Use of the Aromatase Inhibitor 4-Hydroxyandrostenedione in Postmenopausal Breast Cancer: Optimization of Therapeutic Dose and Route


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ABSTRACT

4-Hydroxyandrostenedione (4-OHA) is a potent inhibitor of estrogen production by aromatase and causes suppression of plasma estradiol levels and disease regression in postmenopausal breast cancer patients. Groups of patients were given p.o. or parenteral 4-OHA, and plasma estradiol and 4-OHA levels were measured to enable the delineation of the minimal effective dose and optimal therapeutic regimen. A single injection of 500 mg i.m. suppressed estradiol levels to a mean 36.3 ± 3.3% (SE) (n = 14) of base line after 4 to 7 days and maintained this suppression in six of seven patients for >14 days. The half-life of 4-OHA was approximately 8 days, and when the level had fallen to less than 3 ng/ml, estradiol levels began to rise. Similar suppression was achieved by a single i.m. injection of 125 mg of 4-OHA and by 500 mg of 4-OHA p.o. daily after 1 wk, but escape from suppression was more rapid.

INTRODUCTION

It is widely accepted that estrogen deprivation is the major mechanism by which both medical and surgical endocrine treatment of breast cancer is effective (1). One means by which this can be achieved is by reduction of estrogen production, and during the last few years, we have developed a number of selective inhibitors of aromatase (estrogen synthetase) (2). Several of these appear to act by both competition with the substrate and inactivation of the enzyme. The latter type of activity has been termed suicide inhibition, and 4-OHA is the most potent inhibitor of this type that we have evaluated (3, 4). Another drug, AG, inhibits the peripheral aromatase enzyme system and reduces plasma estradiol levels in postmenopausal women (5), and the clinical effectiveness of AG in postmenopausal breast cancer patients supports the concept that aromatase inhibition is a viable approach to the treatment of breast cancer (6, 7). The inhibition of aromatase by AG is dependent on its interaction with cytochrome P-450 (8) which results in suppression in six of seven patients for >14 days. The half-life of 4-OHA was approximately 8 days, and when the level had fallen to less than 3 ng/ml, estradiol levels began to rise. Similar suppression was achieved by a single i.m. injection of 125 mg of 4-OHA and by 500 mg of 4-OHA p.o. daily after 1 wk, but escape from suppression was more rapid.

4-OHA is a more potent inhibitor of aromatase in vitro than AG (13) and has been shown to inhibit ovarian estrogen synthesis in rats (13, 14) and peripheral aromatization in rhesus monkeys (15). The additional observation that 4-OHA inhibits the growth of estrogen-dependent mammary tumor growth in rats (13, 14) has led to the clinical trial of 4-OHA in postmenopausal breast cancer patients, the first use of a suicide inhibitor of aromatase in humans. We have demonstrated that the drug is clinically effective in these patients and that it leads to marked and prolonged suppression of estradiol levels at a dose of 500 mg weekly given by i.m. injection (16). The local side effects and relative inconvenience associated with this route of administration have led us to examine the use of lower doses and less frequent injections of 4-OHA, as well as the use of p.o. administration. In these studies plasma estradiol levels have been quantified to provide a measure of peripheral aromatase inhibition and, in combination with 4-OHA levels, have been used to determine the minimal effective circulating level of 4-OHA. In this way an approach has been made to delineate the optimal therapeutic regimen while avoiding the large clinical trials which continue to be used to optimize the use of established endocrine treatments for breast cancer.

MATERIALS AND METHODS

Patients. All patients were either postmenopausal (at least 2 yr of amenorrhea) or surgically ovariectomized women who had histologically or cytologically proven progressive metastatic breast cancer. No patient had received endocrine or cytotoxic chemotherapy within 4 wk of starting treatment. Informed consent was obtained for all patients, and the study was approved by the Royal Marsden Hospital's Ethics Committee, the Office for Protection from Research Risks, NIH, and Human Volunteers Research Committee, University of Maryland. Patients were free to withdraw from the study at any time.

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. In patients receiving chronic parenteral therapy the injection sites were varied to minimize local side effects.

Nine studies were conducted in a total of 55 patients, 5 of whom were included in 2 studies. The 5 patients from Study 1 were included in a previous publication (16). The details of the studies are given in Table 1, although in a few instances there were minor variations from the intended schedule of venipuncture. Blood samples were taken into heparinized containers at the same time of day before and during treatment for each patient, and the plasma was separated and stored frozen at −20°C until analysis. All samples from the same patient were included in the same assay batch.

Estradiol Assay. Plasma estradiol concentration was measured by radioimmunoassay using a highly specific rabbit antiserum which had been raised against an estradiol-6-carboxymethylxime-bovine serum albumin conjugate (EIR, Wurenlingen, Switzerland) and estradiol-6-carboxymethylxime-[2,13H]iodohistamine (~2000 Ci/mmole; Amer sham International, United Kingdom).

Duplicate samples (200 μl) of plasma were extracted with 3 ml of diethyl ether which had been freshly purified on an alumina column. Recovery was greater than 90% in all cases (n = 10) when assessed by the addition of [3H]estradiol. Results were therefore not routinely corrected for recovery. The sensitivity of the assay was defined as 0.76 pg/ml by calculation from the 95% confidence limits of the zero standard. The solvent blank was 0.74 ± 0.38 pg/ml (mean ± SD of duplicate blank estimates from 11 assays) and was not deducted from the results (except for Study 1 when blank = 2.2 pg/ml). Four charcoal-stripped postmenopausal plasma samples gave results of 0.38, 0.44, 0.49, and 0.54 pg/ml in an assay with a solvent blank of 0.33 pg/ml. Within-assay variability was assessed in 5 assays, using in each case 5 replicates of different pools which were randomized in the assay and

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1To whom requests for reprints should be addressed.

2The abbreviations used are: 4-OHA, 4-hydroxyandrostenedione; AG, aminoglutethimide.

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was in these latter fractions. The eluate was dried and reconstituted in
and less than 1% between 2.8 and 3.6 ml, while 76% of [3H]4-OHA
were used throughout. Overall recovery of [3H]4-OHA varied between 25 and 50%. The
analyzed blind. The pools gave mean estradiol levels of 10.9, 8.9, 8.7,
7.3, and 3.9 pg/ml with respective coefficients of variation of 9.9, 5.1,
14.3, 9.7, and 4.0%, giving an overall within-assay coefficient of varia-
tion of 9.4%. The between-assay coefficient of variation was 12.8% in
a pool having a mean estradiol level of 7.4 pg/ml (n = 10).

Cross-reaction with estrone was 0.03% and with 4-OHA was <1 ×
10⁻⁸%. The validity of the assay was also assessed in a study of paral-
lelism in samples taken from patients treated with 4-OHA. Ether extracts of 8 ml of plasma from 7 patients on chronic 4-OHA therapy
(500 mg i.m. weekly for at least 2 mo) were made, and the reconstituted
extracts were subjected to double dilutions. This allowed for each
sample the analysis of 6 dilutions which were all above the sensitivity
limit of the assay. The data were linearized using a 2-parameter log-
logit transformation. The standard curve and all samples gave r² >
0.99. The standard curve had a slope of -1.78, and the samples had a
mean slope (±SD) of -1.61 ± 0.40.

4-Hydroxyandrostenedione Assay. 4-OHA was found to have a 25%
cross-reactivity in a previously described radioimmunoasay for andro-
stenedione (17). Use was made of this cross-reaction to measure plasma
4-OHA levels after chromatographic separation from androstenedione
using the antibody against androstenedione (sheep anti-androstenedi-
one-7a-carboethythioether-ovalbumin; HP/S/665/1A; Guildhay, Sur-
rey, United Kingdom) and [1,2,6,7-3H]androstenedione (100 Ci/mmol;
Amersham International, Amersham, United Kingdom). Approximately
1000 cpm of [6,7-3H]4-OHA (18) which had been freshly puri-
ified chromatographically were added to 500 µl of plasma as a recovery
control. The plasma was extracted with diethyl ether (3 × 3 ml, giving
a mean recovery of 75%), and the combined extracts were dried. Residues were redissolved in 2 × 75 µl of trimethylpentane:isopropanol
(1:5) and were applied to 18-cm columns of Lipidex 5000 (Packard
Instruments, Caversham, United Kingdom) in 2-ml disposable glass
pipets (John Poulten, Barking, United Kingdom). Fractions between
2.8 ml and 3.6 ml were collected from the columns on elution with
trimethylpentane:isopropanol (1:5). Preliminary studies had shown that
91% of [3H]androstenedione eluted in fractions between 2 and 2.6 ml,
and less than 1% between 2.8 and 3.6 ml, while 76% of [3H]4-OHA
was in these latter fractions. The eluate was dried and reconstituted in
phosphate-buffered saline. Two × 100 µl were taken for radioimmu-
noassay and 200 µl for recovery estimation. Analytical grade solvents
were used throughout.

Overall recovery of [3H]4-OHA varied between 25 and 50%. The
sensitivity of the assay was 0.3 ng/ml, and this was not significantly
improved by using [3H]4-OHA as tracer ligand rather than [3H]andro-
stenedione. Analysis of buffer and a plasma pool, to which 4-OHA had
been added to give concentrations of 0, 2, and 10 ng/ml, yielded mean
results for buffer of <0.3, 2.1, and 11.3 ng/ml and for plasma of 0.7,
3.2, and 12.7 ng/ml, respectively. The within- and between-assay coef-
ficients of variation were 14.1% (n = 16) and 18.0% (n = 7), respectively,
at a 4-OHA concentration of 10 ng/ml.

Statistical Analyses. Comparisons were performed using paired and
unpaired Student's t test or the Mann-Whitney nonparametric test
when comparisons were made of data expressed as a percentage of base
line. In no case were multiple comparisons made using the same data,
which would have required a Bonferroni correction.

RESULTS

Estradiol Levels. The levels of estradiol in 7 patients who
received a single injection of 4-OHA (500 mg i.m.) and were then studied for 4 wk (Study 3) are shown in Fig. 1 as percent-
ages of the pretreatment level. The mean pretreatment level of
estradiol was 11.6 ± 3.4 pg/ml, and the mean (±SE) suppres-
sion was to 33.6 ± 2.1%, 39.4 ± 4.6%, 50.6 ± 7.7%, and 53.6
± 7.7% of the pretreatment level during the first, second, third,
and fourth wk, respectively. Estradiol levels were reduced to
less than 50% of base line in all patients, and only one (Patient
A, who had a high pretreatment estradiol level: 31 pg/ml)
did not show any escape from this suppression within the first 2 wk.
Thereafter estradiol levels began to rise in an increasing number
of the patients.

Data from Studies 1 to 3 on the estradiol suppression achieved by a single 500-mg injection of 4-OHA have been pool-
ed and are shown in Fig. 2 in comparison to the suppression
found with a single 125-mg injection (Study 4). The suppression
of estradiol was statistically significant 1 day after injection in

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Table 1 Details of studies performed

<table>
<thead>
<tr>
<th>Study</th>
<th>Route of administration</th>
<th>Dosage</th>
<th>Schedule of postadministration blood sampling</th>
<th>No. of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>i.m.</td>
<td>Single 500 mg</td>
<td>1, 3, 5, 7 h, Days 1 to 7</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>i.m.</td>
<td>Single 500 mg</td>
<td>Every 1 to 3 days for 14 days</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>i.m.</td>
<td>Single 500 mg</td>
<td>Wk 1 and 2, every 1 to 3 days; wk 3 and 4, twice weekly</td>
<td>7</td>
</tr>
<tr>
<td>4</td>
<td>i.m.</td>
<td>Single 125 mg</td>
<td>Wk 1 and 2, every 1 to 3 days; wk 3, twice weekly</td>
<td>6</td>
</tr>
<tr>
<td>5</td>
<td>i.m.</td>
<td>500 mg weekly</td>
<td>Weekly, just prior to next injection</td>
<td>11</td>
</tr>
<tr>
<td>6</td>
<td>p.o.</td>
<td>Single 500 mg</td>
<td>Day 1 or 2; Day 5, 6, or 7</td>
<td>3</td>
</tr>
<tr>
<td>7</td>
<td>p.o.</td>
<td>Single 1500 mg</td>
<td>Day 1, 2, or 3; Day 7: at least twice in wk 2</td>
<td>4</td>
</tr>
<tr>
<td>8</td>
<td>p.o.</td>
<td>500 mg daily for 7 days</td>
<td>1, 3, 4 to 6, and 24 h</td>
<td>5</td>
</tr>
<tr>
<td>9</td>
<td>p.o.</td>
<td>Single 250 mg</td>
<td>Weekly</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>p.o.</td>
<td>250 mg daily for 1 mo, transferring to 500 mg daily for 1 mo</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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Fig. 1. Estradiol (E2) levels, expressed as a percentage of pretreatment levels, in 7 patients receiving a single 500-mg i.m. injection of 4-OHA on Day 0 (Study 3). The letters identify the same patients as those in Fig. 5.
patients on both dosage levels ($P < 0.001$ for both groups). There was further suppression of the mean level for the 500-mg group during the next 6 days, but this was only statistically significant between Days 4 and 7 ($P = 0.04$). Thereafter the mean level in the 500-mg group rose, and by the fourth wk after injection, this was significantly higher than the mean level on Days 4 to 7 ($P = 0.025$). Within the group treated with 125 mg of 4-OHA, there was no statistically significant difference in the suppression of estradiol between Day 1, Days 2 to 7, and Days 8 to 14. The only significant difference between the 2 doses in the degree of suppression achieved was between Days 8 and 14 ($P < 0.005$; Days 2 to 7, $P = 0.10$).

To determine whether there was any increase in the degree of estradiol suppression on giving the second and subsequent injections of 500 mg of 4-OHA, samples were taken from 11 patients throughout the first 2 mo of their treatment with weekly 500-mg injections (Study 5; Fig. 3). The mean pretreatment level of estradiol in this group was 8.8 ± 1.7 pg/ml, and this was decreased to a mean 37.1 ± 4.9% of base line by the first injection. There was no significant difference in the degree of suppression during the subsequent 7 wk on treatment.

In the 3 patients who were given a single 500-mg dose of 4-OHA p.o. the mean plasma estradiol level was 60.0 ± 15.7% of base line between 1 and 2 days after ingestion and 68.3 ± 18.0% after 5 to 7 days. In neither case was this a statistically significant suppression. The comparable figures for the 4 patients taking a single 1500-mg dose were 52.3 ± 8.2% ($P = 0.03$ versus base line) and 84.3 ± 4.2% ($P = 0.11$ versus base line), respectively.

Progressive suppression of estradiol levels was found in 6 patients during the 7 days of their treatment with p.o. 4-OHA, 500 mg daily (Study 7; Fig. 4). After 7 days the mean suppression was to 44.5 ± 7.9% of base line ($P < 0.001$ versus pretreatment) which was not significantly different from the suppression between Days 4 and 7 in the patients receiving 500 mg i.m. (36.3 ± 3.3; $n = 14$; $0.10 > P > 0.05$). After a further 2 to 3 days (i.e., Days 9 and 10) the suppression was 66.7 ± 9.0%, which was still a significant reduction on pretreatment levels ($P < 0.02$).

Nine patients received 250 mg p.o. 4-OHA daily for 1 mo and then 500 mg for a further month (Study 9). The first on-treatment blood sample was taken after 7 days. There was no indication that there was increased suppression of estradiol after the first 7 days on each dosage. To allow a comparison between dosages the mean value of estradiol as a percentage of pretreatment was therefore determined for each patient at each dose level. There was no significant difference between the degree of suppression found on the 2 dosages (250 mg: 58.3 ± 6.1% of pretreatment; 500 mg, 50.8 ± 7.9%).

4-Hydroxyandrostenedione Levels. 4-OHA levels were measured in samples from 6 of the 7 patients, who received a single 500-mg injection and were followed for 4 wk (Study 3, insufficient samples on the seventh patient). These levels are shown, and the days on which the estradiol levels returned to above 50% of pretreatment level are marked in Fig. 5. The estradiol levels in Patient F, who had the highest drug levels throughout, did not return to above 50% of base line during the 4 wk. Patient A was the only patient to show any escape from estradiol suppression within 2 wk of injection and had plasma levels of 4-OHA of less than 1 ng/ml by Day 10. Estradiol levels rose above 50% of base line in the other 4 patients at a time when their 4-OHA level was between 1 and 3 ng/ml.
Levels of 4-OHA were also measured during the first 24 h after the p.o. administration of a single 250-mg dose to 5 women (Study 8). The highest levels were found after 1 to 3 h with a mean peak level of 44.6 ± 7.3 ng/ml. Samples were available in only 4 of the subjects after 24 h. At that time levels were undetectable (< 0.3 ng/ml) in 3 of these and 0.8 ng/ml in the fourth.

DISCUSSION

Aromatase inhibition has been demonstrated to be an effective approach to the endocrine treatment of advanced breast cancer in postmenopausal women by the use of aminogluthimide (19). However, aminogluthimide has a number of toxic side effects (20) and is used in combination with glucocorticoid to achieve maximal estrogen suppression (11) and therapeutic safety (12). 4-OHA was selected as the most potent from over 200 potential aromatase inhibitors (4, 14), being approximately 60 times more potent in vitro than aminogluthimide (13). 4-OHA acts as both a competitive reversible inhibitor as well as an irreversible suicide inhibitor (3). We have reported that, at a dose of 500 mg i.m. weekly, 4-OHA was associated with marked suppression of plasma estradiol levels and objective clinical responses (16). No effects on serum gonadotrophin, sex hormone binding globulin, or dehydroepiandrosterone sulfate were found, and somewhat surprisingly, although estrone levels were reduced they were not significantly different from pretreatment levels (21). This is despite the inhibition by 4-OHA of aromatization to estrone in rhesus monkeys as measured by conversion of radiolabeled androstenedione (15). The clinical effectiveness, low toxicity, and lack of any observed endocrine side effects led to the current pharmacokinetic studies in small groups of patients to determine the optimum therapeutic dose, route, and scheduling of 4-OHA administration. The defined aim was to determine the lowest dose of 4-OHA which achieved maximal and maintained estradiol suppression via the most acceptable route.

We have previously demonstrated that a single dose of 500 mg i.m. maintained estradiol suppression at between 40 and 50% of base line in 5 patients for 7 days (16). In this report it has been shown that the suppression achieved during that first week is maximal; i.e., no further suppression occurs with further treatment (Fig. 3), and additionally, that if no further treatment is given this degree of suppression is maintained for at least 2 wk in 6 of 7 patients. The action of the drug as an irreversible inhibitor of aromatase may contribute to this long-lasting suppression, but the relatively rapid escape from suppression shown in the p.o.-treated patients, in whom we have shown that 4-OHA is cleared from the circulation in 24 h, suggests that the maintained suppression in the patients given injections is largely due to the slow clearance of 4-OHA (the half-life in Patients B to F appears to be about 8 days) rather than suicide inhibition. It seems likely that this slow clearance is due to the formation at the injection site of a depot of 4-OHA from which the drug is slowly released.

Overall the data from the patients on 500 mg i.m. suggest that, for the majority of patients, a dose of 500 mg weekly as previously used (16) is unnecessarily high for the maintenance of maximal estradiol suppression. The apparent half-life of 8 days of the drug suggests that more than 50% of the drug may remain after 7 days and be present therefore prior to injection in a weekly schedule. Since a lower dose or less frequent injection schedule might adequately maintain estradiol suppression and may result in a reduced incidence and severity of local side effects (21), the effect of a single injection of 125 mg i.m. on estradiol levels was examined. The suppression achieved during the first week was similar to that in patients receiving 500 mg i.m., but escape was more rapid. Studies of estradiol suppression achieved by 125 mg at weekly intervals and 250 mg at 2-weekly intervals are to be conducted in a larger group of patients, as a further approach to dose optimization.

An important finding in the current report was the observation that 4-OHA given p.o. can also cause marked suppression of estradiol levels despite the rapid glucuronidation of the 4-hydroxyl group by first-pass liver metabolism (22) which is probably largely responsible for the essentially total clearance of 4-OHA from the circulation within 24 h of administration. It is clear that suppression is incomplete after single dosage and during the first few days of daily ingestion of 500 mg of 4-OHA, but there was a progressive fall which after 7 days approached that seen in patients on the parenteral regimen. The action of 4-OHA as a suicide substrate is probably significant in determining the cumulative effect of p.o. therapy. There is a suggestion from the results of Study 9 that chronic p.o. treatment with 500 mg daily achieved greater suppression than 250 mg. This was not statistically significant, however, and further experimentation is required to determine the optimal p.o. dosage in terms of estradiol suppression prior to studies of clinical efficacy and toxicity.

The radioimmunoassay of 4-OHA used an antiserum which was raised to androstenedione rather than to 4-OHA, and the potential cross-reactions of some endogenous steroids and metabolites of 4-OHA were therefore probably greater than if a homologous antiserum was used. While cross-reactions were minimized by chromatography, knowledge of the metabolism of 4-OHA is incomplete, and therefore some cross-reaction with coeluting unknown metabolites cannot be excluded. However, within these limits the assay has allowed estimates to be made of the half-life of 4-OHA after i.m. administration (see above) and of the minimal plasma level of 4-OHA needed to achieve maximal estradiol suppression. That a relationship exists between circulating drug levels and the effectiveness of aromatase inhibition is supported by the observation that the patients who had the fastest and slowest clearance of the drug had the fastest and slowest escape from estradiol suppression, respectively. The results shown in Fig. 5 suggest that, for the majority of patients, escape from maximal suppression does not occur until levels fall below 3 ng/ml.

In conclusion, by a detailed study of small groups of patients, we have made an approach to the definition of the optimal therapeutic route, dose, and scheduling of 4-OHA without the need for large-scale clinical trials which have accompanied the development of some other agents for breast cancer treatment. It has been demonstrated that the dose of 500 mg i.m. weekly, which has been shown to be clinically effective, is probably higher than that required to maintain maximal estradiol suppression. The assay in parallel of levels of estradiol and 4-OHA has enabled an estimate of 3 ng/ml to be made as the minimal effective plasma level of 4-OHA. Confirmation of these data should allow a rapid and logical approach to dose optimization. The p.o. activity of 4-OHA may enable the development of a more convenient form of administration.

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