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Original Paper

Treatment with Formestane Alone and in Combination with Aminoglutethimide in Heavily Pretreated Breast Cancer Patients: Clinical and Endocrine Effects

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We studied the clinical and endocrine effects of the aromatase inhibitor formestane (4-hydroxyandrostenedione, 4-OHA) in heavily pretreated breast cancer patients (median number of previous endocrine treatments 2, range 1–4). Of 30 patients eligible for response evaluation, 3 patients (10%) showed a partial response while 11 patients (36.7%) experienced stable disease over a time period of at least 6 months. Plasma levels of oestrone, oestradiol and oestrone sulphate were found suppressed to a mean of 33.9, 35.6 and 24.2% of control values. 4 of 6 patients experienced a further substantial reduction in plasma oestrone sulphate to < 5% of pretreatment values when aminoglutethimide (AG) was added after relapse on 4-OHA monotherapy. Our findings suggest that these aromatase inhibitors may suppress plasma oestrogen levels by a percentage approaching the percentage inhibition of *in vivo* aromatisation measured by tracer techniques.

Key words: breast cancer, endocrine treatment, aromatase inhibitor, formestane, aminoglutethimide
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INTRODUCTION

AROMATASE INHIBITION is an effective treatment option for advanced breast cancer in postmenopausal women. The first generation aromatase inhibitor, aminoglutethimide (AG, Orimeten®), was introduced for breast cancer treatment more than 20 years ago [1, 2]. While this drug proved effective in breast cancer, its toxicity resulted in efforts to develop novel, less toxic aromatase inhibitors.

Formestane (4-hydroxyandrostenedione, 4-OHA, Lentaron®) is a second generation aromatase inhibitor and the first "steroidal" aromatase inhibitor introduced for clinical use [3]. Results from several phase II studies suggest a response rate comparable to that which may be achieved with AG as second line endocrine therapy [4–6].

Aminoglutethimide has also been shown to be effective in heavily pretreated patients [7, 8]. Because 4-OHA is well tolerated, we used this drug as a late treatment option in heavily pretreated patients with advanced breast cancer. This paper summarises our clinical experience from the first 34 patients treated in our department.

Previous studies have revealed that treatment with AG and 4-OHA suppresses plasma oestrogens by only 40–70% [9–12] despite inhibition of *in vivo* aromatisation by more than 85% [13–15]. Whether this is due to alternative oestrogen sources or caused by non-specific interactions in the radioimmunoassays (RIA) is currently not known. Recently, we developed a new, highly sensitive assay for measurement of plasma oestrone sulphate (E_1S) levels in the low concentration range [16]. This study represents the first evaluation of plasma E_1S with this assay during treatment with an aromatase inhibitor.

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PATIENTS AND METHODS

Patients

The records of all patients treated with 4-OHA in our department from December 1989 to March 1995 were examined. The patient group comprises 34 patients with advanced breast cancer (32 postmenopausal women, one premenopausal woman on continuous treatment with goserelin acetate and one male) treated with 4-OHA because of progressive disease. 21 patients (61.8%) were positive for oestrogen and/or progesterone receptor, either in their primary tumour or metastasis, and in the remaining patients the receptor status was unknown. A locoregional relapse was the treatment indication in 21 patients. 3 patients (8.8%) suffered from bone metastasis as the only manifestation of their disease and 14 patients (41.2%) had two or more different sites of disease, including visceral metastasis in 7 patients. The median age of the patients was 75 years (range 36 to 87 years).

None of the patients received any other hormonal treatment or drugs known to influence drug metabolism.

All patients had previously been treated with at least one

endocrine treatment option (median of 2, range 1–4 regimens). 1 patient had received adjuvant chemotherapy (CMF). 2 other patients had received treatment with doxorubicin weekly, and 1 of them had received 5-fluorouracil in combination with mitomycin (FUMI) after progressing on doxorubicin monotherapy. None of the other patients had received chemotherapy. Previous endocrine treatment was terminated at least 4 weeks before commencing on treatment with 4-OHA in all patients from whom blood samples were obtained for plasma oestrogen measurement.

Drug schedule

All patients received 4-OHA as intramuscular injections of 250 mg per dose. 13 patients had weekly injections during the first 6 weeks on treatment, and thereafter received their injections at 2-weekly intervals. The other patients ($n = 21$) had their injections at 2-weekly intervals from the beginning of therapy.

6 patients had AG added following progression on 4-OHA and were subsequently treated with the two drugs in concert. Aminoglutethimide was given orally at a dosage of 250 mg four times a day in combination with cortisone acetate (50 mg twice daily during the first 2 weeks, thereafter 25 mg twice daily).

Blood sampling for oestrogen measurement

Heparinised blood samples were obtained from 19 patients before commencing 4-OHA therapy and after 36 to 80 days on treatment. All blood samples were obtained after an overnight fast. Plasma was separated with centrifugation and stored at –20°C until processing.

Oestrogen analysis

Plasma levels of oestradiol (E_2) and oestrone (E_1) were determined as previously described [11, 17]. The sensitivity limit of the methods in our laboratory is 2.1 and 6.3 pmol/l, respectively. Plasma levels of oestrone sulphate (E_1S) were determined by a novel highly sensitive assay involving purification and derivatisation into E_2, and RIA analysis using E_2-6-carboxymethyloxime-[2-^{125}I]iodohistamine as tracer ligand [16]. The sensitivity limit of the method is 2.7 pmol/l.

Statistical methods

In a previous study, we found plasma oestrogen levels to be well fitted to a lognormal distribution [17]. Thus, plasma oestrogens were expressed as their geometrical mean values with 95% confidence intervals of the mean. Values obtained before and during treatment were compared using the Wilcoxon matched pair signed rank test. All *P*-values are expressed as two-tailed.

Evaluation of response

Objective response was defined according to the UICC criteria [18]. To classify a response as "stable disease", this should have lasted for a minimum time period of 6 months.

RESULTS

Clinical response

Of a total number of 34 patients, 4 patients had been on treatment for less than 6 months, leaving 30 patients for response evaluation. 3 patients (10%) showed a partial response (PR) while 11 patients (36.7%) experienced stable disease (SD) over a period of at least 6 months. 16 patients (53.3%) had progressive disease (PD).

10 patients had received previous treatment with AG. In this group, 2 patients experienced a PR and 3 patients a SD when treated with 4-OHA as monotherapy. All 5 had obtained at least a stable disease or an objective response when treated with AG. 4 had received AG as their last treatment regimen before commencing on 4-OHA.

Mean time to progression (TTP) for all patients was 5.3 months, and in the subgroup of patients with an objective response (PR and SD), 13.3 months.

Of 6 patients treated with the combination of 4-OHA and AG after relapsing on 4-OHA alone, 2 patients showed a partial remission and 1 patient had stable disease over a period of more than 6 months.*

Plasma oestrogen levels

Plasma levels of E_1 were suppressed from a mean value of 60.3 pmol/l before treatment to 20.5 pmol/l during treatment with 4-OHA alone (Table 1, Figure 1). This corresponded to suppression to 33.9% of pretreatment values (95% confidence interval 28.3 to 40.7%, $P = 0.0001$).

E_2 fell from a mean value of 13.0 pmol/l before treatment to 4.6 pmol/l during treatment (35.6% of control values, 95% confidence interval of 27.8 to 45.5%, $P = 0.0001$), while plasma levels of E_1S decreased from 308 to 75 pmol/l (24.2% of control values, 95% confidence interval of 15.7–37.2%). The arithmetical mean suppression of plasma E_1, E_2 and E_1S was 63.9, 60.8 and 68.5%, respectively.

Plasma samples from 6 patients beginning treatment with 4-OHA in concert with AG, immediately following progression on 4-OHA monotherapy, were available for oestrogen determinations. 5 of these 6 patients experienced a further reduction in plasma E_1, E_2 and E_1S compared to values obtained during 4-OHA monotherapy, with a substantial decrease in plasma E_1S to $< 5\%$ of pretreatment values in 4 of these patients (Table 2).

DISCUSSION

This paper reports clinical and endocrine effects of 4-OHA in a group of heavily pretreated patients. Two of the 34 patients had received one previous antihormone treatment modality only, the others had received 2 or more previous antihormone regiments. Thus, a selection of heavily pretreated patients may explain the low response rate (3 of the 30 patients achieved a PR). However, 11 patients had stable

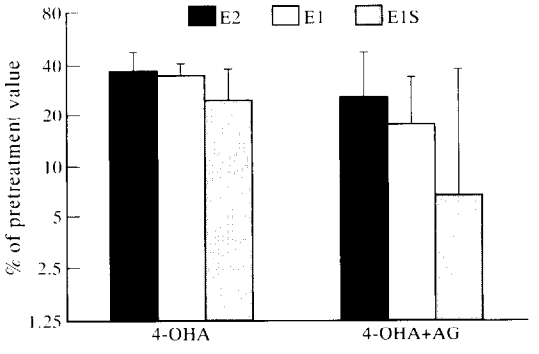

Figure 1. Influence of treatment with 4-OHA alone and in concert with AG on plasma oestrogen levels.

*The clinical response for 2 patients in this trial (1 with a PR and 1 with PD) has previously been reported [19].

Table 1. Effects of treatment with 4-OHA on plasma oestrogens

Patient	Plasma oestrone (pmol/l)			Plasma oestradiol (pmol/l)			Plasma oestrone sulphate (pmol/l)		
	Pretreatment values	4-OHA	% control	Pretreatment values	4-OHA	% control	Pretreatment values	4-OHA	% control
1	54.6	21.5	39.4	6.2	2.1	34.0	288	41	14.2
2	49.3	23.8	48.3	5.2	2.4	46.2	115	21	18.3
3	58.9	32.0	54.3	11.4	6.6	57.9	149	129	86.6
4	46.9	25.4	54.2	5.1	<2.1	41.0	171	48	28.1
5	71.1	22.8	32.1	14.8	2.3	15.5	275	40	14.5
6	168.8	29.3	17.4	43.9	13.2	30.1	7437	88	1.2
7	81.2	21.7	26.7	19.2	4.4	22.9	940	136	14.5
8	43.0	17.7	41.2	35.4	2.8	7.9	109	30	27.6
9	97.6	31.6	32.4	28.5	14.8	51.9	1177	274	23.3
10	74.8	29.0	38.8	14.6	8.8	60.7	577	474	82.1
11	71.3	26.0	36.5	11.8	4.4	37.3	104	32	30.8
12	29.9	11.4	38.1	8.1	5.0	61.7	149	36	24.2
13	51.8	13.4	25.9	14.2	5.4	38.0	391	73	18.7
14	30.4	14.1	46.4	8.8	4.2	47.7	48	13	27.1
15	32.6	13.7	42.0	11.2	5.4	48.2	244	123	50.4
16*	52.0	27.5	52.9	18.1	9.4	51.9	336	137	40.8
17	67.8	13.4	19.8	11.2	2.9	25.9	255	89	34.9
18	89.6	21.6	24.1	15.1	5.4	35.8	439	126	28.7
19	84.1	13.4	15.9	10.6	3.2	30.2	595	191	32.1
Mean	60.3	20.5	33.9	13.0	4.6	35.6	308	75	24.2
95% CI	48.9–74.2	17.4–24.1	28.3–40.7	9.8–17.2	3.5–6.2	27.8–45.5	180–527	48–116	15.7–37.2

95% CI, 95% confidence interval limits. * Premenopausal patient on continuous treatment with goserelin acetate.

Table 2. Plasma oestrogens under treatment with 4-OHA alone and in concert with AG (% of control values)

Patient	Plasma oestrone (pmol/l)			Plasma oestradiol (pmol/l)			Plasma oestrone sulphate (pmol/l)		
	Pretreatment values	4-OHA	4-OHA+AG	Pretreatment values	4-OHA	4-OHA+AG	Pretreatment values	4-OHA	4-OHA+AG
1	54.6	21.5 (39.4%)	10.3 (18.9%)	6.2	2.1 (33.9%)	<2.1 (33.9%)	288.0	40.5 (14.1%)	8.7 (3.0%)
7	81.2	21.7 (26.7%)	9.1 (11.2%)	19.2	4.4 (22.9%)	3.4 (17.7%)	939.7	136.2 (14.5%)	38.8 (4.1%)
9	97.6	31.6 (32.4%)	19.6 (20.1%)	28.5	14.8 (51.9%)	6.7 (23.5%)	1177.0	274.0 (23.3%)	54.0 (4.6%)
10	74.8	29.0 (38.8%)	7.9 (10.6%)	14.5	8.7 (60.0%)	<2.1 (14.5%)	573.4	473.8 (82.6%)	16.4 (2.9%)
11	71.3	26.0 (36.5%)	28.0 (39.3%)	11.8	4.4 (37.3%)	6.0 (50.8%)	104.0	31.5 (30.3%)	83.4 (80.2%)
16*	52.0	27.5 (52.9%)	<6.3 (12.1%)	18.1	9.4 (51.9%)	2.3 (12.7%)	336.0	137.0 (40.8%)	38.0 (11.3%)
Mean	70.2	25.9 (36.9%)	11.7 (16.6%)	14.8	6.1 (40.9%)	3.3 (22.6%)	430.5	120.7 (28.0%)	31.3 (7.3%)
95% CI	54.6–90.3	22.0–30.6 (29.1–46.9%)	6.4–21.3 (9.8–28.2%)	8.6–25.5	2.9–12.6 (28.1–59.6%)	1.9–5.8 (12.9–39.4%)	169.5–1094.4	39.8–365.4 (13.8–56.8%)	13.2–74.4 (1.9–27.7%)

AG, aminoglutethimide; 95% CI, 95% confidence interval limits. * Premenopausal patient on continuous treatment with goserelin acetate.

disease with a median duration of > 13 months. Accordingly, 14 of the 30 patients (46.7%) benefited from treatment with 4-OHA. Of particular interest was the observation that 2 of the 10 patients previously progressing on AG treatment achieved a PR during treatment with 4-OHA, while 3 of the 10 patients achieved a SD. This is in accordance with the observations of others [20] suggesting there may be lack of crossresistance between the two drugs.

We found plasma E_1S to be suppressed to a mean (geometric) value of 24.2% (arithmetical mean of 31.5%) of pretreatment levels during treatment with 4-OHA monotherapy. However, there was substantial variation in the suppression between individuals. 2 patients achieved minor suppression of plasma E_1S of less than 20% during treatment with 4-OHA (patients 3 and 10, Table 1). Both patients had a body weight within the normal range, and neither was exposed to other drugs that might influence plasma oestrogen disposition. One explanation for an inferior response to 4-OHA could be a hormone "escape" phenomenon, in as much as some patients may achieve a rise in their plasma oestrogen levels when approaching 2 weeks from their last injections [21]. However, as the time period between the last injection with 4-OHA and the blood sampling for our 2 patients with a minor suppression of E_1S was 4 and 8 days, respectively, this is not a likely explanation for our findings. One of these patients obtained stable disease while the other did not respond to 4-OHA treatment. When one of these patients received AG in concert, she achieved a pronounced suppression of her E_1S value.

Another possibility is that some patients, for reasons unex-

plained, may have a reduced sensitivity to particular drugs such as 4-OHA. Miller and associates [22], using tumour tissue biopsies, have shown that 4-OHA may have little effect on the tumour aromatase activity in a minority of patients. However, results from studies measuring *in vivo* aromatisation with tracer techniques do not support a hypothesis that some patients may be less sensitive to aromatase inhibitors [13–15].

In this study, 12 of the 19 patients had more than 70% suppression of plasma E_1S with 13 pmol/l as the lowest value recorded. Jones and coworkers [14] found 4-OHA, given in the same drug schedule as used here to inhibit aromatisation of androstenedione to E_1 by a mean value of 85%. Thus, for most of the patients in this study, the percentage of plasma E_1S suppression approached the percentage of aromatase inhibition achieved in the tracer study. This suggests that previous findings of sustained oestrogens at a mean value of 40–50% of pretreatment levels could be due to non-specific crossreactions in the RIAs.

The suppression of plasma E_1 and E_2 was less pronounced and in accordance with previous results obtained by us [23] and others [11]. For E_2, some of the values measured in the on treatment situation was below the sensitivity limit of the method, but this was not the case for E_1. Whether non-specific interactions in the E_1 assay could explain the somewhat smaller suppression of this hormone compared to the suppression of E_1S is not known.

The finding that adding AG to 4-OHA causes further suppression of plasma E_1S is in accordance with previous studies [19]. However, the magnitude of suppression observed in this study, using a highly sensitive RIA, is more pronounced than that previously seen. The substantial reduction in plasma E_1S may be partly caused by enhancement of the metabolic clearance rate of this oestrogen conjugate by AG [12], in addition to the influence on the aromatisation.

We cannot explain why one patient (patient 11, Table 2) experienced an increase in plasma E_1S when AG was added to 4-OHA treatment. As plasma AG levels were not determined, the possibility exists she might not have taken the drug as prescribed. In addition, this patient had a very low initial level of E_1S and should be considered as atypical.

In summary, this study shows that heavily pretreated patients may benefit from treatment with 4-OHA. Evaluation of its effects on plasma oestrogen levels suggests 4-OHA to be more effective suppressing plasma oestrogens and plasma E_1S in particular compared to that which has been suggested from previous studies. The finding of a substantial variation in the degree of plasma E_1S suppression demands further studies to compare plasma oestrogen suppression among responders and non-responders to aromatase inhibitors.

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