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V. Enzyme Inhibitors

SECOND LINE ENDOCRINE TREATMENT OF POSTMENOPAUSAL ADVANCED BREAST CANCER. PRELIMINARY ENDOCRINE RESULTS OF A 5-ARM RANDOMIZED PHASE II TRIAL OF MEDIUM VS LOW DOSE AMINOGLUTETHIMIDE, WITH OR WITHOUT HYDROCORTISONE VS HYDROCORTISONE ALONE (EORTC 10861)

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Summary—The supposed mechanism of action of aminoglutethimide (AG), medical adrenalectomy, has been challenged. AG is now considered to act as an inhibitor of the aromatization of mainly adrenal androgens to estrogens in peripheral tissues and/or breast cancer itself. To further establish the AG dose required to sufficiently reduce estrogen levels in plasma and the possible role of hydrocortisone (HC) in combination with AG or by itself, postmenopausal advanced breast cancer patients received AG low (125 mg bid) or medium (250 mg bid) dose alone or combined with HC (20 mg bid) or HC alone (20 mg bid). Preliminary hormonal data show a similar reduction of serum estrone and estrone sulphate by at least some 50% at 8 wk in all treatment groups. At 6 months these effects persist except for patients treated with HC alone. In the latter a normalization of estrone levels is observed with effective suppression of adrenal androgen precursors, suggesting increased aromatase activity with prolonged glucocorticoid treatment.

INTRODUCTION

Aminoglutethimide (AG) originally introduced as an anticonvulsant in the 1960s was found to impair the synthesis of cortisol by the adrenal cortex [1, 2]. Subsequent use of AG in advanced postmenopausal breast cancer patients yielded response rates that were not different from those obtained by ablative surgery of the pituitary or the adrenal gland [3, 4]. For such "medical adrenalectomy" a dose of AG 250 mg qid was used in combination with hydrocortisone (HC) 20 mg bid. HC was thought to be required for two reasons: (1) to prevent a reflex rise of ACTH causing an increment of the adrenocortical precursor of oestrone (E_1) androstenedione and (2) to prevent a possible cortisol deficiency syndrome [5]. The latter syndrome may very rarely occur [6] under extreme circumstances such as volume depletion from protracted vomiting or dehydration from heat, or in patients in whom the drug is metabolized very slowly. However, we [7] and others [8, 9] have demonstrated that AG, when given alone in doses ranging from 250 to 1000 mg/day during at least 6 months, does not lead to decreased plasma cortisol levels. Also, withdrawal of AG 1000 mg daily plus HC after prolonged administration is followed by normal adrenal secretion within 36 h [10]. The finding that plasma E_1 levels were effectively reduced to less than 50% with androstenedione levels becoming even elevated demonstrated that AG plus HC does not act by

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"medical adrenalectomy". In fact, it was found that AG inhibits aromatase *in vitro* at a much lower concentration than required for the inhibition of steroid synthesis by adrenal cells [11]. Also *in vivo* aromatase activity was shown to be almost completely inhibited by low dose AG treatment [2]. It was therefore logical to study the effect of lower doses of AG and to investigate whether the addition of HC contributes to the reduction of plasma estrogens and the clinical tumor response. HC by itself could suppress ACTH and consequently adrenocortical secretion of estrogen precursors [13].

In 1986 the EORTC Breast Cancer Cooperative Group undertook a randomized Phase II study to investigate the endocrine and tumor responses to medium-dose vs low-dose AG, with or without HC vs HC alone. Preliminary endocrine results of this study are presented here.

PATIENTS AND METHODS

Postmenopausal patients with advanced breast cancer were randomized for one of five treatment modalities as second-line endocrine therapy. Patients had to be less than 75 yr old. They should have had their last menstrual cycle more than 1 yr before entry into the study or they should be over 55 if they had undergone hysterectomy with one or two ovaries left in place. Performance status had to be <3 (WHO).

Serum samples for hormonal assays were collected before ($t = 0$) treatment and after 2 ($t = 2$) and 6 ($t = 6$) months of therapy. Serum aliquots were kept frozen at $-20°C$ until analysis. Radioimmunoassays of serum oestrone (E_1), dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulphate (DHEA-SO_4), delta 4-androstenedione (delta 4) and thyrotropin (TSH) were performed in The Netherlands Cancer Institute using methods described previously [14, 15]. Oestrone sulphate (E_1-SO_4) was measured according to Samojlik *et al.* [16].

For statistical analysis comparisons were made within treatment groups using Student's paired t-test. The unpaired t-test was applied for comparison between groups. The analysis was done on log values to correct for skewness of the distribution of data and also to account for proportional rather than absolute differences. Due to limitations to the amounts of serum available per patient paired comparisons could be done only in a restricted number of patients. The mean values presented in the figures come from all determinations done.

RESULTS

Figure 1 shows that plasma E_1 levels at 2 months were very significantly reduced to a similar degree in all groups. In the HC alone treatment group, however, at 6 months E_1 levels

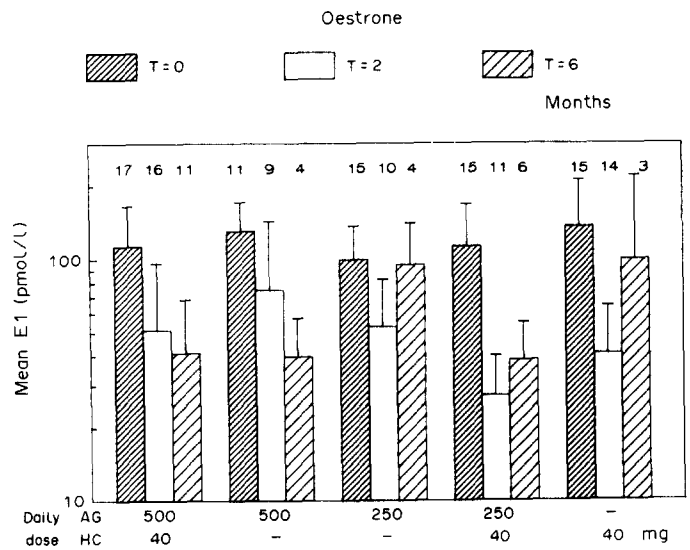

Fig. 1. Serum levels of oestrone (mean + SD) before treatment ($t = 0$), after 2 ($t = 2$) and 6 ($t = 6$) months of treatment, respectively, presented for each of five treatment groups. The different doses of aminoglutethimide (AG) and hydrocortisone (HC) administered daily are indicated along the abscissa. Serum concentrations are given on a log scale. The figures above each bar denote the number of patients whose serum levels were measured at that particular point of time.

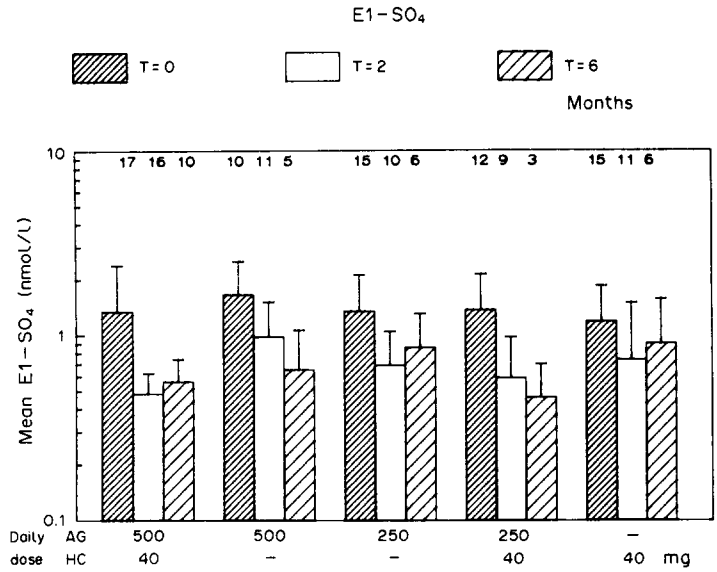

Fig. 2. Serum levels of oestrone sulphate (mean ± SD). For further explanation see legend to Fig. 1.

returned towards values that were statistically not different from normal. In the AG 250 mg/day alone group a similar trend was observed but without a statistical significance between $t = 2$ and $t = 6$.

E_1S levels followed a very similar pattern as demonstrated in Fig. 2. The influence of therapy on serum androgens is different: DHEA (Fig. 3) and its sulphate (Fig. 4) do not change much in the AG alone groups, but are effectively decreased in the groups receiving HC alone or in combination with AG. Somewhat surprisingly, the decrease of DHEA and DHEA-SO_4 at $t = 2$ is significant ($P = 0.002$) in the AG 250 mg/day alone, but not in the AG 500 mg/day alone group.

As could be expected, androstenedione levels are significantly ($P = 0.02$) increased by AG alone, showing a dose–response relationship, which, however, is not statistically significant (Fig. 5). HC alone effectively suppresses androstenedione so that the net result of combined AG plus HC is no change at 500 mg and a slight but significant ($P = 0.01$) decrease at 250 mg AG daily. In Fig. 6 the E_1/androstenedione ratio clearly underlines the aromatase inhibiting effect of AG in the AG alone groups. The ratio did not change in the HC alone treatment group at 2 months, but increased at 6 months of treatment, although the latter effect was not significant.

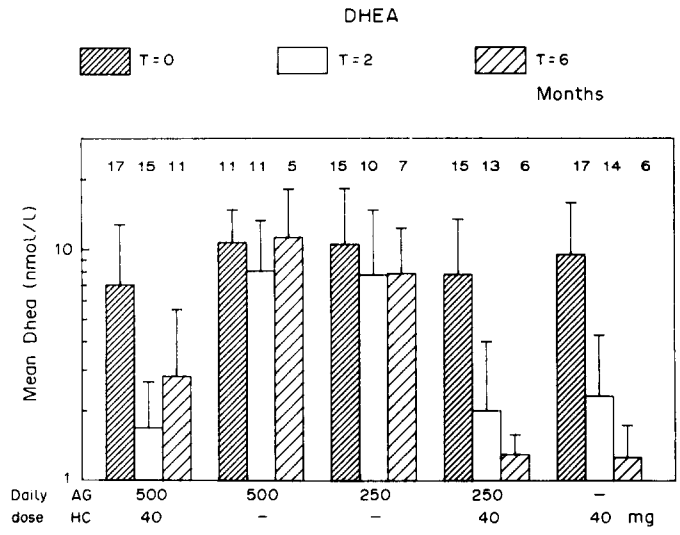

Fig. 3. Serum levels of dehydroepiandrosterone (mean + SD). For further explanation see legend to Fig. 1.

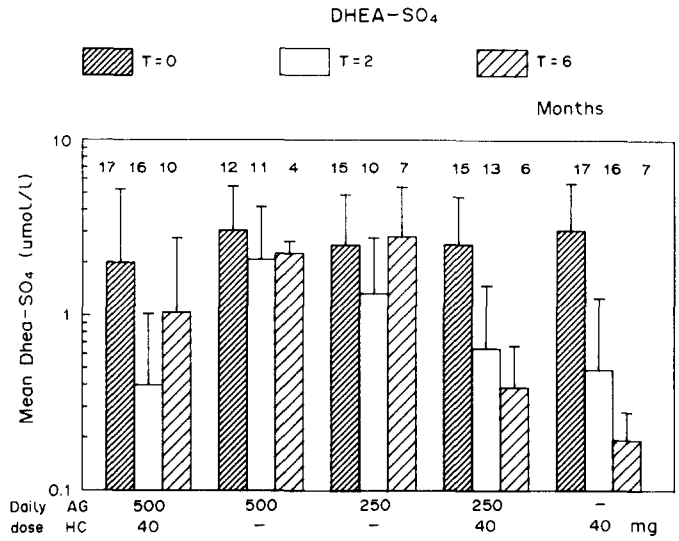

Fig. 4. Serum levels of dehydroepiandrosterone sulphate (mean + SD). For further explanation see legend to Fig. 1.

It has been known since long that AG impairs thyroid hormone synthesis at conventional doses of 1000 mg daily [17, 18]. At low or medium doses of AG we observed only a slight but significant rise of the mean TSH levels in serum, as demonstrated by Fig. 7.

DISCUSSION

We have investigated the endocrine effects of prolonged treatment with medium-dose AG vs low-dose AG, with or without a near physiological replacement dose of HC vs HC alone in randomized postmenopausal women with advanced breast cancer. This treatment was given as second-line hormonal therapy, mostly after tamoxifen. At 2 months the significant decrease of serum E_1 and E_1-SO_4 approximating 50% or more was similar for the four AG containing regimens and the decrease obtained by HC alone was not less. After 6 months the effects of treatment persisted except for the HC alone group, which had E_1 levels returning to base-line.* DHEA, DHEA-SO_4 and androstenedione remained significantly depressed by HC alone at 6 months, indicating that the normalization of E_1 during prolonged HC administration is not due to a waining suppression of adrenocortical function. The rising E_1/ delta 4 ratio also strongly suggests an increasing aromatase activity after 6 months of treatment with a dose of HC that is only some 25% more than full replacement of adrenal function would require. To our knowledge this is the first *in vivo* observation of aromatase stimulation in humans by glucocorticoid. Mendelson *et al.* [19, 20] reported aromatase induction by glucocorticoids *in vitro* in human adipose stromal cells. It is too early to communicate on the tumor responses in the present trial. Literature data on breast cancer responses to glucocorticoid treatment are few, varied and mostly based on response criteria that are no longer considered as adequate [21–23]. It remains to be seen whether the duration of responses in the present trial is related to the short-lasting reduction of serum E_1 levels with HC alone.

Although AG has been used for breast cancer treatment since almost a generation, its mode of action and optimal dose schedule are still insufficiently known. If AG would be therapeutic by reducing serum estrogen levels as the result of aromatase inhibition in peripheral tissues, then the simple administration of HC 20 mg bid could be as effective during at least a few months. This we could observe in confirmation of earlier reports [13, 24, 25]. Our comparisons gave no indication that either dose of AG or combination with HC cause significantly different reductions of serum estrogens. This is also in agreement with findings done by others [26–28] and ourselves [7] and indicates that AG 250 mg per day causes maximal inhibition of peripheral aromatase activity and that even less AG may prove sufficient.

*Too few data were available for paired analysis of E_1-SO_4 levels at 2 and 6 months in the HC alone group.

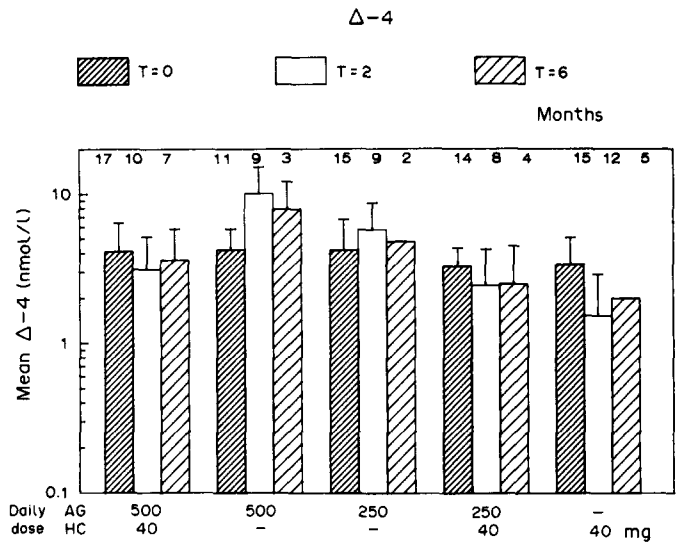

Fig. 5. Serum levels of androstenedione (mean + SD). For further explanation see legend to Fig. 1.

It has recently been reported that AG can increase the metabolic clearance rate of E_1-SO_4 [29]. From our data is seems very unlikely that this mechanism has a significant influence on serum levels. With the doses used here, which are known to be compatible with objective tumor regression, similar E_1-SO_4 reductions are seen in all groups.

Aromatase activity has been found in a variable proportion of breast cancers in very different amounts [30–32]. If tumor responses to treatment with AG are due to inhibition of tumor aromatase the dose of AG required is still unknown and could be less than 250 mg per day. In this respect it seems important to note that reduction of AG dose leads to proportionally less reduction of AG plasma concentrations as it has been shown that the relationship between dose and plasma concentration is not linear. The latter is the result of AG increasing its own metabolic clearance rate in a dose–responsive manner [8].

From our preliminary data we may conclude that:

1. If the therapeutic effect of AG is mediated by a reduction of serum E_1 levels a low dose of 125 mg bid without HC is sufficient for a reduction of 50%.
2. HC alone reduces serum E_1 levels at 2 months to less than one-third by suppression of ACTH.

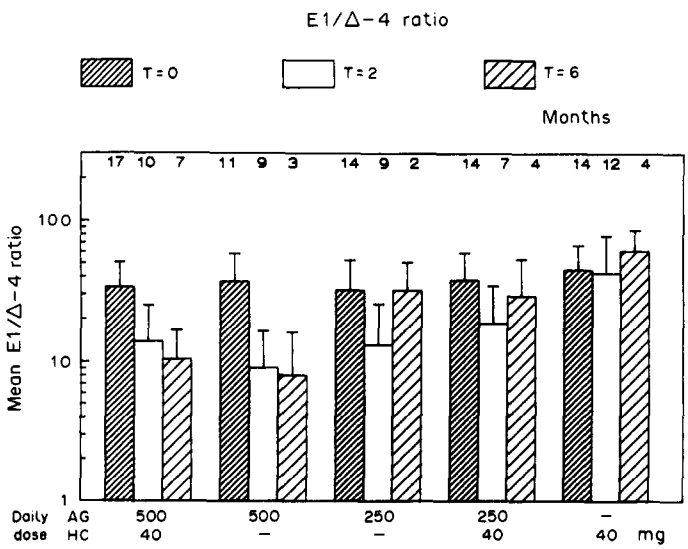

Fig. 6. The ratios of the serum levels of oestrone/androstenedione (mean + SD). For further explanation see legend to Fig. 1.

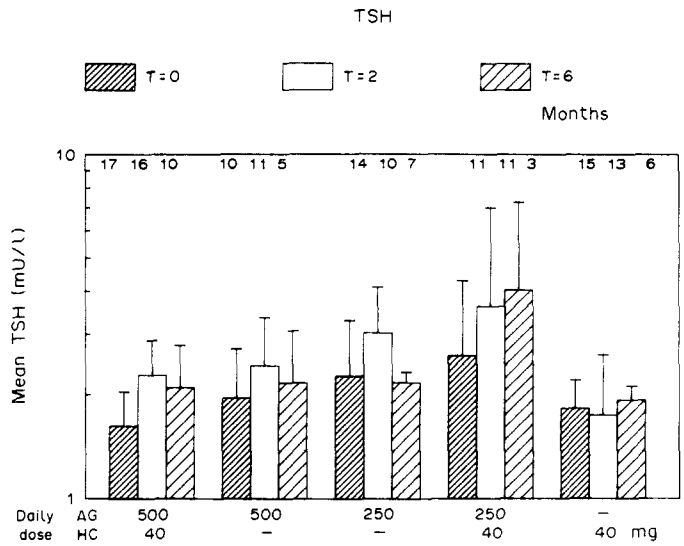

Fig. 7. Serum levels of thyrotropin (mean + SD). For further explanation see legend to Fig. 1.

3. Administration of HC alone during 6 months still causes diminished secretion of adrenocortical androgens, but E_1 levels tend to return to normal suggesting increased aromatase activity.
4. Thyroid function is much less affected by medium- or low-dose AG than by the original 250 mg bid dose.

The adverse effects of AG known to cause withdrawal of the drug in some 5–8% of patients given conventional doses of 1000 mg per day [15, 34] are much less frequently observed with low-dose AG [7, 33, 34]. Whether tumor response rates and duration are as good with low dose AG or HC alone as with medium dose has to be awaited from the present and other randomized prospective trials.

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