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ORIGINAL ARTICLE

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The effects of oral 4-hydroxyandrostenedione on peripheral aromatisation in post-menopausal breast cancer patients

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Abstract This study investigated the influence of the aromatase inhibitor 4-hydroxyandrostenedione (4OHA, formestane), given orally, on peripheral aromatase activity and plasma oestradiol (E_2) levels in post-menopausal women with advanced breast cancer. The aim was to establish whether an optimal dose could be identified that had a pharmacological effectiveness comparable with that of parenteral 4OHA. A total of 13 post-menopausal women were studied before treatment and after a minimum of 4 weeks on treatment with one or more of the following doses: 125 mg once daily (od), 125 mg b.i.d. (bd) and 250 mg od. In all, seven aromatase studied were performed at 125 mg od; four, at 125 mg bd; and ten, at 250 mg od. Three patients were studied at all doses. E_2 was measured concurrently and was available at all dose increments for seven patients. Given at doses of 125 mg od, 125 mg bd and 250 mg od, treatment with formestane inhibited in vivo aromatisation by 62.3% ± 9.5%, 70.0% ± 5.1% and 57.3% ± 5.3%, respectively (mean ± SEM). Corresponding values for plasma E_2 suppression were 30.7% ± 6.5%, 43.4% ± 4.5% and 42.9% ± 6.7%, respectively. Thus, apart from a somewhat better suppression of plasma E_2 levels by the two higher doses as compared with 125 mg od, no significant difference in the degree of aromatase inhibition or plasma E_2 suppression was observed. The suppression of E_2 by oral 4OHA at 125 mg bd or 250 mg od approaches that achieved by the recommended parenteral schedule of 250 mg fortnightly, but inhibition of aromatase at this dose was substantially inferior. The findings are consistent with a hypothesis that 4OHA given orally may cause substantial plasma oestrogen suppression during part of the day, but neither the od nor the bd regimens investigated in the present study were capable of producing optimal aromatase inhibition.

Key words Breast cancer · Aromatase inhibitors · 4OHA

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Introduction

Inhibition of the peripheral aromatase enzyme complex, which is responsible for production of oestrogen after the menopause, is now a well-established treatment modality in advanced postmenopausal breast cancer [1]. At present, commercially available inhibitors are aminoglutethimide (AG) and 4-hydroxyandrostenedione (formestane, 4OHA). AG is a non-specific inhibitor causing adrenal and CNS toxicity, a combination that limits its clinical use [2]. 4OHA is more aromatase-specific and has minimal systemic toxicity but is conventionally given by intramuscular injection [3].

4OHA has previously been shown to be a clinically and endocrinologically effective aromatase inhibitor with minimal toxicity when given by the oral route [4, 5], but as compared with parental administration, oral 4OHA needs to be given in large daily doses because of extensive hepatic first-pass metabolism [6–8]. This feature may potentially result in less consistent and efficacious aromatase inhibition. Although a direct comparison of oestrogen suppression achieved by oral and parental 4OHA revealed no significant difference in efficacy between the two forms of administration [7], a review of our data from a series of studies involving a substantial number of patients [6, 7, 9] indicated that the oral route may be inferior

(unpublished comparison). Recent endocrine studies [9] using the new microcrystalline formulation, which improves 4OHA bioavailability over that of the micronized drug [8], have demonstrated that the optimal therapeutic dose of oral 4OHA is probably 125–250 mg once daily (od), as a 4OHA dose of 500 mg od was found not to enhance oestrogen suppression as compared with 125 mg od [9]. The bulkiness of the microcrystalline formulation makes it difficult to achieve an acceptable pharamaceutical preparation for a dose above 250 mg. In this study we therefore confined measurements to doses ranging between 125 and 250 mg, including a b.i.d. (bd) dose, to ascertain if this would improve efficacy by overcoming pharmokinetic deficiencies.

Extended clinical use of oral 4OHA in the management of advanced breast cancer would be acceptable only after confirmation that its pharmacological efficacy was comparable with that of parenteral 4OHA. Consequently, in vivo peripheral aromatase inhibition was measured in conjunction with further E_2 analysis in patients treated with 125 mg od, 125 mg bd and 250 mg od of oral 4OHA. The results were compared with similar studies undertaken on parenteral 4OHA at the recommended dose of 250 mg fortnightly [10], which revealed a mean aromatase inhibition of 85%. This technique has proved its sensitivity and usefulness by identifying significant dose-related differences for other aromatase inhibitors thus far investigated, including rogletimide (RG) [11], parental 4OHA [10] and fadrzole [12].

Patients and methods

Patients

Demographic data are given in Table 1. A total of 13 women with advanced metastatic breast cancer suitable for endocrine therapy were recruited. Standard legal and ethical considerations were applied and written informed consent was obtained. All patients were post-menopausal (spontaneously, > 2 years). No systemic anti-cancer treatment had been given for a minimum of 4 weeks prior to trial entry. All patients had a life expectancy of greater than 6 months and were entered on an "intention to treat" basis, were fully staged at study initiation and completion and were assessed fortnightly/monthly. Treatment responses and toxicity were documented using International Union Against Cancer (UICC) and WHO criteria, respectively. All patients had received prior treatment with tamoxifen and five women had received one or more further treatment manoevres. Four of these patients had received previous treatment with another aromatase inhibitor in the adjuvant setting or due to advanced disease.

Study design

The protocol was designed to examine the effects of all three doses of formestane (125 mg od. 125 mg bd and 250 mg od) on in vivo aromatisation in all patients to allow for a dose comparison within individual patients. However, in the majority of patients the study was not completed according to the protocol due to disease progression and an unexpected shortage of commercially available [^{14}C]-E_1. Thus, only three women (patients 3, 6 and 9) successfully completed the full dose comparison and all aromatase/endocrine studies. The other patients completed a variety of aromatase and endocrine analyses such that seven received 125 mg od; four, 125 mg bd; and ten, 250 mg. In all, seven patients completed the full comparative analysis for plasma E_2. Three women (patients 11–13) were started and maintained on 4OHA at 250 mg until remission.

4OHA was provided by Ciba-Geigy Pharmaceuticals as a sterile microcrystalline formulation in vials of 62.5 and 250 mg, stored at 4 °C, and required mixing in 10 ml of 0.9% saline prior to ingestion. For the 125-mg dose, two vials of 62.5 mg were given concurrently. Patients received each dose of oral 4OHA for a minimum of 4 weeks before dose change. Patients in whom continued treatment with oral 4OHA was indicated remained on 250 mg daily.

Aromatase activity was assessed as previously described [13] using a double-tracer bolus i.v. injection of 10 μCi [4-^{14}C]-oestrone (E_1) and 500 μCi [6, 7-^{3}H]-androstendione (A) pre-treatment and again after a minimum of 4 weeks on treatment with any dose of oral 4OHA. Blood samples for endocrine analysis were taken pre-treatment (control), at 14 days and 4 weeks after each dose change, and thereafter at intervals determined by outpatient attendance according to clinical judgement. The blood was centrifuged and the serum, stored at − 20 °C.

Table 1 Demographic indices for patients on oral 4OHA (*r* responder, *Q/I* Quetelet's index = weight/height^2, *ER* oestrogen receptor, *UK* unknown, *T* tamoxifen, *RG* rogletimide, *MPA* medroxyprogesterone acetate, *POND* progestin/AG/T/danazol, *AG* aminoglutethimide, *adj* aduvant, *chemo* chemotherapy)

Patient number	Age (years)	Q/I	ER status	Metastatic site	Treatment	Duration (months)
1	53	31.2	UK	Bone	T(adj)	7
2	83	24.5	UK	Local, nodal	T(adj), AG	9
3r	78	31.4	+	Local	T	18
4	70	25.0	+	Local	T, chemo	2
5	65	24.8	+	Bone, local, visceral	T(adj), RG,	5
6r	63	28.7	UK	Skin	T(adj)	14
7	60	23.5	UK	Local, bone	T	12
8	59	28.7	UK	Nodal	T(adj)	8
9r	64	35.6	−	Local	AG(adj), POND	9
10	61	20.4	+	Bone, visceral	T(adj)	4
11	64	22.2	+	Bone, nodal	T	9
12	55	25.4	+	Local, bone	T, chemo, MPA, AG	4
13	68	26.9	UK	Local, bone, visceral	T(adj)	4
Mean	64.8	26.7				7.5

Methods

Peripheral aromatase activity was measured in vivo by determining the [^{3}H]/[^{14}C] isotope ratio in the major urinary oestrogen metabolites (E_1 and oestriol, E_3) in 4-day urine samples collected after injection of [^{3}H]-A and [^{14}C]-E_1. Procedural details for isotope injections, urine collection, reagents, and urinary high performance liquid chromatography (HPLC) have been extensively described [13, 14] and were unchanged for this study. The values are expressed as the percentage of conversion of androstenedione to oestrone.

Serum E_2 was measured by a radioimmunoassay (RIA) with a detection limit of 3 pmol/l as previously described [6]. Mean values were calculated from multiple on-treatment values measured after a minimum of 14 days of treatment at any dose and are expressed as the percentage of suppression from the control value. The magnitudes of E_2 suppression achieved by the different doses of formestane were compared using the Friedman test. If this revealed any difference of statistical significance, a paired comparison was performed using the Wilcoxon matched-pair sign-rank test.

Results

Pre- and on-treatment E_2 levels and the percentage of suppression are shown in Table 2 and Fig. 1. Treatment with formestane suppressed plasma E_2 levels significantly ($P < 0.005$, Friedman test). Doses of 125 mg od, 125 mg bd and 250 mg od suppressed plasma E_2 levels by 30.7% ± 6.5% (mean ± SEM, $P < 0.025$), 43.4% ± 4.5% ($P < 0.005$) and 42.9% ± 6.7% ($P < 0.001$), respectively. Thus, although treatment with formestane at 125 mg od caused somewhat less suppression of plasma E_2 as compared with the two higher doses, this difference was not of statistical significance. It is noteworthy that patients who successfully completed all dose increments and aromatase studies did not display a relationship between E_2 suppression and aromatase inhibition.

Pre- and on-treatment values for the percentage of aromatisation and the percentage of inhibition caused by treatment with different doses of formestane are shown in Table 3 and Fig. 1. Pre-treatment aromatase activity was 1.27% ± 0.10% ($n = 13$). A maximal aromatase inhibition of 70.0% ± 5.1% ($n = 4$; range, 62.9%–84.6%) was seen at 125 mg bd, with inhibition of 62.3% ± 9.5% ($n = 7$; range, 18.4%–83.6%) and 57.3% ± 5.28% ($n = 10$; range, 20.5%–76.9%) being recorded for 125 mg od and 250 mg od, respectively. Further statistical analyses were not performed because of the small patient numbers, but patients who successfully completed all dose increments displayed no increase in aromatase inhibition with increasing doses of

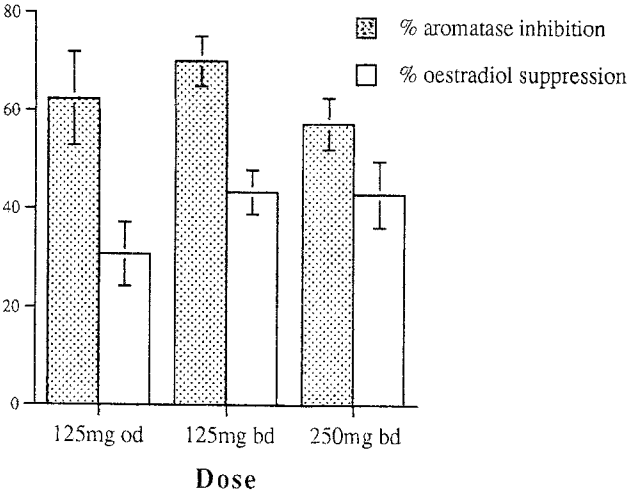

Fig. 1 Aromatase inhibition and serum oestradiol suppression by difference doses of 4-OHA given orally. Data represent mean values ± SEM (*od* once daily, *bd* b.i.d.)

Table 2 Serum E_2 levels and percentage of suppression in oral 4OHA dose comparison (*Pre* Pre-treatment, *od* once daily, *bd* b.i.d., *% S* percentage of suppression)

	Pre	125 mg od		125 mg bd		250 mg od	
Patient number	E2 (pmol/l)	E2 (pmol/l)	%S	E2 (pmol/l)	%S	E2 (pmol/l)	%S
1	29	30	− 5.3	22	23	29	− 2.5
2	27	[a]	[a]	18	33	14	56
3	27	21	24	14	48	15	44
4	42	[a]	[a]	21	50	[a]	[a]
5	31	25	20	21	33	19	38
6	59	36	39	18	70	31	48
7	33	21	38	18	47	19	44
8	29	12	58	15	49	23	21
9	39	25	36	24	39	24	39
10	22	14	36	[a]	[a]	19	13
11	20	[a]	[a]	[a]	[a]	6.7	66
12	18	[a]	[a]	[a]	[a]	4.9	72
13	25	[a]	[a]	[a]	[a]	6.5	74
Mean	30.8	22.9	30.7	18.7	43.4	17.5	42.9
SD	11.4	7.9	18.5	3.4	13.4	8.8	23.1
SEM	3.3	2.8	6.5	1.1	4.5	2.5	6.7
n	13	8	8	9	9	12	12

[a] No on-treatment sample available

Table 3 Aromatase activity and percentage of inhibition for oral 4OHA dose comparison (*Pre* Pre-treatment, *od* once daily, *bd* b.i.d., *AA* aromatase activity, *%I* percentage of inhibition)

Patient number	Pre *AA*	125 mg od *AA*	*%I*	125 mg bd *AA*	%	250 mg od *AA*	*%I*
1	1.32	0.78	40.8	[a]	[a]	1.05	20.5
2	0.96	[a]	[a]	[a]	[a]	0.39	60.0
3r	1.24	0.53	57.1	0.41	67.0	0.62	50.1
4	2.11	[a]	[a]	0.33	84.6	[a]	[a]
5	1.13	[a]	[a]	[a]	[a]	0.42	63.2
6r	1.61	0.45	72.2	0.60	62.9	0.72	55.2
7	1.69	0.28	83.6	[a]	[a]	0.39	76.9
8	1.11	0.18	83.4	[a]	[a]	[a]	[a]
9r	0.97	0.19	80.7	0.35	63.7	0.40	58.6
10	1.13	0.92	18.4	[a]	[a]	[a]	[a]
11	1.11	[a]	[a]	[a]	[a]	0.30	73.3
12	1.38	[a]	[a]	[a]	[a]	0.41	70.4
13	0.81	[a]	[a]	[a]	[a]	0.47	41.8
Mean	1.27	0.48	62.3	0.42	70.0	0.52	57.3
SEM	0.10	0.11	9.45	0.06	5.09	0.07	5.28
n			7		4		10

[a] Results not used for calculations

formestane. One other patient who had tracer injection on drug doses of 125 mg od and 250 mg od did not have a better aromatase inhibition on the higher dose.

This study was not conducted to assess clinical response, although this was documented. There were five cases of disease stabilisation, two partial responses and one minimal response. The overall median duration of treatment was 7.5 months. Tolerability was good except for one patient, who vomited after ingestion of 4OHA because of its salty taste and declined to continue the trial.

Discussion

Parental 4OHA has a well-proven pharmacological [6, 7, 10] and clinical efficacy [5, 15] with minimal toxicity and the additional benefit of excellent compliance. Once-daily oral administration of 4OHA has never been established because of technical difficulties with formulation, compounded by the high doses required to offset its rapid first-pass metabolism by the liver [7].

For the reasons outlined above, most of the patients in this study did not complete aromatase measurement in all of the three "on-treatment" situations. Although this excluded the possibility of comparing the efficacy of the different doses in statistical terms, it did not invalidate the conclusion of the study. As mentioned, there is uncertainty as to whether formestane given orally is as efficacious as the parenteral drug in causing plasma oestrogen suppression [6, 7, 9, 16]. In this study we found formestane at 125 mg od to cause a somewhat smaller suppression of plasma E_2 (30.7%) as compared with the suppression value of 40%–60% generally recorded on treatment with parenteral fomestane, but the suppression achieved with 125 mg bd (mean value, 43.4%) and 250 mg od (mean value, 42.9%) is in the lower range of what is observed on parenteral therapy. However, it is evident that formestane given orally on any of the three dosing schedules investigated in the present study (125 mg od, 125 mg bd and 250 mg od) did not inhibit in vivo aromatisation to the same extent as formestane given as i.m. injections at 250 mg every 2 weeks (mean inhibition, 84.8%) or 500 mg every 2 weeks (mean inhibition, 91.9%) [10]. Other aromatase inhibitors such as CGS 16949A (fadrzole) [12] or aminoglutethimide [11] produce a mean aromatase inhibition of 82%–93% when given at different doses orally. Thus, any difference in aromatase inhibition between the oral doses of formestane used in this study is moderate and of only academic interest as compared with the difference between the inhibitory action of formestane given orally, on the other hand, and that of other contemporary aromatase inhibitor regimens, on the other.

The finding that the percentage of oestrogen suppression did not correspond to the percentage of aromatase inhibition (i.e. that the degree of aromatase inhibition was more pronounced than the oestrogen suppression) is similar to what has been recorded for all the aromatase inhibition regimens investigated thus far [7, 10–12, 17–19]. Possible explanations for these observations have been discussed elsewhere [1, 16]. In this study, 4OHA given orally at 125 mg bd or 250 mg od caused suppression of plasma E_2 levels approaching that seen on treatment with parenteral 4OHA, despite insufficient aromatase inhibition. This underlines what we have previously argued: that measuring serum oestrogen suppression is insufficient for comparing the pharmacological efficacy of different aromatase inhibitors [20]. One explanation could be that current methods of oestrogen measurement lack the sensitivity necessary to discriminate between plasma oestrogen

levels in the low range found among patients on treatment with different aromatase inhibitors [1]. However, all patients in this study had on-treatment E_2 values well above the sensitivity limit of the RIA method.

A more likely explanation in this case is that by measuring aromatisation with a urine technique we get an integral picture of aromatisation throughout the day, in contrast to plasma oestrogen measurements, which give a value at a fixed time point in relation to drug dosing. In this study as well as in previous investigations [7, 9], serum samples obtained during treatment were drawn at approximately 2–5 h following ingestion of 4OHA, i.e. at the time of maximal 4OHA concentration [7, 9]. Earlier findings that formestane orally produces similar suppression of plasma E_2 when given in the 125- to 500-mg-daily dose range [9], despite a linear increase in plasma drug area under the curve (AUC) in relation to dose, suggest that at the time of peak drug concentration, all these drug regimens produce plasma drug levels above those required for optimal oestrogen suppression. When plasma levels of 4OHA were measured over a period after i.m. dosing, the results suggested an "escape" phenomenon of E_2 suppression when plasma drug levels fell under 2–3 ng/ml [6]. The terminal half-life of 4OHA following oral administration is dose-independent and approximately 3 h [7, 9], with serum concentrations of 4OHA falling to undetectable levels before 24 h. Given these plasma pharmacokinetic data, a twice-daily dosing may be expected to be advantageous over od dosing, but the results obtained in this study did not substantiate such a hypothesis. There are several explanations for this observation. Little is known about tissue levels or the volume of distribution of 4OHA, and the possibility exists that 40HA may be concentrated in peripheral compartments or have a slow tissue turnover not reflected in its plasma pharmacokinetic profile. However, a more likely explanation is that plasma levels of 4OHA may reach suboptimal levels at the end of the dosing interval regardless of whether patients receive 125 mg bd or od, allowing for an "escape" of oestrogen synthesis.

It is noteworthy that the pre-treatment values of aromatisation (1.27% $\pm$ 0.10%) recorded in this group of patients were somewhat lower than the mean values reported in our previous investigations [10–12]. However, data from these studies revealed no evidence for a relationship between pre-treatment values of aromatisation and the percentage of inhibition in groups of patients treated with the same drug regimens. Although four patients in this study had received previous treatment with other aromatase inhibitors (aminoglutethimide or rogletimide), it is not likely that this would have influenced pre-treatment values of aromatisation. All patients had been off any endocrine treatment for at least 4 weeks before entering this trial, and previous studies have shown the estrogen-suppressive effect of aminoglutethimide to be lost within days of treatment termination [21].

An interesting clinical observation is that one of four patients previously treated with an aromatase inhibitor (patient 9) subsequently responded to treatment with oral 4OHA. The question of cross-resistance to different aromatase inhibitors or of whether they may have partially different mechanisms of action is currently a subject of debate, but on the basis of theoretical considerations it is justified to explore the use of different drugs sequentially in selected patients [22].

In conclusion, 4OHA given orally at 125 mg od, 125 mg bd, or 250 mg od causes aromatase inhibition and (probably) E_2 suppression inferior to what is seen on treatment with parenteral 4OHA.

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