23. Dean JC, Griffith KS, Cetas TC, Mackel CL, Jones SE, Salmon SE. Scalp hypothermia: a comparison of ice packs and the Kold Kap in the prevention of doxorubicin-induced alopecia. *J Clin Oncol* 1983, **1**, 33–37.
24. Dean JC, Salmon SE, Griffith KS, Cetas TC, Mackel CL. A comparison of ice packs and Kold Kap in the prevention of adriamycin induced alopecia. *Proc ASCO* 1981, **22**, 415.
25. Benjamin RS, Wiernik PH, Bachur NR. Adriamycin chemotherapy—efficacy, safety and pharmacologic basis of an intermittent, single, high dosage schedule. *Cancer* 1974, **33**, 19–27.
26. Grochow LB, Colvin M. Clinical pharmacokinetics of cyclophosphamide. *Clin Pharmacokinet* 1979, **4**, 380–394.
27. Giaccone G, Di Giulio F, Morandini MP, Calciati A. Scalp hypothermia in the prevention of doxorubicin-induced hair loss. *Cancer Nurs* 1988, **11**, 170–173.
28. Wheelock JB, Myers MB, Krebs HB, Goplerud DR. Ineffectiveness of scalp hypothermia in the prevention of alopecia in patients treated with doxorubicin and cisplatin combinations. *Cancer Treat Rep* 1984, **68**, 1387–1388.
29. Witman G, Cadman E, Chen M. Misuse of scalp hypothermia. *Cancer Treat Rep* 1981, **65**, 507–508.
30. Middleton J, Franks D, Buchanan RB, Hall V, Smallwood J, Williams CJ. Failure of scalp hypothermia to prevent hair loss when cyclophosphamide is added to doxorubicin and vincristine. *Cancer Treat Rep* 1985, **69**, 373–375.
31. Dean JC, Salmon SE, Griffith KS. Prevention of doxorubicin-induced hair loss with scalp hypothermia. *New Engl J Med* 1979, **301**, 1427–1429.
32. Timothy AR, Bates TD, Hoy AM. Influence of scalp hypothermia on doxorubicin related alopecia letter. *Lancet* 1980, **1**, 663–663.

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Endocrine Changes With the Aromatase Inhibitor Fadrozole Hydrochloride in Breast Cancer

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Fadrozole hydrochloride is a potent aromatase inhibitor with proven clinical effectiveness. However, its optimal dose and its effects on serum aldosterone levels/electrolyte balance have been disputed. To resolve these issues, a double-blind randomised endocrine study of three doses of fadrozole hydrochloride [0.5 mg twice daily (bd); 1.0 mg bd; 2.0 mg bd] was conducted in 80 (68 evaluable) postmenopausal patients with advanced breast cancer over a period of 3 months. There were substantial falls in the serum levels of oestradiol, oestrone and oestrone sulphate. For oestrone only, there was a significant effect of dose (on-treatment means: 0.5 mg, 38.0 pmol/l; 1.0 mg, 25.0 pmol/l; 2.0 mg, 23.9 pmol/l). All oestrogens showed a similar pattern in relation to time, with the 3-month mean being higher than those at 1 and 2 months, and this was significant for oestradiol ($P = 0.012$). There was an indication that complete suppression of oestradiol and oestrone was not maintained throughout the 12-h dosing period, but the data and its interpretation are complicated by a minor diurnal rhythm in these parameters. There were significant increases in 17-hydroxyprogesterone and androstenedione which may be due to a block of 11β-hydroxylase. There was a statistically non-significant fall in aldosterone levels ($P = 0.06$) during treatment (median pretreatment, 446 pmol/l; median decrease, 125 pmol/l). However, the concurrent significant fall in the plasma sodium : potassium ratio indicated that changes in aldosterone secretion did occur. None of these effects on adrenal pathways was of a degree which is likely to have clinically relevant consequences. It is concluded that fadrozole hydrochloride achieves near maximal suppression of oestrogens at 1 mg bd, and that its effects on aldosterone synthesis are unlikely to be of clinical significance.

Key words: fadrozole, aromatase inhibition, breast cancer
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INTRODUCTION

THE WELL-KNOWN oestrogen dependence of a proportion of human breast cancers allows effective therapeutic intervention by oestrogen antagonism or deprivation. In premenopausal women, deprivation is generally achieved by some form of ablation or suppression of ovarian steroidogenesis [1], but this is largely ineffective in postmenopausal women [2, 3] in whom the ovaries are devoid of aromatase. In postmenopausal women, oestrogen is synthesised by extraovarian aromatase which is expressed at a low level in numerous peripheral tissues. Inhibition of aromatase is now widely accepted as an effective treatment in postmenopausal breast cancer patients [4].

Aminoglutethimide is an efficient inhibitor of aromatase, achieving over 95% inhibition of the enzyme, as assessed by isotopic infusion techniques [5, 6]. However, this drug also inhibits a number of other cytochrome P_{450} enzymes involved in

steroidogenesis, e.g. 11β-hydroxylase, 21-hydroxylase and 18-hydroxylase, which has led to its combined use with replacement doses of glucocorticoid [7]. In addition, the drug leads to a number of side-effects including a rash and a series of neurological problems, such as somnolence and ataxia [8]. This has resulted in the development of a number of other aromatase inhibitors with the aim of finding a drug which will completely suppress aromatase activity at a dose which lacks significant clinical side-effects and retains high selectivity [4]. These new inhibitors may be subdivided into two groups: firstly, steroidal substrate analogues, which compete with the androgen substrate at the enzyme binding site (e.g. formestane, exemestane, atamestane); secondly, non-steroidal compounds which bind through a basic nitrogen atom directly to the enzyme's prosthetic haem group, which is intimately involved in the catalytic process. Selectivity for aromatase with the latter group of compounds has been more difficult to achieve since binding to the prosthetic haem group might occur with other members of the cytochrome P_{450} superfamily of enzymes. This potential problem of a lack of selectivity is well illustrated by the numerous enzymes with which aminoglutethimide interacts [7].

Preclinical work with the highly potent aromatase inhibitor, fadrozole hydrochloride, indicated that this compound was far more selective for aromatase than aminoglutethimide [9], but it is now known that fadrozole hydrochloride also inhibits 11β-hydroxylase and 18-hydroxylase, enzymes, which are involved in the synthesis of cortisol and aldosterone, respectively [10, 11]. Our preliminary investigations with this compound indicated that there was a dose-related suppression of oestradiol levels between the doses of 0.3 and 2.0 mg twice daily (bd) and that aldosterone levels were suppressed by approximately 50% at the 2.0 mg bd dose [12]. The indication that a dose of 2.0 mg bd was required for maximum oestrogen suppression was supported by *in vivo* aromatisation measurements by isotopic infusion techniques [13]. There were changes in plasma electrolyte levels consistent with aldosterone suppression [14], but the clinical significance of these observations is not clear, since the electrolyte changes were quantitatively only minor. These observations were in some respects in contrast with those of Santen and colleagues [15], who in a dose-incremental study, found that aldosterone suppression occurred only at substantially higher doses than those required for maximum oestrogen suppression. We have sought to clarify the comparative selectivity of fadrozole hydrochloride for aromatase by conducting a broad spectrum of endocrine analyses in association with a double-blind randomised clinical trial of 0.5, 1.0 and 2.0 mg bd of fadrozole hydrochloride in patients with advanced breast cancer. The study included an assessment of the ability of the compound to maintain full suppression between doses and during prolonged therapy.

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PATIENTS AND TREATMENT

This was a double-blind, between-patient comparison of three doses of fadrozole hydrochloride carried out in four centres: Royal Marsden Hospital, London (centre 1), Guy's Hospital, London (centre 2), St George's Hospital, London (centre 3) and Royal Marsden Hospital, Sutton (centre 4). Treatment allocation was random, 0.5, 1.0, or 2.0 mg orally bd, with the doses being coded until completion of the trial. Patient numbers were balanced within dosage group and within centre. The target number of patients was 96, i.e. 32/treatment group and 24/centre. The double-blind nature of the study was maintained until after statistical analysis of the clinical and endocrine data. Tumour response and tolerability are not considered in this report.

Patients to be enrolled were to be postmenopausal with this status being defined by one of the following criteria: 5 years or more since spontaneous menopause; serum follicle stimulating hormone (FSH) > 20 U/l if less than 5 years after spontaneous menopause; bilateral oophorectomy; radiation castration. Eligibility criteria included all patients having previously been treated with tamoxifen, followed by a treatment-free period of at least 4 weeks before starting fadrozole hydrochloride. No concurrent endocrine or other cancer treatment was to be given during the study. Patients under treatment with diuretics and/or ACE inhibitors were also excluded. Additional exclusions were patients with endocrine disorders such as diabetes mellitus, confirmed hypo- or hyperthyroidism, Cushing's syndrome or Addison's disease, patients with significant renal or hepatic dysfunction, cardiac decompensation or neurological disorders such as epilepsy.

The endocrine component of the trial lasted for 3 months. Two blood samples were taken before treatment and single samples after 2, 4, 8 and 12 weeks of treatment. All blood samples were taken between 9.00 and 10.00 am before the first tablet of the day was taken, except in centre 2, where the second pretreatment sample was taken 6 h after the first pretreatment sample (3.00 to 4.00 pm). In the other centres, the pretreatment samples were taken 24 h apart. In centres 1 and 2, additional blood samples were taken at 2 weeks and 3 months, 6 h after the morning tablet was taken.

All the endocrine analyses were conducted in the same laboratory (at centre 1). All samples were analysed for oestrone and oestradiol. Other endocrine analyses were performed only on samples from centre 1. The following analyses were conducted on the first pretreatment sample and the predrug samples on weeks 2, 4, 8 and 12: oestrone sulphate, androstenedione, cortisol, testosterone, aldosterone, 17-hydroxyprogesterone, dehydroepiandrosterone (DHEA) and dehydroepiandrostone sulphate (DHEAS). The following analyses were performed on the same samples other than those at 8 weeks: luteinising hormone (LH), FSH, prolactin (Prl), sex hormone binding globulin (SHBG), triiodothyronine (T3), thyroxine (T4) and thyroid stimulating hormone (TSH). The following methods have previously been described in detail: oestrone [16], oestradiol [17], androstenedione [18], cortisol [18], testosterone [19], aldosterone [18], 17-hydroxyprogesterone [18], TSH [18], LH [20], FSH [20] and Prl [21]. The performance characteristics and source of reagents for the other analyses are given in Table 1. The sensitivity of the oestradiol assay, which was the major measure of pharmacological efficacy, was 3 pmol/l [17].

Oestrone sulphate was analysed after hydrolysis, ether extraction and column chromatography. One millilitre of serum was spiked with approximately 1000 cpm of [6, 7-^{3}H(N)] oestrone

Table 1. Source of kits and performance data for previously unpublished methods

Hormone	Method	Kit manufacturer	Detection limit	Within assay cv	Between assay cv
SHBG	IRMA	Farmos (Oulunsalo, Finland)	0.5 nmol/l	3.2%	8.3%
DHEA	Liquid-phase RIA	Radioassay Systems (Carson, California U.S.A.)	0.3 nmol/l	5.8%	9.8%
DHEAS	Liquid-phase RIA	Diagnostic systems (Webster, Texas, U.S.A.)	0.02 μmol/l	8.1%	10.9%
T3	Enzyme immunoassay	Immunotech (Boston, Massachusetts U.S.A.)	0.25 nmol/l	7.1%	8.6%
T4	Enzyme immunoassay	Immunotech (Boston, Massachusetts, U.S.A.)	4 nmol/l	7.2%	9.0%

cv, coefficient of variation. See text for abbreviations.

sulphate (recovery control), was extracted with ether to remove unconjugated oestrone and then subjected to overnight hydrolysis with sulphatase. The ether extracts were dried and chromatographed on Sephadex LH-20 (dichloromethane : ethyl acetate : methanol; 95 : 5 : 1). The fractions containing oestrone were pooled, evaporated and reconstituted in assay buffer. Two aliquots of 200 μl were assayed by the same method as for oestrone [16] and 300 μl was taken to estimate recovery. This was generally between 45 and 67% and the results were corrected for loss. Reagent and solvent blanks were included in all batches and were undetectable. The sensitivity of the assay was 25 pmol/l and intra and interassay coefficient of variation values were 8.7 and 10.7%, respectively.

Statistical methodology

Analyses of the endocrine data have been carried out on patients considered evaluable for the analysis of endocrine parameters (see Results, paragraph 1 for criteria of evaluability). The statistical analysis was completed prior to the code being broken. The primary efficacy objective of the study was to assess the degree of oestrogen suppression (oestrone, oestradiol and oestrone sulphate). The oestrogen data were pooled across all centres for analysis. In centres 1, 3 and 4, the baseline for the oestrogen data was taken to be the second of the two baseline values if this was present, but the first basal value was used in the absence of a second pretreatment value. The first baseline value was always used for patients from centre 2, since the two baseline samples were taken 6 h apart on the same day in this centre. Logarithmic transformation was required since the raw data were not normally distributed. Repeated measures analyses of covariance with baseline as covariate were used to investigate changes over time and differences between treatment groups. If the overall statistic for one parameter was significant ($P < 0.05$), paired t-tests were used to compare time points. The P-values cited are unadjusted for multiple comparisons. The same statistical approach was taken for data on serum sodium, potassium and sodium/potassium ratio.

All other endocrine data were analysed by comparing the log of the baseline value with the mean log-transformed on-treatment values. The taking of the on-treatment *mean* for comparison was considered valid since the half-life of fadrozole is approximately 10 h [22], indicating that steady state should be achieved in 50 h, and since there was no consistent time-related change in any of the parameters between 2 and 12 weeks. Two statistical tests were performed. The Wilcoxon signed-rank test was used to test for significant changes from baseline. The Kruskal–Wallis test was used to investigate if there were dose differences. The number of comparative tests conducted was used to correct the P-values to critical levels of overall significance ($P \leq 0.002$).

RESULTS

80 patients were recruited from the four trial centres. 25 of these received 0.5 mg bd, 27 were treated with 1.0 mg bd, and 28 with 2.0 mg bd. All patients were reviewed carefully to check if they were evaluable for the endocrine parameters measured. 12 patients were excluded on the basis of not being confirmed as postmenopausal ($n = 3$), having had no endocrine measurement after the start of fadrozole ($n = 3$) or having had a washout of less than 25 days prior to fadrozole treatment subsequent to a treatment known to affect hormone analyses under consideration ($n = 6$).

The median age of the patients was 66.6 years (range 36.7–80.1) and the median weight was 63.5 kg (range 43–96 kg). Of the evaluable patients, 21 were treated with 0.5 mg bd, 25 with 1.0 mg bd and 22 with 2.0 mg bd. The repeated-measures analyses of covariance carried out on the $\log_e$ transformed data for the 3-month on-treatment period (2, 4, 8 and 12 weeks) showed no significant treatment by time interactions for any of the three variables analysed (oestrone, oestrone sulphate, oestradiol). Therefore, the effects of *treatment* can be assessed averaged over the four time points and similarly the effect of *time* can be assessed averaged over the three treatments. The results showed clear decreases in the levels of all three oestrogens following treatment ($P < 0.0002$; paired t-tests on changes from baseline at 2 weeks and 3 months on data pooled over dosage groups). There were no significant differences between doses for oestradiol or for oestrone sulphate, but for oestrone, the between-treatment effect was marginally significant (F ratio test $P = 0.048$). The overall geometric on-treatment means for each dose were 0.5 mg bd, 38.0 pmol/l; 1.0 mg bd, 25.0 pmol/l;

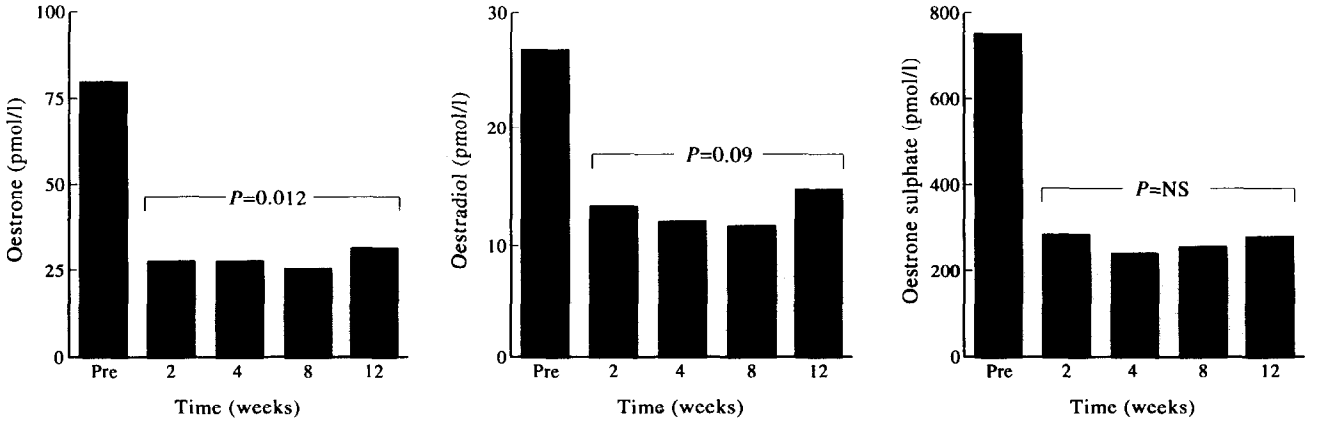

Figure 1. The effect of fadrozole on plasma oestrogen levels (geometric means) during the first 12 weeks of treatment. The *P*-values refer to the change during treatment.

2.0 mg bd, 23.9 pmol/l. Thus, the response for oestrone appears to be dose related.

The overall geometric means before and during treatment for each oestrogen are shown in Figure 1. All three oestrogens showed a similar pattern, with the 1-month and 2-month means being a little lower than the 2-week mean and substantially lower than the 3-month means. The differences between the four time points were not statistically different for oestrone sulphate (based on relatively small numbers) or oestrone ($P = 0.09$), but were significant for oestradiol ($P = 0.012$).

At centre 2, the two pretreatment blood samples were taken 6 h apart, and these were therefore used to assess the oestrogen levels for changes during the day at baseline. Samples were also taken 6 h apart at the 2-week and 3-month assessments in both centres 1 and 2 to investigate whether once oestrogen levels were suppressed a further suppression occurred 6 h postdose. Samples were taken prior to the morning dose and approximately 6 h after the morning dose. The differences between the 0 h and 6 h levels were assessed using paired *t*-tests on the differences between the $\log_e$ (values) pooled over dosage groups and centres. The back-transformation of the data results in the mean differences taking the form of ratios as follows: (predose value)/(6-h postdose value). The data are summarised in this form in Figure 2. For oestrone, there was no significant difference between the two values for the samples taken prior to treatment or after 2 weeks ($P = 0.06$), but at 12 weeks after treatment, there was a significantly greater suppression 6 h after the dose than just prior to the morning dose ($P = 0.03$). For oestradiol, there was a significant difference between the two samples taken prior to treatment starting. The magnitude of the difference was greater, however, 2 weeks and 12 weeks after starting treatment. There was no indication that these differences were dose related.

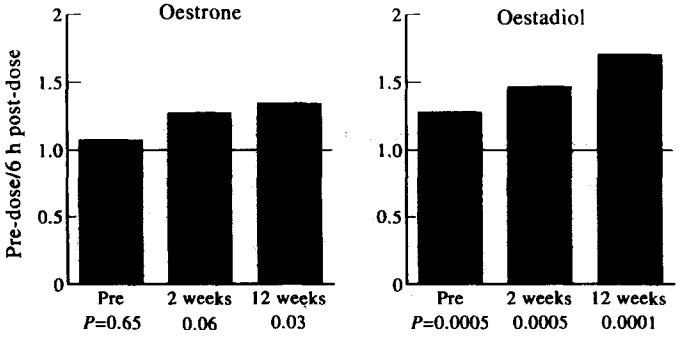

Figure 2. The difference between oestrone and oestradiol levels prior to and 6 h after the morning dose. The data are expressed as mean ratios (see Results for statistical treatment). Values above unity indicate that there was a decrease in oestrogen levels after the administration of the dose of fadrozole. Only the overall data (i.e. irrespective of dose) are shown.

Other endocrine effects

The median pretreatment values and the median change during treatment are shown for all parameters in Table 2. Serum levels of 17-hydroxyprogesterone and androstenedione increased significantly but in neither case was this significantly related to dose. Other changes, including those for aldosterone, failed to meet the level of statistical significance set to account for multiple testing.

Electrolytes

Small but consistent changes from baseline were found in sodium, potassium and the sodium : potassium ratio at 1 and 3 months. Sodium fell slightly and potassium increased slightly such that the sodium : potassium ratio therefore fell during treatment (paired *t*-tests on the data pooled over centres at 1 month, $P < 0.001$; at 3 months, $P < 0.05$).

DISCUSSION

The demonstration that aminoglutethimide exerted its antiproliferative effects in breast cancer by inhibiting the enzyme aromatase was made about a decade ago [8]. This identified aromatase as a new target for the endocrine treatment of breast cancer, and, in the ensuing period, a series of inhibitors have been developed and have entered early clinical studies. The steroidal compound formestane (4-hydroxyandrostenedione) is now widely available in some countries as an injectable given every 2 weeks. Fadrozole hydrochloride has shown clinical activity in a number of studies [14, 23–25], but the optimum dose has yet to be defined. Earlier studies [12, 13] indicated that a dose of 2 mg bd was required for maximum suppression of oestradiol and of peripheral aromatisation. The current study confirmed that fadrozole suppresses the plasma level of all three oestrogens measured, but did not detect a significant difference between doses for oestradiol. In contrast, there was evidence for greater suppression of oestrone at the higher two doses with very little difference between doses of 1 and 2 mg bd. The different

Table 2. Pretreatment hormone levels and on-treatment effects of fadrozole hydrochloride. The median change was calculated from the back-transformed on-treatment mean for each patient minus that patient's baseline value. Since multiple comparisons (28 tests) were made the overall level of significance is set at P = 0.002.

Parameter	Unit/l	n	Median baseline	Median change on treatment	P-value Overall	P-value Dose
Aldosterone	pmol	22	446	− 125	0.06	> 0.10
Cortisol	nmol	22	405	+ 10	> 0.10	> 0.10
Androstenedione	nmol	22	3.60	+ 1.71	0.001	0.07
Testosterone	nmol	21	1.70	+ 0.31	0.03	> 0.10
DHEA	nmol	17	4.90	+ 0.47	> 0.10	> 0.10
DHEAS	μmol	21	1.10	+ 0.06	0.06	> 0.10
17-OH progesterone	nmol	20	1.10	+ 0.35	< 0.0001	> 0.10
LH	IU	22	29.4	+ 3.6	0.08	> 0.10
FSH	IU	22	32.1	+ 6.0	0.02	> 0.10
SHBG	nmol	22	101	− 18	0.004	> 0.10
T3	nmol	22	1.49	− 0.16	0.09	> 0.10
T4	nmol	22	114	− 9.6	> 0.10	> 0.10
TSH	mIU	19	2.10	+ 0.03	> 0.10	> 0.10
Prolactin	mIU	21	119	+ 17	0.005	> 0.10

See text for abbreviations.

findings between this and our previous two studies may indicate that there is a small number of patients who have greater suppression of oestrogens at the higher dose of 2 mg bd, but that in the population as a whole, the higher dose has minimal advantage.

The observation that oestrogen levels were suppressed to a greater degree 6 h after a dose than just prior to dosing has not been described before. The implication is that maximum oestrogen suppression is not maintained by dosing every 12 h. However, the data are somewhat surprising given that the half-life of fadrozole hydrochloride is 10 h [22], and that we found only a small differential in oestrogen suppression between a four-fold difference in dose. The study conducted here involved the taking of blood samples at approximately 9.00 am and 3.00 pm, a time difference which is known to have a major effect on the secretion of adrenal androgens [26], and this might be expected to have an effect on the levels of oestrogens which are derived from these adrenal androgens. Indeed, in this study, a pretreatment difference was noted for oestradiol, but not for oestrone, and it is therefore probable that the observed on-treatment difference in suppression was at least, in part, due to such a diurnal rhythm. It would be preferable to repeat this measurement after taking the evening dose at a time when adrenal steroidogenesis is more stable. However, this would mean taking blood samples at 3.00 am which is clearly logistically difficult, particularly in this group of patients with breast cancer who are generally treated on an outpatient basis. An additional factor which may have influenced these data is that the control observations were made in only one centre and the on-treatment observations were made in that centre plus another. Examination of the data does not reveal a systematic difference between the centres, but it cannot be excluded that this may be a confounding factor. This is an important issue and further studies should be conducted to clarify whether full suppression is or is not maintained by the bd dosage.

The minor recovery of oestrogen levels after 3 months of treatment was not expected, but was consistent for all three oestrogens measured. This observation should be viewed with caution since it is in contrast with other reports on oestrogen levels during the use of fadrozole for at least 3 months [23, 27, 28]. The sensitivity of the plasma assays for oestradiol in these reports was not as good as our assay, and may therefore not have detected a difference. However, the oestrone and oestrone sulphate assays in two of the reports [27, 28] were equivalent to ours, and their additional finding of no change in urinary oestrone and oestradiol levels strengthens the observed absence of a recovery of oestrogen secretion in these studies. Whilst clarification of this issue will require further prospective study, it should be noted that even if the apparent recovery phenomenon is real, the absolute changes are small and therefore of doubtful significance.

A number of studies have previously demonstrated the significant suppression of aldosterone by fadrozole hydrochloride [12, 15, 16, 29]. The critical issue is whether fadrazole hydrochloride has a clinically significant effect on aldosterone and mineralocorticoid balance at the dose which is needed for maximum clinical efficacy. The data from this double-blind study showed reduced levels of aldosterone at the 1- and 2-mg bd doses. Although these changes from pretreatment levels were not statistically significant, the significant concurrent change in sodium : potassium ratio would suggest that aldosterone secretion is impaired. However, the small magnitude of the changes in electrolyte levels suggests that any effects will be of negligible clinical significance. Indeed, no clinically relevant changes in blood pressure were recorded.

In an earlier study, we observed increases in androstenedione, 17-hydroxyprogesterone and testosterone levels during treatment with fadrozole, but in no case were the on-treatment levels statistically significantly different from pretreatment. The present study has confirmed that there are significant increases in 17-hydroxyprogesterone and androstenedione levels, whilst testosterone levels increased to a lesser and statistically non-insignificant degree. Santen and colleagues [15] also reported increases in 17-hydroxy-progesterone and androstenedione levels at doses of 2 mg bd and above, and for testosterone at 4 mg bd and above. The data from Lambert and colleagues [11], indicating that fadrozole is an inhibitor of 11β-hydroxylase, suggests that this is the underlying explanation for the increase

in the level of these steroids: 17-hydroxyprogesterone would be expected to increase as a result of a partial block of cortisol synthesis, and this would then result in increased levels of androgens. The changes, however, do not approach those seen with aminoglutethimide when used alone at dosages of 250 mg/day or above [30,31], and the increased levels are unlikely to be of clinical significance, either in increasing the concentration of the substrates for aromatisation to oestrogens or in terms of androgenic side-effects.

We have confirmed that fadrozole has no significant effect on thyroid function. SHBG levels, which are a determinant of the bioactivity of oestradiol, showed a tendency to fall but this was not significant according to the criteria set. Decreases in SHBG in this context may be influenced by many patients having been treated in the recent past with tamoxifen. This causes increases in SHBG levels, and its very long tissue half-life leads to prolongation of its pharmacological effects after cessation of treatment.

In conclusion, fadrozole hydrochloride in this randomised double-blind study has been shown to be an effective suppressant of plasma oestrogen levels and to have only minor effects on other steroidogenic pathways, which are unlikely to be of clinical significance. Any increase in effectiveness of the 2 mg bd dose above 1 mg bd was undetectable by serum oestrogen assay. Apparent small deficits of total suppression between doses and after 3 months treatment merit further study.

1. Dowsett M. Reproductive endocrinology and endocrine effects of therapy. In Powles TJ, Smith IE, eds. *Medical Management of Breast Cancer*. London, Martin Dunitz, 1991, 19–26.
2. Harris AL, Carmichael J, Cantwell BMJ, Dowsett M. Zoladex: Endocrine and therapeutic effects in postmenopausal breast cancer. *Br J Cancer* 1989, **59**, 97–99.
3. Crighton IL, Dowsett M, Lal A, Man A, Smith IE. Use of a luteinizing releasing hormone agonist (Leuprorelin) in advanced postmenopausal breast cancer: clinical and endocrine effects. *Br J Cancer* 1989, **60**, 644–648.
4. Dowsett M. Clinical development of aromatase inhibitors for the treatment of breast and prostate cancer. *J Steroid Biochem* 1990, **37**, 1037–1041.
5. Santen RJ, Santner S, Davis B, Veldhuis J, Samojlik E, Ruby E. Aminoglutethimide inhibits extraglandular estrogen production in postmenopausal women with breast carcinoma. *J Clin Metab* 1978, **47**, 1257–1265.
6. MacNeill FA, Jones AL, Jacobs S, Lonning PE, Powles TJ, Dowsett M. The influence of aminoglutethimide and its analogue rogletimide on peripheral aromatisation in breast cancer. *Br J Cancer* 1992, **66**, 692–697.
7. Santen & Henderson. Pharmanual. *A Comprehensive Guide to the Therapeutic Use of Aminoglutethimide*. S. Karger AG Basel, New York, 1981.
8. Stuart-Harris R, Smith IE. Low-dose aminoglutethimide in treatment of advanced breast cancer. *Lancet* 1984, **ii**, 604–607.
9. Steele RE, Mellor LB, Sawyer WK, Wasvary JM, Browne LJ. *In vitro* and *in vivo* studies demonstrating potent and selective estrogen inhibition with the non-steroidal aromatase inhibitor CGS 16969A. *Steroids* 1987, **50**, 147–161.
10. Hausler A, Monnet G, Borer C, Bhatnagar AS. Evidence that corticosterone is not an obligatory intermediate in aldosterone biosynthesis in the rat adrenal. *J Steroid Biochem* 1989, **34**, 567–570.
11. Lamberts SWJ, Bruining HA, Marzouk H, *et al.* The new aromatase inhibitor CGS 16949A suppresses aldosterone and cortisol production by human adrenal cells *in vitro*. *J Clin Endocrinol Metab* 1989, **69**, 896–901.
12. Dowsett M, Stein RC, Mehta A, Coombes RC. Potency and selectivity of the non-steroidal aromatase inhibitor CGS 16949A in postmenopausal breast cancer patients. *Clin Endocrinol* 1990, **32**, 623–634.
13. Lonning PE, Jacobs S, Jones A, Haynes B, Powles T, Dowsett M. The influence of CGS 16949A on peripheral aromatisation in breast cancer patients. *Br J Cancer* 1991, **63**, 789–793.
14. Stein RC, Dowsett M, Davenport J, *et al.* Preliminary study of the treatment of advanced breast cancer in postmenopausal women with the aromatase inhibitor CGS 16949A. *Cancer Res* 1990, **50**, 1381–1384.
15. Santen RJ, Demers LM, Adlercreutz H, *et al.* Inhibition of aromatase with CGS 16949A in postmenopausal women. *J Clin Endocrinol Metab* 1989, **68**, 99–106.
16. Trunet PF, Mueller PH, Girard F. The effects of fadrozole hydrochloride on aldosterone secretion in healthy male subjects. *J Clin Endocrinol Metab* 1992, **74**, 571–576.
17. Dowsett M, Goss PE, Powles TJ, *et al.* Use of the aromatase inhibitor 4-hydroxyandrostenedione in postmenopausal breast cancer: optimization of therapeutic dose and route. *Cancer Res* 1987, **47**, 1957–1961.
18. Iveson TJ, Smith IE, Ahern J, Smithers DA, Trunet PF, Dowsett M. Phase I study of the oral nonsteroidal aromatase inhibitor CGS 20267 in postmenopausal patients with advanced breast cancer. *Cancer Res* 1993, **53**, 266–270.
19. Dowsett M, Mehta A, Mansi J, Smith IE. A dose-comparative endocrine-clinical study of leuprorelin in premenopausal breast cancer patients. *Br J Cancer* 1990, **62**, 834–837.
20. Ferguson K, Hayes M, Jeffcoate SL. A standardized multicentre procedure for plasma gonadotrophin radioimmunoassay. *Ann Clin Biochem* 1982, **19**, 358–361.
21. Dowsett M, McGarrick GE, Harris AL, Coombes RC, Smith IE, Jeffcoate SL. Prognostic significance of serum prolactin levels in advanced breast cancer. *Br J Cancer* 1983, **47**, 763–769.
22. Kochak GM, Mangat S, Mulagha MT, *et al.* The pharmacodynamic inhibition of estrogen synthesis by fadrozole, an aromatase inhibitor, and its pharmacokinetic disposition. *J Clin Endocrinol Metab* 1990, **71**, 1349–1355.
23. Raats JI, Falkson G, Falkson HC. A study of fadrozole, a new aromatase inhibitor in postmenopausal women with advanced metastatic breast cancer. *J Clin Oncol* 1992, **10**, 111–116.
24. Lipton A, Harvey HA, Demers LM, *et al.* A phase I trial of CGS 16949A, A new aromatase inhibitor. *Cancer* 1990, **65**, 1279–1285.
25. Beretta KR, Hoeffken K, Kvinnsland S, *et al.* CGS 16949A a new aromatase inhibitor in the treatment of breast cancer—a phase I study. *Ann Oncol* 1990, **1**, 421–426.
26. Lonning PE, Dowsett M, Jacobs S, Schem B, Hardy J, Powles TJ. Lack of diurnal variation in plasma levels of androstenedione, testosterone, estrone and estradiol in postmenopausal women. *J Steroid Biochem* 1989, **34**, 551–553.
27. Santen RJ, Demers LM, Lynch J, *et al.* Specificity of low dose fadrozole hydrochloride (CGS 16949A) as an aromatase inhibitor. *J Clin Endocrinol Metab* 1991, **73**, 99–106.
28. Demers LM, Lipton AL, Harvey HA, *et al.* The effects of long term fadrozole hydrochloride treatment in patients with advanced stage breast cancer. *J Clin Endocrinol Metab* 1993; **44**, 683–685.
29. Demers LM, Melby JC, Wilson TE, Lipton A, Harvey HA, Santen RJ. The effects of CGS 16949A, an aromatase inhibitor on adrenal mineralocorticoid biosynthesis. *J Clin Endocrinol Metab* 1990, **70**, 1162–1166.
30. Harris AL, Dowsett M, Jeffcoate SL, Smith IE. Aminoglutethimide dose and hormone suppression in advanced breast cancer. *Eur J Cancer Clin Oncol* 1983, **19**, 493–498.
31. Stuart-Harris R, Dowsett M, D'Souza A, *et al.* Endocrine effects of low dose aminoglutethimide as an aromatase inhibitor in the treatment of breast cancer. *Clin Endocrinol* 1985, **22**, 219–226.

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