

# Dose-related Endocrine Effects and Pharmacokinetics of Oral and Intramuscular 4-Hydroxyandrostenedione in Postmenopausal Breast Cancer Patients<sup>1</sup>

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## ABSTRACT

4-Hydroxyandrostenedione (CGP32349; 4-OHA) is a clinically effective treatment for advanced postmenopausal breast cancer by both the parenteral and p.o. routes, as a result of its inhibition of aromatase and consequent suppression of plasma estrogen levels. Thirty patients were randomized to treatment with 250 mg 4-OHA orally once, twice, and 4 times daily for 2 weeks and 29 of these plus a further 11 patients were then randomized to treatment with 250 or 500 mg i.m. every 2 weeks to determine the optimal dose for each route according to the suppression of serum estradiol levels. There was no significant difference between the 3 oral doses in their suppression of estradiol levels indicating that the maximum required p.o. dose of 4-OHA is probably 250 mg daily. Suppression by the parenteral dose of 250 mg every 2 weeks was marginally suboptimal but clinical considerations of response and tolerability indicate this as the optimal dose for i.m. injection. 4-OHA had no effect on serum levels of androstenedione, testosterone, or 5 $\alpha$ -dihydrotestosterone when given by either route but p.o. treatment with 4 doses of 250 mg daily reduced sex hormone-binding globulin levels by a mean of 34%. Serum levels of estrone as measured by gas chromatography-mass spectrometry were suppressed to approximately 40% of baseline by parenteral treatment. The half-life of 4-OHA p.o. was approximately 3 h, whereas the apparent half-life of injected drug was between 5 and 10 days after a more rapid clearance during the first 4 days after injection.

## INTRODUCTION

Inhibition of aromatase, the enzyme complex which converts androgens to estrogens, was first established as an effective measure in the endocrine treatment of advanced breast cancer in postmenopausal women by the use of aminoglutethimide (1-3). There are a number of disadvantages to the use of this drug. These include its inhibition of some other steroid hydroxylases (4, 5), which necessitates its combination with a glucocorticoid for maximum effectiveness (6) and possibly therapeutic safety (7), and its side effects such as subclinical hypothyroidism in some patients (8) and the frequent occurrence of a skin rash (9). This has led to efforts on the part of many research groups to derive more specific and more potent aromatase inhibitors, which lack significant clinical side effects.

We have previously reported that 4-OHA<sup>3</sup> (Ciba-Geigy, CGP 32349) is such an inhibitor and is the first selective inhibitor to be studied clinically (10, 11). The compound has been characterized as a suicide inhibitor *in vivo* (12) and has been shown to be clinically effective by both the i.m. and p.o. routes (10, 13, 14). Serum estradiol levels are consistently and markedly suppressed by both p.o. and parenteral 4-OHA (11). For the p.o. route daily administration is required to maintain suppres-

sion, whereas a single 500-mg injection led to maximal suppression for over 14 days in 6 of 7 patients. Repeated weekly injections of 500 mg i.m. resulted in persistent estrogen suppression but at this and higher doses sterile abscesses and lumps at the injection site were noted in some patients (13). It was therefore important to determine to what degree the dose of 4-OHA could be reduced without loss of efficacy.

Suppression of estradiol levels has been used to determine the optimum dose by the i.m. route (11). This approach is utilized in the current study to compare the effectiveness of 250 and 500 mg 4-OHA i.m. every 2 weeks, and of 250 mg 4-OHA p.o. once, twice, or four times a day. Pharmacokinetic profiles were obtained for both routes of administration.

We previously reported that estrone levels, as measured by radioimmunoassay after chromatography, were not significantly affected by i.m. 4-OHA treatment (13). To clarify this unexpected result, samples from parenterally treated patients were measured in this study by the definitive technique of GCMS. 4-OHA has no effect on LH, FSH, or SHBG levels when given i.m. at a dose of at least 500 mg weekly (13), but no report has been made of the effects of p.o. 4-OHA on these parameters or of the effects on androgen levels of 4-OHA administration by either route. Both of these effects have been documented in this study.

## MATERIALS AND METHODS

**Patients.** All patients were either postmenopausal (at least 2 years of amenorrhea) or surgically ovariectomized women and all had histologically or cytologically proven progressive metastatic breast cancer. No patient had received endocrine or cytotoxic chemotherapy within 4 weeks of starting treatment. Informed consent was obtained from all patients, and the study was approved by the Ethics Committee at St. George's Hospital. Patients were free to withdraw at any time. Disease response was assessed according to standard Union Internationale Contre le Cancer criteria (15).

**Drug.** 4-OHA was provided by Ciba-Geigy Pharmaceuticals as a sterile microcrystalline formulation (CGP 32349) in ampuls and was stored at 4°C. The powder was suspended in physiological saline (125 mg/ml) immediately prior to i.m. injection and in water or saline (50 mg/ml) for p.o. administration.

**Oral Study.** Thirty patients were randomized from random number tables to receive 250 mg 4-OHA p.o. once, twice, or four times daily for 2 weeks. Four patients were withdrawn from the study because of rapidly progressive disease and they were replaced by four others who were assigned in a random manner to the appropriate groups. The mean ages were 60.2  $\pm$  10.4 (SD), 63.4  $\pm$  10.4, and 68.4  $\pm$  8.4 years and the mean weights were 59.3  $\pm$  6.3, 68.6  $\pm$  20.3, and 65.9  $\pm$  11.9 kg for the once, twice, and four times daily treatment groups, respectively, and were not statistically significantly different between the groups. No treatment was given for at least 7 days after the last p.o. dose of 4-OHA. At least one clotted blood sample was drawn from each patient on the day before starting treatment and a further pretreatment sample was obtained immediately prior to the first dose of 4-OHA. On-treatment blood samples were collected 5, 10, 15, 20, 30, 45, 60, and 90 min; 3, 4, 6, 8, 10, 12, and 24 h; and 2, 4, 7, and 14 days after starting treatment. A further sample was taken 7 days after

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<sup>3</sup> The abbreviations used are: 4-OHA, 4-hydroxyandrostenedione; GCMS, gas chromatography-mass spectrometry; LH, luteinizing hormone; FSH, follicle-stimulating hormone; SHBG, sex hormone-binding globulin; 5 $\alpha$ DHT, 5 $\alpha$ -dihydrotestosterone; 4-OHT, 4-hydroxytestosterone; 4-OHA-G, 4-OHA-glucuronide.



finishing p.o. treatment. The on-treatment blood samples at 6, 12, and 24 h were taken immediately prior to administration of 4-OHA. Samples were unobtainable at one or more time points in some patients, as indicated in the figures.

**Parenteral Study.** Forty patients were randomized into groups and received either 250 mg ( $n = 22$ ) or 500 mg ( $n = 18$ ) i.m. injections of 4-OHA every 2 weeks. The first 29 patients were the same patients that completed the p.o. study which is detailed above. The mean ages were  $60.2 \pm 12.3$  (SD) and  $66.2 \pm 9.3$  years and the mean weights were  $66.2 \pm 14.5$  and  $68.6 \pm 16.7$  kg, for the 250- and 500-mg groups, respectively, and were not statistically significantly different between the groups. The time between finishing the oral study and beginning the parenteral study was between 7 and 14 days for all except one patient who had a 35-day gap. Clotted blood samples were drawn on the day before and on the day of the first injection immediately before the injection was given. Samples were then taken at 1, 2, 4, 7, 10, and 14 days after the first injection and at weekly intervals thereafter. The samples which were taken on the same day as the drug was given were drawn immediately prior to injection. In a number of patients samples were unavailable at some time points.

**Hormone Assays.** The assays for estradiol (11), LH, FSH (16), and SHBG (17) were performed according to methodologies which have been described in detail previously. Androstenedione, testosterone, and  $5\alpha$ DHT were analyzed by radioimmunoassays after ether extraction and thin layer chromatographic purification to avoid cross-reaction with the drug or its putative metabolites. The solvent system used was benzene/ethyl acetate/methanol, 80/20/2, and the support was silica gel on plastic-backed plates. Recovery was monitored and corrected for with tracer quantities of the appropriate tritiated steroids. The radioimmunoassay system for testosterone was the St. Thomas's Hospital (London, United Kingdom) ether extraction, iodinated tracer kit, which uses an antibody raised against a 3-linked conjugate. The androstenedione antibody was raised to an androstenedione-19-carboxymethyl-bovine serum albumin conjugate and was used with an iodinated ligand (both from Radioassay Systems Laboratories, Carson, CA). The antibody to  $5\alpha$ DHT was raised to a 3-linked conjugate (Chelsea Hospital for Women) and was used with a tritiated tracer. After chromatography there was no significant interference in any of the androgen assays when serum was spiked with 300 ng/ml 4-OHA or with 30 ng/ml 4-OHT. In this study 4-OHA levels were measured in over 700 samples. In two samples values were  $>200$  ng/ml, but no values  $>300$  ng/ml were found. There is little information on the metabolism of 4-OHA in humans, but in rhesus monkeys circulating levels of 4-OHT were approximately 15% of those of 4-OHA (18). These data therefore indicate that the assays are not subject to significant interference by circulating levels of 4-OHA or its potential metabolite, 4-OHT. In all assays the within- and between-assays coefficients of variation were less than 9 and 15%, respectively.

Estrone levels were measured by GCMS basically as described previously (19). Some minor modifications were made: extraction of 2 ml plasma with dichloromethane and liquid chromatography was performed on Sephadex LH-20 columns (120 x 4 mm) with hexane/ethanol/acetic acid (80/20/1) as an eluent. The first 4 ml were discarded and estrone was eluted in the next 4 ml. After evaporation to dryness, estrone was derivatized to the trimethylsilyl ether.

Accuracy was checked by standard additions to a plasma pool (10–200 pg/ml range). Linear regression of added ( $x \pm$  SD) on found ( $y$ ) estrone gave the equation

$$y = 1.03(\pm 0.09)x + 12.4(\pm 1.3)$$

The interassay coefficient of variation was 10.4% at the 30-pg/ml level.

Samples from the same patient were analyzed in the same batch for all assays.

**4-Hydroxyandrostenedione Assay.** Levels of 4-OHA were measured with a cross-reacting androstenedione antibody after prior organic extraction and chromatographic purification, as previously described and validated (11), with the minor modification that extraction was performed with 2 doses of 3 ml diethyl ether/dichloromethane (4/1).

4-OHA-G levels were measured after enzymic hydrolysis of the conjugate. Serum (50  $\mu$ l) was extracted with 2 doses of 3 ml diethyl

ether/dichloromethane (4/1) to remove the unconjugated 4-OHA and was then treated with 0.5 mg bovine liver  $\beta$ -glucuronidase (type B-3; Sigma, Poole, United Kingdom) in 500  $\mu$ l acetate buffer, pH 4.5, for 24 h at 37°C. Experiments were conducted which established that these hydrolytic conditions achieved  $>90\%$  of 4-OHA-G. After hydrolysis [ $^3$ H]4-OHA (donated by Dr. R. Wade, Ciba-Geigy, Horsham, United Kingdom) was added (about 1000 cpm) as a recovery control and the mixture was extracted with 2 doses of 3 ml diethyl ether/dichloromethane. Thereafter the technique was identical to that for unconjugated 4-OHA.

**Statistical Tests.** Within- and between-dose comparisons were made applying analysis of variance to the logarithms of the original values. Two-sided tests of significance were applied, with a significance level of 5%. Whenever possible a repeated-measures analysis of variance was applied, with the Greenhouse-Geisser correction for within-dose comparisons; otherwise, within- and between-dose comparisons were tested for significance, in the event of a statistically significant  $F$  test applying the Bonferroni correction.

## RESULTS

**Oral Treatment.** The effect of p.o. 4-OHA on serum estradiol levels is shown in Table 1 for all three doses throughout the 2 weeks of treatment. The effect of doses on estradiol levels on days 7 and 14 of treatment are compared in Fig. 1. The mean levels were significantly reduced within 3 h of starting treatment. After 4 days the mean levels were less than 50% of pretreatment levels in all three groups. During the next 10 days of treatment there was little variability in the degree of suppression, but within 7 days of stopping p.o. therapy the mean estradiol levels returned to within 16% of pretreatment values in all groups. The mean pretreatment estradiol level for the 500-mg group was over 10 pmol/liter higher than those for the other two groups. Although this was not statistically significant it is likely that this may have some effect on the levels during treatment. It is therefore difficult to perform meaningful comparisons with this group. However, comparisons between the lowest and highest dose groups showed no significant difference in suppression at any point during treatment.

The concentrations of 4-OHA in serum were measured for the first 24 h after the first dose of 4-OHA. 4-OHA was detected in 9 of 10 patients at 5 min postdosing. For most subjects the subsequent serum profiles were irregular, with more than one peak or trough in the profile. Maximum serum concentrations ranged from 23 to 235 ng/ml and were reached 0.5 to 4 h postdosing. The mean levels are shown in Fig. 2. Where the elimination phase could be determined the decline in plasma concentrations was adequately described by a monoexponential decay with a half-life of about 3 h. Seven of the patients who had a pharmacokinetic profile had either overt liver metastases or abnormal liver function. The mean level of 4-OHA at 1.5 h after the first dose was  $65.4 \pm 63.5$  (SD) ng/ml in these patients compared with  $55.6 \pm 40.6$  ng/ml in the rest of the group ( $P$ , not significant).

Serum levels of 4-OHA-G were measured in the same 24 patients 90 min after administration of the first dose of 4-OHA. The mean level was  $2.40 \pm 1.07$   $\mu$ g/ml and at the same time point the mean level of 4-OHA was  $55.2 \pm 43.6$  ng/ml. The mean ratio of conjugated to free 4-OHA (determined after conversion to molar equivalents) was  $36.3 \pm 19.8$  (range, 5–96). The coefficients of variation of these three measurements at 90 min were thus 79% (4-OHA), 44% (4-OHA-G), and 55% (4-OHA-G/4-OHA). There was no statistically significant dose relationship between the free and conjugated forms when examined by linear regression analysis ( $r = 0.38$ ,  $0.05 < P < 0.10$ ).



Table 1 Effect of p.o. 4-OHA treatment on serum estradiol levels (mean  $\pm$  SEM) in postmenopausal women

Daily dose of 4-OHA (mg)	Units	Time on treatment										
		Pre	3 h	6 h	12 h	1 day	2 days	4 days	7 days	14 days	7 days post-treatment	
250	pmol/liter	26.3 $\pm$ 3.8	20.4 $\pm$ 2.7	18.6 $\pm$ 2.4	19.4 $\pm$ 2.9	14.4 $\pm$ 2.0	14.6 $\pm$ 3.1	12.2 $\pm$ 1.5	11.1 $\pm$ 1.9	11.6 $\pm$ 1.9	22.3 $\pm$ 3.5	
	% of pretreatment level	100.0	85.9 $\pm$ 6.9	72.2 $\pm$ 4.9	71.1 $\pm$ 6.6	56.0 $\pm$ 4.3	56.0 $\pm$ 12.3	48.0 $\pm$ 6.4	43.7 $\pm$ 4.4	47.0 $\pm$ 6.6	89.8 $\pm$ 9.4	
500	pmol/liter	37.6 $\pm$ 7.8	29.7 $\pm$ 6.4	26.1 $\pm$ 7.6	28.4 $\pm$ 8.2	21.0 $\pm$ 6.3	11.8 $\pm$ 3.8	10.8 $\pm$ 2.0	9.6 $\pm$ 2.0	14.0 $\pm$ 3.0	27.3 $\pm$ 8.7	
	% of pretreatment level	100.0	84.8 $\pm$ 7.4	72.1 $\pm$ 9.9	78.3 $\pm$ 14.7	69.8 $\pm$ 15.0	41.4 $\pm$ 7.9	35.7 $\pm$ 6.6	28.6 $\pm$ 4.9	40.9 $\pm$ 5.1	95.5 $\pm$ 19.4	
1000	pmol/liter	27.2 $\pm$ 5.5	22.3 $\pm$ 5.6	20.5 $\pm$ 3.1	16.8 $\pm$ 2.6	12.4 $\pm$ 2.4	12.0 $\pm$ 2.2	8.7 $\pm$ 1.6	11.0 $\pm$ 1.9	8.3 $\pm$ 1.1	22.5 $\pm$ 4.8	
	% of pretreatment level	100.0	86.8 $\pm$ 5.5	85.3 $\pm$ 8.9	73.0 $\pm$ 8.8	57.2 $\pm$ 11.0	59.8 $\pm$ 13.4	35.5 $\pm$ 8.7	50.4 $\pm$ 10.7	41.5 $\pm$ 8.2	84.5 $\pm$ 14.2	

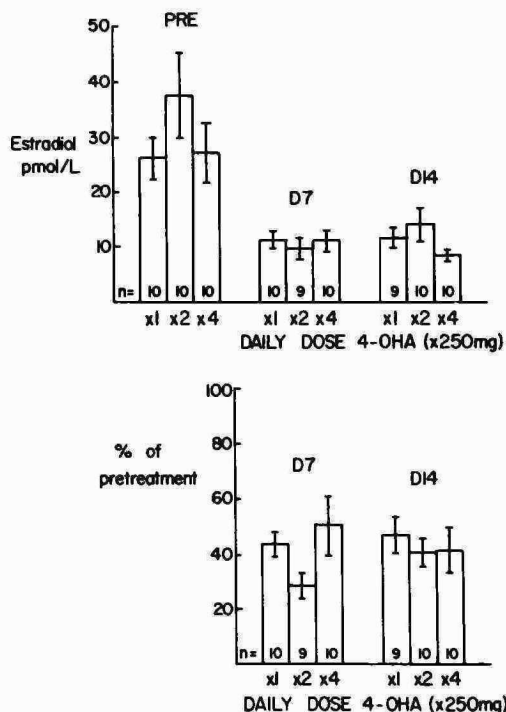


Fig. 1. Effect of 2 weeks of p.o. 4-OHA on mean serum estradiol levels in 30 postmenopausal breast cancer patients. Bars, SEM. Top, estradiol levels expressed as pmol/liter; bottom, estradiol levels expressed as percentage of pretreatment levels. D, day.

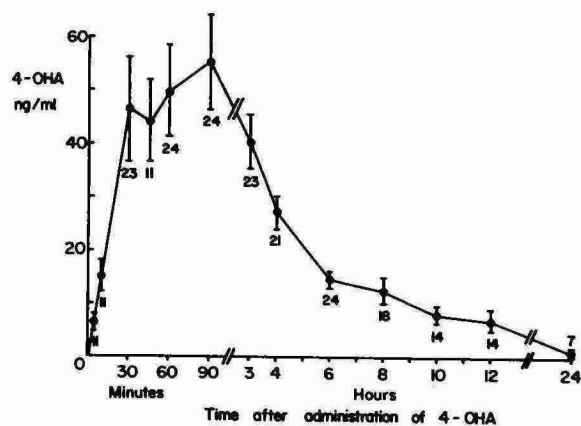


Fig. 2. Mean serum levels of 4-OHA after a single 250-mg p.o. dose. Bars, SEM. The numbers of observations made at each time point are indicated under bars.

The effect of p.o. 4-OHA treatment in LH, FSH, SHBG, testosterone, 5 $\alpha$ DHT, and androstenedione levels is shown in Table 2. There were no significant differences between the groups for any of the analytes before treatment. LH, testosterone, 5 $\alpha$ DHT, and androstenedione levels were unaffected by p.o. 4-OHA treatment. For FSH there was a minor significant increase in levels (by a mean 16% above pretreatment levels,  $P < 0.05$ ) in patients treated with 250 mg twice daily. SHBG levels were significantly suppressed at both the lowest and highest doses, by means of 9.5% ( $P < 0.05$ ) and 34.0% ( $P < 0.01$ ), respectively.

**Parenteral Treatment.** The mean serum levels of estradiol before and during treatment with i.m. 4-OHA are shown for each dose in Fig. 3. Levels were significantly suppressed to approximately 60% of baseline levels for both doses after 24 h. On day 7 the mean ( $\pm$ SEM) levels were  $9.2 \pm 1.5$  pmol/liter (26.5  $\pm$  3.0% of baseline) and  $8.7 \pm 0.9$  pmol/liter (42.4  $\pm$  7.2% of baseline) for the lower and higher doses, respectively. Thereafter, levels were maintained below 50% of baseline throughout the next 9 weeks in both groups. There was no statistically significant difference in estradiol levels between the two doses at any point during treatment. However, the suppression was more variable between time points with 250 mg than with 500 mg. For the lower dose the estradiol levels were significantly higher on day 14 than on day 7 and on day 28 than on day 21. This phenomenon of recovery just prior to the next injection also appeared to occur prior to the fifth and sixth injections but this was not statistically significant.

It can be seen from Fig. 4, in which individual patient values are plotted during the first 4 weeks of treatment, that the recovery of estradiol levels in the 250-mg group was most apparent in those patients with pretreatment values  $>35$  pmol/liter. However, of the 6 patients treated with 250 mg 4-OHA every 2 weeks who showed an objective clinical response to therapy, 3 had pretreatment estradiol levels  $>35$  pmol/liter. Only 1 of these 3 responders had on-treatment estradiol levels which were maintained below 20 pmol/liter. There were 5 patients in the 500-mg group with pretreatment estradiol levels  $>35$  pmol/liter, but there was little evidence of a recovery phenomenon even within that subgroup.

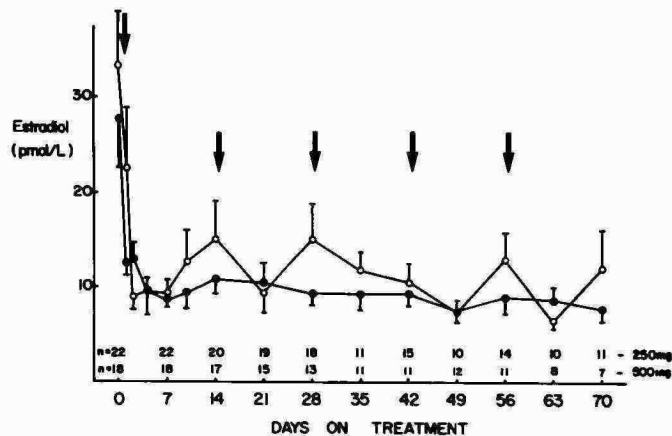
A mathematical estimate of the quantitative importance of the recovery phenomenon in the patients in the 250-mg group with pretreatment estradiol values  $>35$  pmol/liter is made in Fig. 5. The day 7 and day 21 estradiol values are taken as minimal on-treatment levels after the first and second injections and the lines connecting these points with the day 14 and day 28 values, respectively, are taken to represent the intermediate estrogen levels. The nonshaded areas then indicate the degree



Table 2 Effect of p.o. 4-OHA treatment on mean ( $\pm$ SEM) serum level of LH, FSH, SHBG, testosterone (testo), 5 $\alpha$ DHT, and androstenedione ( $\Delta$ 4A) in postmenopausal women

The numbers in parentheses indicate the number of observations. Pre- and on-treatment samples were analyzed for the same patients in all cases.

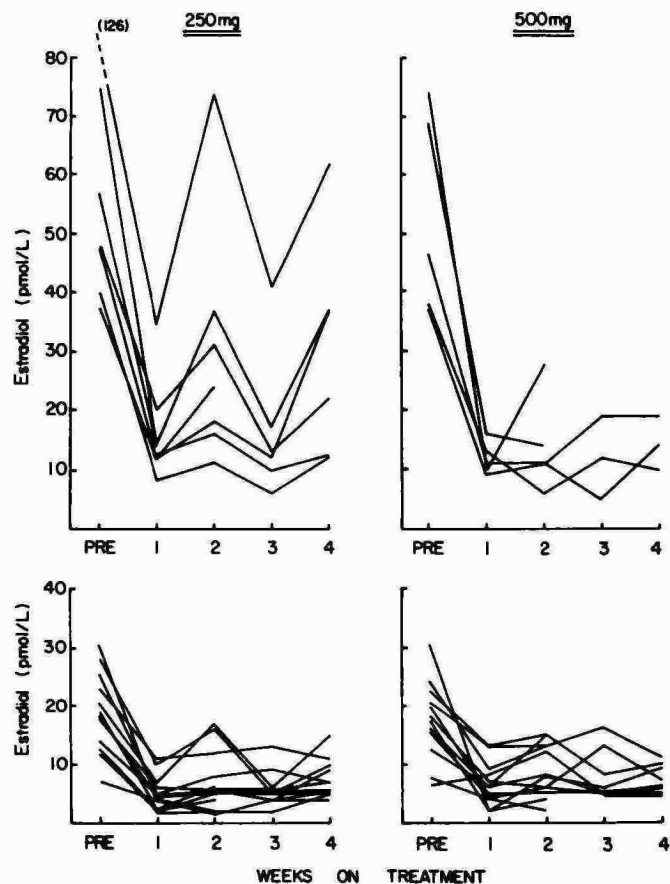
Hormone	Units	250 mg in 1 dose		250 mg twice daily		250 mg 4 times daily	
		Pre	On	Pre	On	Pre	On
LH	IU/liter	27.8 $\pm$ 3.3 (10)	27.9 $\pm$ 4.1	33.1 $\pm$ 6.5 (9)	35.7 $\pm$ 6.3	40.2 $\pm$ 10.5 (10)	35.0 $\pm$ 8.2
FSH	IU/liter	36.8 $\pm$ 3.0 (10)	36.1 $\pm$ 3.5	37.9 $\pm$ 7.6 (9)	42.7 $\pm$ 8.2 <sup>a</sup>	40.6 $\pm$ 9.5 (10)	39.4 $\pm$ 9.2
SHBG	nmol/liter	81.5 $\pm$ 12.2 (10)	74.5 $\pm$ 12.1 <sup>a</sup>	60.0 $\pm$ 9.5 (9)	55.8 $\pm$ 8.7	77.0 $\pm$ 12.4 (10)	46.4 $\pm$ 5.5 <sup>b</sup>
Testo	nmol/liter	1.08 $\pm$ 0.30 (8)	1.18 $\pm$ 0.28	1.15 $\pm$ 0.30 (6)	1.24 $\pm$ 0.25	0.89 $\pm$ 0.20 (5)	1.40 $\pm$ 0.24
5 $\alpha$ DHT	nmol/liter	0.40 $\pm$ 0.06 (10)	0.41 $\pm$ 0.10	0.33 $\pm$ 0.07 (7)	0.31 $\pm$ 0.06	0.40 $\pm$ 0.07 (8)	0.42 $\pm$ 0.07
$\Delta$ 4A	nmol/liter	1.73 $\pm$ 0.28 (10)	1.76 $\pm$ 0.31	1.62 $\pm$ 0.38 (7)	2.08 $\pm$ 0.22	1.94 $\pm$ 0.43 (8)	2.99 $\pm$ 0.59

<sup>a</sup>  $P < 0.05$ .<sup>b</sup>  $P < 0.01$ .Fig. 3. Effect of i.m. 4-OHA every 2 weeks on mean serum estradiol levels in 40 postmenopausal breast cancer patients. Bars, SEM.  $\circ$ , 250 mg;  $\bullet$ , 500 mg. Arrows, time of injection.

of suppression during the second and fourth weeks as being 84.5 and 83.7% maximal. An equivalent estimate for all patients in the 250-mg group gives values of 85.5 and 87.6% maximal suppression, respectively. The subgroup with the higher pretreatment estradiol levels is thus little different from the whole group in this respect.

Twenty-nine of the patients that received p.o. therapy were also included in the parenteral study. A comparison of the estradiol suppression achieved by the two routes is made in Fig. 6. It can be seen that there was little difference in any of the six subgroups after the change from p.o. to i.m. treatment. The subgroups are too small to allow meaningful statistical analysis, but an overall comparison of p.o. versus parenteral treatment showed no statistically significant difference ( $P = 0.18$ ).

After the first injection serum levels of 4-OHA were consistently lower in the 250-mg group than in the 500-mg group [approximately one-half (Fig. 7)]. Peak levels occurred between 1 and 2 days after the first injection, but by day 4 levels had fallen to less than one-half of the peak values. Thereafter the fall in serum 4-OHA levels was slower and approximately log-linear with an apparent half-life of between 5 and 10 days. After the second and subsequent injections serum drug levels were measured at weekly intervals and were found to show an inverted V pattern with lowest levels just prior to the next injection (Fig. 8). For the 250-mg group the mean levels ( $\pm$  SEM) at the midpoint between injections became progressively higher (day 7, 2.4  $\pm$  0.2 ng/ml; day 63, 4.5  $\pm$  0.7 ng/ml). The 10 patients who completed 10 weeks of treatment on 250 mg had similar serum 4-OHA levels during the first month of therapy as the whole group. There was therefore no evidence that a patient selection might be responsible for the progressively higher serum levels.

Fig. 4. Individual serum estradiol levels in patients during the first 4 weeks of treatment with 250 mg (left) or 500 mg (right) 4-OHA i.m. every 2 weeks. Top and bottom, levels in patients with pretreatment serum estradiol levels  $>35$  pmol/liter and  $<35$  pmol/liter, respectively.

Nine patients had either overt liver metastases or abnormal liver function. The mean level of 4-OHA in the five treated with 250 mg was 2.9  $\pm$  0.9 (SD) ng/ml after 7 days and in the four treated with 500 mg was 6.2  $\pm$  6.6 ng/ml. The comparable values of the other patients in the two groups were 2.3  $\pm$  0.9 and 5.6  $\pm$  2.5 ng/ml, respectively. There was no significant difference in the levels according to the presence or absence of liver disease.

The data were examined to determine whether there was any correlation on days 1, 7, 14, 21, and 28 between 4-OHA concentrations and the suppression of estradiol levels, when the latter were expressed as absolute concentrations or as percentages of pretreatment. No significant relationship was found for either dose on any of these days.

Serum levels of estrone were measured by GCMS in 9 patients before and during treatment with either 250 mg ( $n = 5$ )



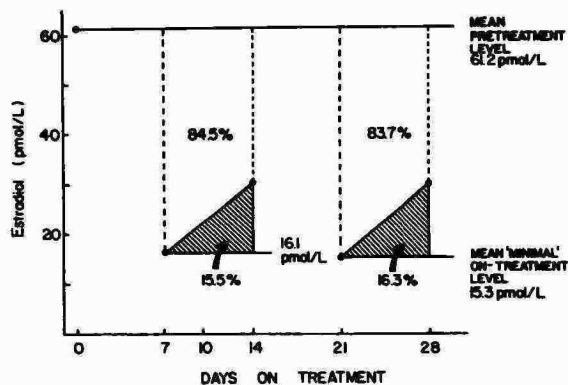


Fig. 5. Estimation of mean quantitative importance of recovery phenomenon in patients treated with 250 mg 4-OHA i.m. every 2 weeks and pretreatment estradiol levels >35 pmol/liter. The points at days 7 and 21 are taken as being the mean minimal on-treatment estradiol level after the first and second injection. ■, degree by which suppression is submaximal during the second week after injection, quantified as a percentage of the oblongs enclosed by the dashed lines.

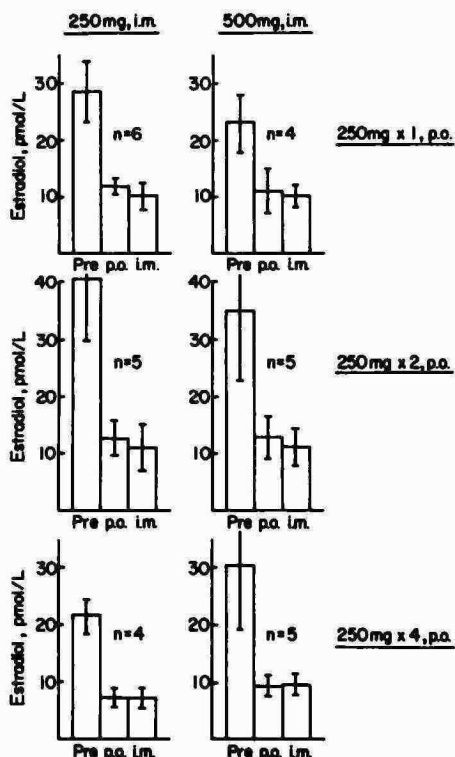


Fig. 6. Comparison of the effect of p.o. and parenteral 4-OHA treatment on mean serum estradiol levels. Bars, SEM. Top, middle, and bottom, patients who received 250 mg in 1 dose, 2 doses or 4 doses p.o. daily, respectively. Left and right, those who transferred to 250 or 500 mg i.m. every 2 weeks, respectively.

or 500 mg 4-OHA every 2 weeks. On-treatment levels were made after at least 21 days of therapy (range, 21–36 days). Levels were suppressed in all patients, the mean fall being from  $82.3 \pm 10.3$  [SEM] to  $33.0 \pm 9.2$  pmol/liter, i.e., to a mean  $40.3 \pm 7.0\%$  of baseline [ $P = 0.001$  (Fig. 9)]. The serum estradiol levels in these same patients were suppressed to a mean 29.4% of baseline at the same time points. There was no statistically significant difference in any of the androgen levels between samples taken prior to treatment and those taken on day 21 of i.m. therapy with either dose of 4-OHA (Table 3).

DISCUSSION

Endocrine treatment is the favored first line therapy for breast cancer in tumors identified as estrogen receptor positive, largely

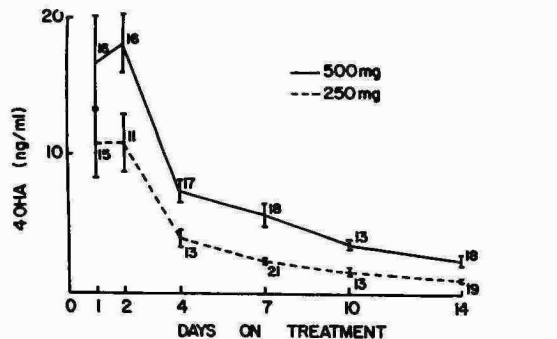


Fig. 7. Mean serum levels of 4-OHA after a single i.m. injection of 250 mg (---) or 500 mg (—) 4-OHA. Bars, SEM. The numbers of observations made at each time point are indicated alongside the bars.

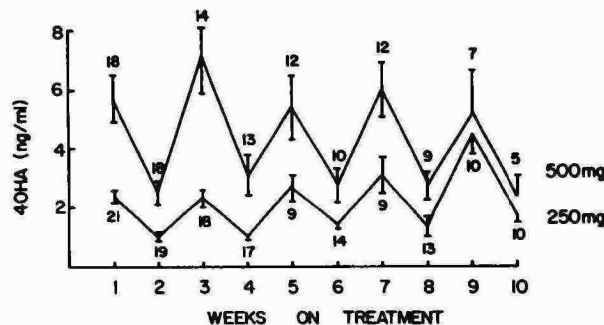


Fig. 8. Mean serum levels of 4-OHA 7 and 14 days after repeated 2 weekly i.m. injections with 250 or 500 mg 4-OHA. Bars, SEM. The numbers of observations made at each time point are indicated alongside the bars.

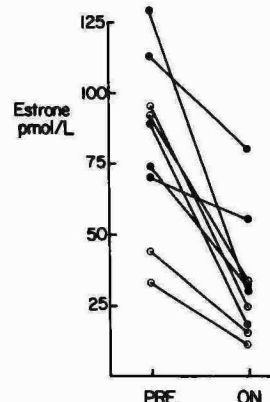


Fig. 9. Effect of 4-OHA i.m. every 2 weeks on serum estrone as measured by GCMS. ●, 250 mg; ○, 500 mg.  $t$  test,  $P = 0.001$ .

Table 3 Effect of i.m. 4-OHA treatment on mean ( $\pm$ SEM) serum levels of testosterone, androstenedione ( $\Delta 4A$ ), and 5 $\alpha$ DHT (nmol/liter)

On-treatment levels were measured on day 21 of therapy. The numbers in parentheses indicate the number of observations.

Hormone	250 mg		500 mg	
	Pre	On	Pre	On
Testosterone	$0.78 \pm 0.09$ (10)	$0.75 \pm 0.07$	$1.35 \pm 0.29$ (9)	$1.09 \pm 0.17$
5 $\alpha$ DHT	$0.38 \pm 0.06$ (10)	$0.38 \pm 0.04$	$0.41 \pm 0.05$ (9)	$0.41 \pm 0.07$
$\Delta 4A$	$1.67 \pm 0.26$ (10)	$1.97 \pm 0.40$	$1.87 \pm 0.35$ (9)	$1.59 \pm 0.27$

as a result of the low toxicity of the medical regimens now available. Aromatase inhibition with aminoglutethimide achieves a similar profile in terms of clinical efficacy to the antiestrogen tamoxifen (20, 21), which has become the benchmark for new endocrine treatments, but aminoglutethimide has significant side effects (8, 9) and requires concurrent glucocorticoid administration for maximal effectiveness (6). Our earlier

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