Endocrine effects of low dose aminoglutethimide alone in advanced postmenopausal breast cancer

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Summary The site of action of aminoglutethimide (AG) has been investigated. An initial study was performed on 10 postmenopausal patients with advanced breast cancer who had taken 1000 mg AG per day and 20 mg hydrocortisone (HC) twice daily (b.d.) for >3 months. There was a 15.5 ± 6.6 s.e.-fold rise in 17-OH progesterone and a 4.9 ± 0.9 s.e.-fold rise in Δ4 androstenedione but no rise in cortisol or oestrone 30 min after short Synacthen tests. These results suggested that peripheral aromatisation was a more important site of AG action than adrenal desmolase, and that adrenal 11β hydroxylase was inhibited. Since aromatase is more sensitive than desmolase to AG in vitro, lower doses of AG alone (i.e. without HC) were assessed for endocrine effects in 13 further post-menopausal women with advanced breast cancer. All of these patients tolerated 125 mg AG b.d., but 3 could not tolerate the conventional maximum dose. Oestrone levels on 125 mg AG b.d. were suppressed below pretreatment levels and were not significantly different from those on 500 mg AG b.d. alone, or with the addition of HC. Oestradiol levels were suppressed to a similar extent. Dehydroepiandrosterone sulphate (DHA-S) levels were not suppressed by AG alone, but fell on addition of HC. The endocrine results show low dose AG alone is an effective and well tolerated inhibitor of the peripheral production of oestrogens in postmenopausal patients. Therapeutic trials are now possible. DHA-S is not a marker of AG effect.

Aminoglutethimide (AG) in combination with hydrocortisone (HC) is an effective endocrine therapy in advanced postmenopausal breast cancer, producing a response rate and duration similar to tamoxifen (Smith et al., 1981). AG was introduced into the treatment of breast cancer as an inhibitor of adrenal steroid production. One site of action is the earliest step in the adrenal conversion of cholesterol to pregnenolone (20,22 desmolase) (Dexter et al., 1967). AG treatment regimes are currently designed to inhibit this step and HC is added in replacement doses to prevent a reflex rise in ACTH secretion (Santen et al., 1974). However, AG has another site of action, the inhibition of the conversion of androgens to oestrogens in peripheral tissues tissues (Santen et al., 1978) (Figure 1), which is the main source of oestrogen in the post-menopausal woman (Grodin et al., 1973). AG can also inhibit 11β-hydroxylase (Faglia et al., 1971).

One of the factors limiting the use of AG is the side effects that occur at conventional dose levels (250 mg 4 times a day). In one series of 190 patients, 58% had transient side effects, 9.5% needed to reduce the dose and 5% discontinued the drug (Harris et al., 1982). Similar results are reported by Santen et al. (1977). Graves & Salhanick (1979) showed that aromatisation in vitro is at least 10 times more sensitive than desmolase to inhibition by AG. We have therefore studied the site of action of AG in postmenopausal patients with advanced breast cancer receiving AG and HC therapy in conventional doses and investigated the endocrine effects of low doses of AG alone.

Patients and methods

Synacthen tests

Ten postmenopausal patients with advanced breast cancer, who were taking AG 250 mg 4 x daily and hydrocortisone 20 mg b.d. (8am, 8pm), were studied after 3 months of therapy. Tetracosactrin (250 μg; Synacthen, Ciba) was given i.m. in the gluteus maximus. The patient was resting before the injection and for 30 min afterwards. Blood samples were taken before and 30 min after the injection.

These tests were performed to assess the need for cortisol replacement during stress, but oestrone, dehydroepiandrosterone sulphate (DHA-S), Δ4 androstenedione and 17 OH progesterone were also measured.

A normal cortisol response was considered to be an initial cortisol level > 138 nM L⁻¹ and a rise of ≥ 200 nM L⁻¹, with a plasma level of ≥ 500 nM L⁻¹ at 30 min, irrespective of initial levels.

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Sites of action of aminoglutethimide

![Diagram of hormone pathways](image)

**Figure 1** Sites of action of aminoglutethimide. 20,22 desmolase (DE); 3 βol dehydrogenase, Δ4-Δ5 isomerase (3b); aromatase (AR) = means inhibition by aminoglutethimide. 17α OH progesterone is converted to 11 deoxycorticisol by 21 hydroxylase; 11 deoxycorticisol is converted to cortisol by 11β hydroxylase.

**Low dose aminoglutethimide alone**

Thirteen postmenopausal patients (not the 10 already described) with progressive advanced breast cancer were studied. Ten were spontaneously menopausal. Nine had been given previous endocrine therapy with 3 partial responses and 2 patients showing disease stabilisation. Two had received adjuvant chemotherapy. Their ages ranged from 37-76 yr (median 58 yr). The last menstrual period was from 1 to 15 yr previously (median 10 yr). The tumour free interval ranged from 0-12 yr, median 20 months. Sites of disease were soft tissue (4), pleura (4), bone (5), nodes (3), lung (1) and ascites (1). Weight ranged from 47-79 kg (median 65 kg).

Each of the patients had a blood sample taken between 9.30 and 11 am before the start of treatment with AG, after they had been off any other endocrine therapy for at least 1 month. They then started treatment with AG 125 mg b.d. (8 am, 8 pm) for one week. For the second week the dose was doubled to 250 mg b.d. for the third and fourth weeks it was doubled to 500 mg b.d. Those patients who could not tolerate this dosage took AG 250 mg, 8am, 500 mg 8pm. HC 20 mg b.d. (8am, 8pm) was added for the 4th week. Blood samples were taken weekly for 4 weeks and all samples from each patient were measured in one assay. Thus the hormone values measured are those occurring after 1 week of therapy with each dose increment. The patients were to be withdrawn from the study if there was any evidence of disease progression or unexpected side effects.

**Hormone assays**

All blood samples were collected in lithium heparin tubes and the plasma was stored at $-20^\circ$C until assay.

Oestrone, oestradiol, DHA-S, $^4$Δ androstenedione and 17 OH-progesterone were all measured by immunoassay as described previously (Harris et al., 1982a, b). However, in this work a chromatography step was included in the oestrone analysis prior to immunoassay. This involved the use of Sephadex LH-20 columns (7 cm long, in short-form pasteur pipettes) with methylene chloride:methanol (95:5) as solvent (Murphy, 1971). The results were corrected according to the recovery of $\sim 10^3$ c.p.m. of [2,4,6,7-3H] oestrone (Amersham International), which was added to serum samples 18 h before extraction. Cortisol was measured using reagents provided by the WHO Matched Reagent Scheme and according to WHO recommended methodology (WHO Manual 1981). The intra- and inter-assay coefficients of variation were 7.2 and 14.6 respectively.

**Results**

**Synacthen tests**

$^4$Δ androstenedione and 17 OH progesterone had risen markedly (Figure 2) 30 min after Synacthen. Their respective rises as a percentage of baseline levels were 489 ± 85 s.e. (median 450) and 1546 ± 563 s.e. (median 810) ($P<0.01$, paired t-tests, Mann–Whitney U tests). There was a correlation of
Figure 2 Δ4 androstenedione and 17OH progesterone levels after Synacthen. Samples were taken before and 30 min after 250 μg Synacthen i.m. All patients were receiving 250 mg AG 4 × daily plus 20 mg HC b.d.

Figures 3 and 4 show the relationship of Δ4 androstenedione and 17OH progesterone to the change in progesterone levels before and after Synacthen tests. 17OH progesterone v Δ4 androstenedione in individual patients before Synacthen tests (●). 17OH progesterone v Δ4 androstenedione in individual patients after Synacthen tests (○) (r = 0.562, P = 0.055).

Marginal significance between the rise in Δ4 androstenedione and the rise in 17 OH progesterone (Figure 3) (r = 0.562, P = 0.055).

Only 1 patient showed a normal rise in cortisol levels. As a group, these did not change significantly (paired t-test, Mann–Whitney U test). In 4/10 patients, the post-stimulation levels were ≤500 nM l⁻¹ (Figure 4). Oestrone and DHA-S levels did not change significantly. These results suggested that the desmolase enzyme (Figure 1) was not the main site of AG action as there was a large rise in the steroids beyond the supposed site of block.

**Low dose aminoglutethimide alone**

Side effects and response Thirteen patients entered the study. One withdrew after 1 week because she developed supraventricular tachycardia (not known to be drug-related). The other 12 patients had their dose increased to 250 mg twice a day. One developed a progressive pleural effusion and was withdrawn. Another patient had “blackouts” but tolerated the lower dose of 125 mg b.d., to which HC was added. She has had stable disease for >6 months. Ten patients had their dose increased to 500 mg b.d. Two could not tolerate the dose and reduced the dose to 250 mg a.m., 500 mg p.m.

The results are thus described for 13 patients in week 1, 12 patients in week 2, 10 patients in week 3 and 8 patients in week 4. Three patients were maintained on lower doses of AG and had HC added.

Because patients with recurrent disease were being studied, the protocol of drug administration was designed so that all the patients would be on maximally tolerated doses of AG plus HC by 1 month from the start of the study. Thus, response to low dose AG alone could not be assessed but 4/11 patients who continued AG responded (1 complete response, 1 partial response, 2 disease stabilisations for >6 months).

**Low dose aminoglutethimide alone**

Endocrine results Oestrone levels were significantly suppressed in all 13 patients (P = 0.015) by the lowest dose of AG alone (125 mg b.d.) (Figure 5). Increasing doses and addition of HC did not suppress oestrone further (Table 1). Oestradiol suppression paralleled oestrone suppression (Figure 5).
Figure 4  Cortisol, oestrone and DHA-S levels after Synacthen. Samples were taken before and 30 min after 250 μg Synacthen i.m. All patients were receiving 250 mg AG 4 x daily plus 20 mg HC b.d.

Figure 5  Endocrine effects of incremental low dose aminoglutethimide alone and after the addition of hydrocortisone. Results are means ± s.e. at each point. P values are unpaired t tests comparing the effects of the given dose with the pretreatment values. The hormone levels were measured after the patients had been on the given dose of drugs for 1 week. Dosage was increased at weekly intervals.
Table 1  Oestrone results as a percentage of pre-treatment levels

| Aminoglutethimide dosage (mg b.d.) | 125 | 250 | 500 | hydrocortisone |
|-----------------------------------|-----|-----|-----|----------------|
| Oestrone levels (% of baseline)   |     |     |     |                |
| Mean                             | 50  | 63  | 49  | 49             |
| SD                               | 20  | 24  | 29  | 22             |

There are no significant differences between any columns.

DHA-S levels were not suppressed by any dose of AG, but fell by 75% when HC was added (Figure 5). Cortisol levels were not affected by AG alone and did not rise when HC was added (Figure 5). Δ4 androstenedione and 17 OH progesterone levels rose progressively as the dose of AG was increased (Figures 5 and 6). After the addition of HC, the levels fell to levels which were not significantly different to basal values.

Patients not tolerating all dosage increments

The 3 patients who did not have the maximum dose of AG of 1000 mg per day were given HC as well as continuing on their maximum tolerated dose. Their results were quantitatively and qualitatively similar to other patients. Thus, HC did not produce further oestrone or oestradiol suppression and DHA-S concentrations only fell on addition of HC. Δ4 androstenedione and 17 OH progesterone fell to pretreatment values 1 week after addition of HC.

Discussion

The results of Synacthen tests on patients receiving conventional dose AG and HC show that one putative site of AG action (at the 20,22 desmolase) is readily overcome by exogenous adrenal stimulation. The study was undertaken to assess whether patients on AG therapy could synthesise additional cortisol when under stress. Within 36 h of stopping HC and AG, the pituitary-adrenal axis returns to normal responsiveness to stress (Worgul et al., 1982). However, we have shown that there is no increase in cortisol levels after Synacthen, which suggests that additional HC may be necessary in the interim period.

The marked rise in 17 OH progesterone with no increase in cortisol suggests that 11β hydroxylase is inhibited (Figure 1). Faglia et al. (1971) also suggested this may occur. The block could also be at the 21 hydroxylase site, since this enzyme converts 17 OH progesterone to 11 deoxycortisol, the substrate that is converted to cortisol 11β.

Figure 6  Effects of incremental aminoglutethimide dose on Δ4 androstenedione levels. Each symbol represents a different patient. Pre treatment values are shown to the left of each column and values after 1 week of treatment with the given dose are shown on the right. Dosage was increased weekly.
hydroxylase. However, Taylor et al. (1978) showed that 11 deoxycorticisol levels rise in patients taking AG, which suggests that 11β hydroxylase is the more likely site of block.

Heterozygotes for congenital adrenal hyperplasia with partial 21 hydroxylase deficiency show a marked rise in 17 OH progesterone after Synacthen tests, although not to the levels found in our patients (Lee & Gareis, 1975; Mauseth et al., 1980).

Samojlik & Santen (1978) showed that Δ4 androstenedione and 17 OH progesterone were not significantly suppressed by AG and HC, although the precursors DHA and 17 OH pregnenolone were markedly suppressed. They suggested that this phenomenon could be explained by increased activity of 3βol dehydrogenase. However, Taylor et al. (1978) showed that 11 deoxycorticisol levels rise in patients taking AG, which suggests that 11β hydroxylase is the more likely site of block.

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These studies provide the endocrine basis for further investigation of the clinical response to low dose AG alone. This dosage regimen should be less toxic, as well as producing a novel manipulation of the endocrine environment (a rise in androgens and fall in oestrogens). The twice daily low dose regime may be useful in adjuvant therapy and combination endocrine therapy as well as in advanced disease.

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