Ciba Geigy (Copenhagen, Denmark). Patients were seen as outpatients before inclusion, after 2, 4, 8 and 12 weeks and then every 3 months. A physical examination including blood pressure, measurements of evaluable lesions and the following blood tests, was carried out at all visits: haemoglobin, haematocrit, leucocytes (+ differential), platelets, sodium, potassium, calcium, phosphate, alkaline phosphatase, creatinine, lactic dehydrogenase (LDH), SGOT, bilirubin, blood sugar and total protein. Urinary analysis included albumin, glucose and blood. Chest X-ray, X-ray and/or ultrasound of suspicious areas were performed every 3 months.

The following hormones were measured before inclusion, after 1 and 3 months and then every 3 months until progressive disease: oestradiol (E_2), oestrone (E_1), oestrone sulphate (E_1S), per cent of non-SHBG-bound oestradiol, testosterone (T), androstendione (4-AD), dehydroepiandrosterone sulphate (DHAS) and SHBG.

Measurement of hormones

Unconjugated, E_1 and E_2 were measured by specific RIA (radioimmune assay) as described by Emment and colleagues [18]. Two-millilitre aliquots of serum were extracted with diethyl ether. After evaporation and redissolution, separation of E_1 and E_2 was performed on columns of Sephadex LH-20, equilibrated

with hexane-methanol-ethyl acetate (85:10:5 by volume), and each compound was measured by RIA. E_1S was measured as unconjugated E_1. Serum aliquots (0.5 ml) were extracted with diethyl ether. The ether phase was discarded. After addition of 1.5 ml of 3 mol/l sodium chloride, the water phase containing E_1S was extracted twice with ethyl acetate. The ethyl acetate extract was evaporated to dryness, and to the residue was added 2 ml of distilled water and 300 μl of 3% sulphuric acid. Hydrolysis was performed for 15 min at 127°C. The resulting unconjugated E_1 was then extracted with diethyl ether, and the ether phase was washed with 8% sodium hydrogen carbonate solution and distilled water, and evaporated to dryness. Chromatography and RIA were performed as described for E_1.

T and 4-AD were measured by specific RIA as described by Parker and colleagues [19]. Serum aliquots (0.5 ml) were extracted with diethyl ether, and after evaporation of the ether subjected to chromatography on columns of celite/ethylene glycol 2:1 by weight. T and 4-AD was eluted with mixtures of toluene in iso-octane and, after evaporation of the solvent, measured by RIA. DHAS was measured by RIA as unconjugated dehydroepiandrosterone. Serum (0.05 ml) was extracted with diethyl ether and ethyl acetate as described for E_1S. The pH of the ethyl acetate was lowered to 2.0 by addition of acetic acid, and solvolysis was performed overnight at 70°C. After evaporation, chromatography and RIA were performed as described for T and 4-AD. The binding capacity of SHBG was determined as described by Hertz and colleagues [20]. Serum was incubated with [^{3}H]dihydrotesterone and increasing amounts of unlabelled dihydrotestosterone. After ammonium sulphate precipitation of SHBG, part of the supernatant was counted and the binding capacity calculated by Scatchard analysis. Measurement of the percentage of non-SHBG-bound E_2 was performed by incubating serum with [^{3}H]-labelled E_2 and precipitation of the [^{3}H]-E_2-SHBG complex with ammonium sulphate, as described by Tremblay and Dube [21].

For E_1, E_2, E_1S, T, 4-AD, DHAS, SHBG and the percentage of non-SHBG-bound E_2 interassay coefficients of variation were 9.6, 8.5, 10.3, 9.4, 9.3, 9.5, 7.5 and 6.4%, respectively. The assay sensitivities were 30 pmol/l for E_1 and E_2, 200 pmol/l for E_1S, 0.25 nmol/l for T, 4-AD and DHAS, and 5 nmol/l for SHBG.

Response criteria

The UICC criteria were employed. Complete response (CR) was defined as disappearance of all evidence of disease for at least 4 weeks. In patients with bone metastases, complete disappearance of all lesions on X-ray was required. Partial response (PR) required a 50% or more decrease in measurable lesion persisting for a minimum of 4 weeks, no new lesions and no lesion increasing by 25% or more. No change (NC) was designated as patients with a < 50% decrease in total tumour size and/or a < 25% increase in one or more measurable lesions. Patients with new lesions or an increase of > 25% in one or more lesions were defined as having progressive disease (PD). An objective response was defined as lasting from the first day of treatment until PD (overall response). In case of bone metastases, NC was only applied after a minimum of 12 weeks.

Statistics

Statistical analysis was carried out on logarithms using the paired Wilcoxon test at a 5% level of significance. Assuming normal logarithmic distribution, Fisher's Z transformation was used to calculate confidence limits for correlation coefficients. The 1-, 3-, 6- and 9-month levels of SHBG were analysed together in a two-way analysis of variance, and were compared using a Bonferroni type *t*-test of equality.

Ethics

The study applied to the Helsinki II declaration and was approved by the Danish Health Authorities and by the ethical committee in Copenhagen County.

RESULTS

In all patients, E_2 levels were under the detection limit (< 30 pmol/l) of the RIA used. Figure 1 shows the serum concentration of E_1, E_1S and 4-AD during treatment with CGS 169 49A. Data from patients on all three doses were pooled as the number of patients decreased rapidly due to deaths. The treatment decreased the serum concentration of E_1 and E_1S

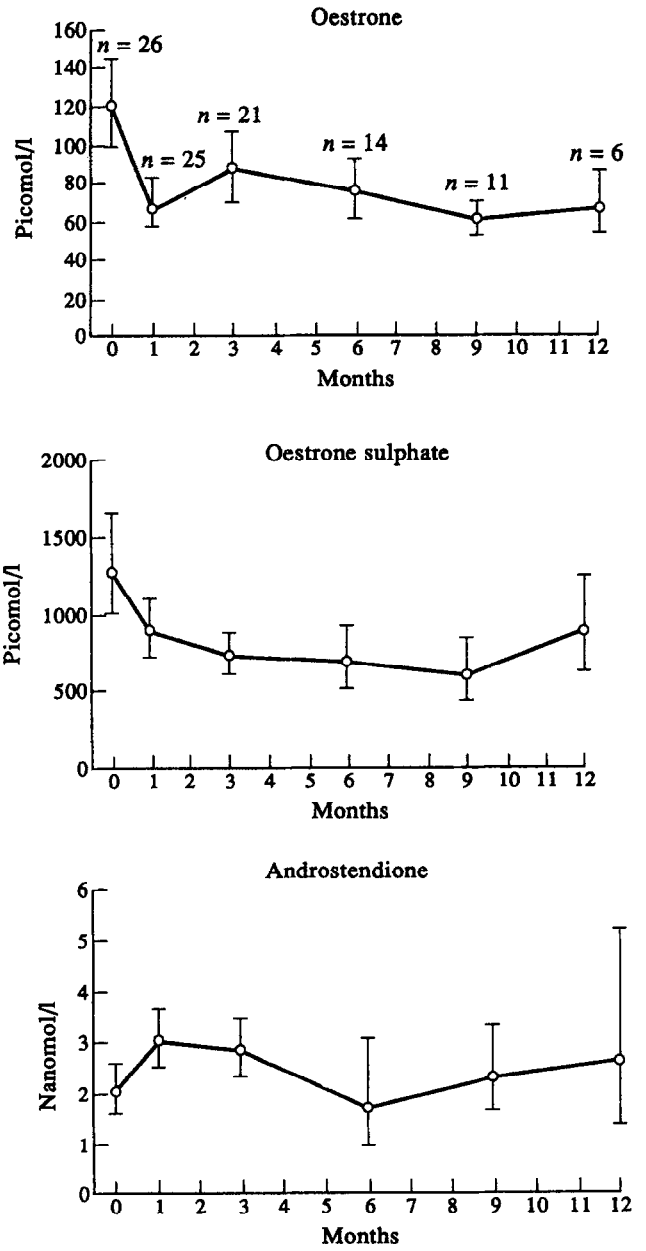

Figure 1. E_1, E_1S and 4-AD levels (± 2 S.E.) during treatment with CGS 169 49A. Data from all treatment groups are pooled. *n*, number of women whose determinations are included.

significantly ($P < 0.001$), but an upward trend was seen after 9 months of therapy. Only after 1 month were sufficient data available to examine dose-related differences in the hormonal changes. For E_1, the serum concentration was found to be significantly different from basal levels ($P < 0.01$) in all treatment groups. No differences in suppression of E_1 were observed between groups. However, for E_1S, only in the group receiving 4 mg CGS 169 49A was a significant reduction in serum concentration observed after the first month of treatment ($P < 0.01$). A trend towards elevated serum concentrations of 4-AD was observed during treatment, but the elevation was only significant ($P < 0.01$) after 1 month, and only in patients receiving the 2 mg dose. As seen from Figure 2, the SHBG levels decreased, and were significantly different from pretreatment levels after 1, 3, 6 and 9 months of treatment ($P < 0.05$). After 1 month, the decrease in SHBG was not related to dose. Concomitant with the decrease in SHBG, an elevation in the amount of non-SHBG-bound E_2 was seen ($P < 0.001$) (Figure 2). No dose relationship was seen after 1 month of treatment. No changes were found in the concentrations of T and DHAS during treatment with CGS 169 49A.

28 of the 30 patients were evaluable for response (Table 2). No patient achieved a CR, 3 patients had PR (11%), NC was observed in 11 patients (39%), and 14 patients (50%) showed PD within 4 months. The median duration of PR was 16 months, and of NC 9 months. Apparently, the response was not related to dose, but the number of patients was too small to draw any conclusions. In no patient was severe toxicity observed during treatment with CGS 169 49A, and except for hormones, no significant variations were observed in the biochemical parameters measured.

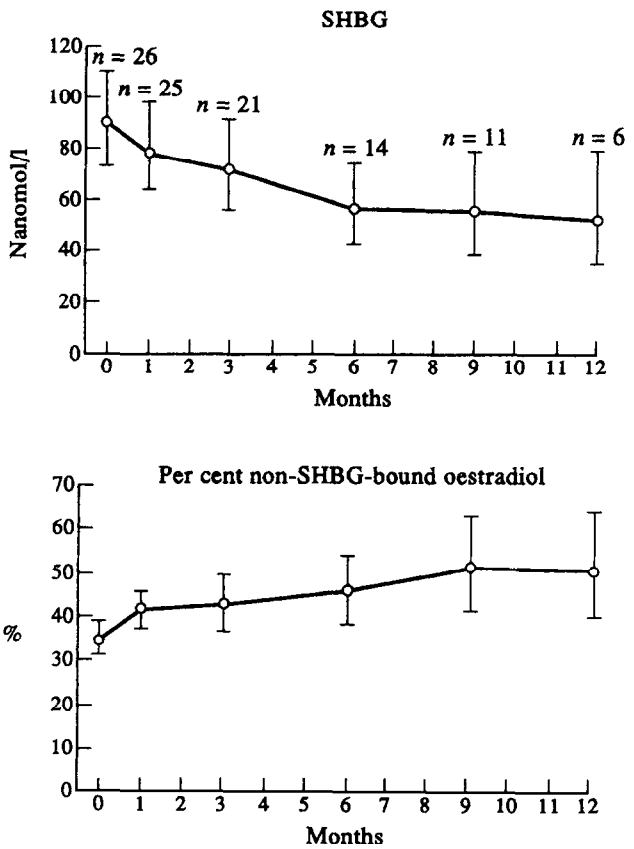

Figure 2. Levels of SHBG and the percentage of non-SHBG-bound E_2 (± 2 S.E.) during treatment with CGS 169 49A. Data from all treatment groups are pooled. *n*, number of women whose determinations are included.

Table 2. Result of therapy with CGS 169 40 A

	No. of patients	Duration (months)	Dose		
			1 mg	2 mg	4 mg
PR	3 (11%)	16 (9–18)	1	1	1
NC	11 (39%)	9 (5–21)	4	4	3
PD	14 (50%)	—	4	4	6

PR, partial response; NC, no change; PD, progressive disease.

There were no differences in hormonal changes after 1 month of treatment when levels in patients with PD were compared with levels in patients with PR or NC.

DISCUSSION

In this study, postmenopausal women with advanced metastatic breast cancer were treated with three different doses of CGS 169 49A. Decreased levels of E_1 and E_1S were seen as a consequence of inhibition of the aromatase enzyme system, as levels of precursor androgens were not correspondingly decreased, but, on the contrary, marginally elevated, probably caused by a mild blockade of 11β-hydroxylase activity [16]. Considering the suppression of E_1S after 1 month of treatment, only the 4 mg dose gave a significant reduction in serum levels. Significant suppression of E_1S is also reported with 2 mg CGS 169 49A [14, 15], but the different findings may be related to the limited number of patients in the dosages groups in all studies. However, Lønning and colleagues [22] reported in tracer studies, in agreement with our observations, a difference in aromatase inhibition between the 2 and 4 mg doses. The serum levels of E_2 were under the detection level of the RIA used (< 30 pmol/l), but we observed an increase in the percentage of non-SHBG-bounded E_2 (Figure 2), concomitant with a decrease in the concentration of SHBG. This implies that a higher proportion of the circulating E_2 must be considered to be biologically active. The decline in SHBG concentration during treatment with CGS 169 49A could be ascribed to cessation of tamoxifen treatment, as this drug is known to increase SHBG levels substantially [23]. However, subdividing patients according to previous tamoxifen treatment into two groups, one with no tamoxifen treatment for at least 6 months and another stopping tamoxifen treatment 1–3 months before inclusion, showed no difference in the initial SHBG levels.

It is unclear if the clinical response is related to dose [16, 15, 24] or if it is related to the degree of oestrogen suppression. The wide variation in oestrogen suppression in patients, reflected in high standard deviations (Figure 1), which were also reported by Stein and colleagues [25], makes it difficult to decide which dose gives maximal suppression. This variability can be related to the aromatase enzyme system and/or to the uptake of CGS 169 49A into oestrogen-producing cells. Recently, Kochak and colleagues [26] investigated the pharmacodynamic inhibition of oestrogen synthesis by CGS 169 49A in postmenopausal women and demonstrated different inhibitory constants for biosynthesis of E_1 from 4-AD and for biosynthesis of E_2 from T, indicating two synthetic pathways which are not equivalent in terms of their inhibition by CGS 169 49A. Osawa and colleagues [27] proposed the existence of two aromatase enzyme

systems acting specifically on either 4-AD or T. Another possibility, as suggested by Fishman and Goto [28], is that each of the steps in the aromatisation sequence using different substrates may be catalysed by different catalytic sites within the same protein. Corbin and colleagues [29] isolated a full-length cDNA insert encoding human placental aromatase. The expression in COS1 monkey kidney tumour cells, which normally lack steroidogenic capacity, showed that the transfected cells were capable of catalysing the aromatisation of androgens, independently of differences in ring D substitution. These findings suggest that only one aromatase enzyme is present in human tissue. Recently, Zhou and colleagues [30] transfected breast cancer cells (MCF-7), non-cancerous breast cells (HBL-100) and Chinese hamster ovary cells with human placental cDNA encoding aromatase. The activity of the enzyme in all three cell lines was identical to what has been found for the enzyme existing in human placenta. However, inhibition of the aromatase enzyme system with aminoglutethimide or 4-hydroxyandrostendione showed different inhibition profiles in different types of cells, suggesting that other factors, such as uptake of inhibitor or different regulation of steroid metabolism may also play a role in determining the inhibitory efficiency. These observations may, in part, explain the differences in suppression of oestrogens between patients treated with aromatase inhibitors, and may explain the lack of correlation to dose of CGS 169 49A, and the lack of correlation to oestrogen levels in patients responding to therapy.

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