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Aminoglutethimide in advanced breast cancer: plasma levels and clinical results after low and high doses

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Summary. Drug plasma levels, metabolism data and clinical results were evaluated after the daily administration of either 500 or 1,000 mg aminoglutethimide (AG, Orimeten, Ciba-Geigy) plus hydrocortisone acetate (20 mg b. i. d.). A total of 34 patients with advanced breast cancer entered the study: 17 were given 1,000 mg/day and 17 received 500 mg/day for at least 3 months. A novel HPLC method was developed to determine the levels of AG and its known metabolites [*N*-acetyl-AG (NAG), formyl-AG, nitroglutethimide, hydroxy-AG] in the biological samples. AG plasma concentration was significantly higher during the 1,000-mg/day regimen. NAG was the only metabolite observed in plasma, always occurring at concentrations lower than those of the parent drug. The ratios between NAG and AG levels distinguish two statistically different groups of patients. Irrespective of the dose, a partial response was observed in 44% of the patients; no change in 32% of cases; and progressive disease had an incidence of 24%. The probability of response was not dependent on the drug AUC or on the NAG/AG ratio and did not significantly depend on previous hormone treatment. Neither the plasmatic level of the AG or metabolite concentrations nor the NAG/AG ratio seemed to affect the incidence of side effects.

Introduction

When combined with hydrocortisone, aminoglutethimide (AG) is an effective agent in the therapy of advanced postmenopausal breast cancer at a conventional dose of 1,000 mg/day, which achieves a 25%–39% response in unselected postmenopausal patients [9, 11, 18, 19]. AG inhibits hydroxylations needed for steroid hormone synthesis, including 20α-hydroxylase in the adrenocortical tissue and aromatase in extra-adrenal and breast cancer tissues. This inhibition also takes place at low AG doses of 500 mg [7, 12, 17] and the therapeutic efficacy does not seem to be affected [2, 16, 21]. Furthermore, AG toxicity has been reported to be dose-related [2, 13].

Despite the large number of pharmacodynamic and clinical studies published thus far, there are few data regarding the metabolism and pharmacokinetics of this drug. *N*-Acetylaminoglutethimide (NAG) has been identified as the major AG metabolite in plasma [8, 20]; the acetylation process is phenotype-dependent, with different plasma levels being induced by fast and slow acetylators [1, 3].

AG is a potent inducer of hepatic metabolic enzymes [14], and a substantial change in half-life (from about 15 to 9 h) and a parallel increase in drug clearance have been reported in several studies during continuous AG treatment [1, 10, 14, 15, 20]. This has prompted the use of a dose-escalating AG treatment schedule to prevent initial side effects.

The aim of present study was to assess plasma levels of AG and its metabolites during prolonged treatment with 500 or 1,000 mg/day so as to evaluate differences between these two regimens as well as the possible relationship between clinical response and/or toxicity and bioavailability.

Patients and methods

Patient selection. The present study is part of a randomized phase IV clinical multicentric study comparing daily treatment with 500 and 1,000 mg AG. After their informed consent had been obtained, 34 postmenopausal women with advanced breast cancer were randomly allocated to receive one of the two AG doses. Eligibility criteria were as follows: age, <75 years; postmenopausal status; the presence of progressive breast cancer and measurable lesions; estrogen receptor status positive or unknown; prognosis, better than 3 months; performance status, WHO grade 3 or better; no other antitumor treatment within the 60 days prior to the study. The characteristics of the subjects are reported in Table 1.

Treatment schedule. AG tablets (Orimeten; Ciba-Geigy, Origgio) were given in increasing doses according to the following schedules. Patients allocated to receive 500 mg/day were given 125 mg AG and 20 mg

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Table 1. Patients characteristics

		AG dose	
		500 mg/day (17 patients)	1,000 mg/day (17 patients)
Age (years):	Mean	58	60
	Range	44–71	46–72
Weight (kg):	Mean	70	67
	Range	46.5–99	50–91
WHO performance status:	Grade 0	9 patients	3 patients
	Grade 1	4 patients	7 patients
	Grade 2	3 patients	6 patients
	Grade 3	1 patient	1 patient
Metastases:	Soft-tissue	3 patients	1 patient
	Osseous	3 patients	3 patients
	Viscera	4 patients	5 patients
	Mixed	7 patients	8 patients
Estrogen receptors	Positive	7 patients	2 patients
	Unknown	10 patients	15 patients
Previous treatment	None	6 patients	6 patients
	Chemotherapy	8 patients	6 patients
	TAM	5 patients	7 patients
	MPA	3 patients	3 patients

hydrocortisone acetate (HCA) twice daily (b. i. d., at 8 a. m. and 8 p. m.) on days 1–7. Beginning on day 8, they received 250 mg AG and 20 mg HCA b. i. d. until the development of tolerance or progressive disease. Patients scheduled to receive 1,000 mg AG daily were given 125 mg AG and 20 mg HCA (b. i. d.) on days 1–7. On days 8–14 patients received 250 mg AG and 20 mg HCA b. i. d. On days 15–21 they received 250 mg AG three times daily (at 8 a. m., 4 p. m. and 10 p. m.) plus 20 mg HCA b. i. d. Beginning on day 22, they were given 250 mg AG four times per day (at 8 a. m., 12 a. m., 4 p. m. and 10 p. m.) and 20 mg HCA b. i. d. until the development of intolerance or progressive disease. Treatment had to be given for a period of at least 3 months before evaluation and was continued until disease progression became apparent or unacceptable toxicity occurred.

Response criteria. After 3 months, clinical response was evaluated in accordance with International Union Against Cancer (UICC) criteria: a complete response (CR) was defined as the disappearance of all clinical evidence of disease; a partial response (PR) involved a reduction of at least 50% in the sum of the products of the diameters of all measurable lesions without the appearance of new lesions; no change (NC) was defined as a response of <50% or an increase of <25% in measurable disease; progressive disease (PD) was defined as an increase of >25% in the measurable lesions and/or the appearance of new lesions.

Quantitative analysis of AG and its metabolites. Blood samples were drawn before treatment and after 7, 14, 21, 30, 60 and 90 days. Each sample was taken before the first daily AG administration at 8 a. m., 12 h after the previous administration on the 500-mg/day regimen and 10 h after the preceding administration on the 1,000 mg/day arm. The plasma samples, separated after centrifugation, were kept frozen at –20° C until analysis.

Analytical methods. A novel HPLC assay using spectrophotometric detection was used for the quantitative determination of AG in plasma and other biological fluids. The method was specific for the simultaneous assay of unchanged drug and its known metabolites NAG, hydroxylaminoglutethimide (HAG), formylglutethimide (FG) and nitroglutethimide (NG).

After the addition of 2-(*p*-*N*-acetylaminophenyl)-2-methyl-glutarimide as the internal standard, 1 ml plasma samples were solid-phase-extracted (C_{18} bonded silica cartridges, Bond-Elut, Analytichem) following the addition of 1 ml methanol and 1 ml phosphate buffer (pH 8). Unchanged drug and metabolites were recovered by elution with 3 ml CH_3CN following washing with 4 ml H_2O. The solution was evaporated under vacuum and, after the addition of mobile phase (0.5 ml), it was analyzed by HPLC (Supelcosil Cianopropyl chromatographic column; in side diameter, 25 cm × 4.6 mm; particle size, 5 µm). The chromatographic system consisted of a Merck-Hitachi 4200 ternary liquid chromatograph and a variable-wavelength detector (set at 235 nm). Complete separation of the compounds was achieved using a mobile phase consisting of 85% KH_2PO_4 (10 mM) and 15% CH_3CN at a flow rate of 1.2 ml/min. Chromatographic peaks of 1,000 area counts (Varian Vista CDS 401 system) were considered to represent the lowest detectable quantity and corresponded to a sensitivity limit of 0.05 µg/ml compound. The mean coefficient of variation (CV) under these analytical conditions was 9.5%. Under these analytical conditions, the metabolites HAG, FG and NG were not present in detectable concentrations in plasma samples from our patient group.

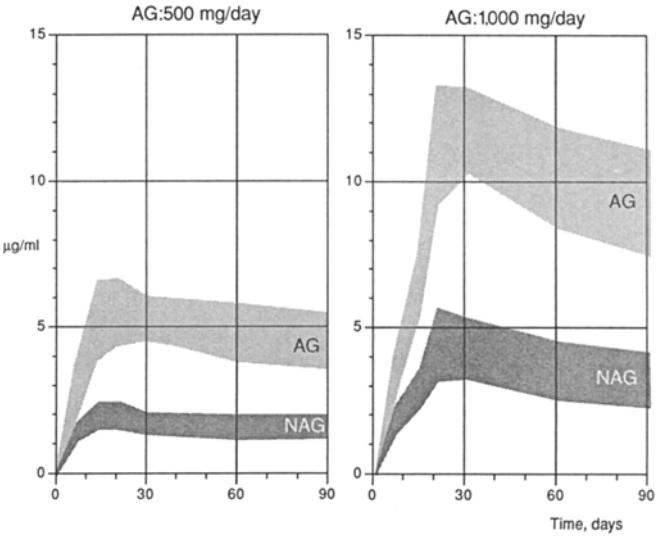

Fig. 1. Mean plasma levels (µg/ml ± SEM) of AG and NAG after the administration of (*left panel*) 500 and (*right panel*) 1,000 mg AG/day plus 20 mg hydrocortisone b. i. d.

Pharmacokinetics and statistical analysis. The area under the time-concentration curve (AUC) was computed for the drug and its main metabolite, NAG, by means of the trapezoidal rule (0- to 90-day interval). Statistical analysis of the results was carried out using the BMDP P7D, PKM, P3S, PLR and P7M programs for analysis of variance, cluster analysis, non-parametric statistics, logistic regression and discriminant analysis, respectively [6].

Results and discussion

AG plasma levels and bioavailability

Mean AG and NAG plasma level profiles (± SEM) are shown in Fig. 1. AG and NAG plasma levels (µg/ml) continuously increased for up to 15 and 21 days during 500 and 1,000 mg/day treatments, respectively. A large interpatient variability in AG and NAG plasma concentrations after both regimens was present (CV: 500 mg AG, 74%; 1,000 mg AG, 61%; 500 mg NAG, 76%; 1,000 mg NAG, 85%.). Notwithstanding that spread, the statistical analysis carried out on AG and NAG plasma profiles after the two regimens indicated a significant difference between the

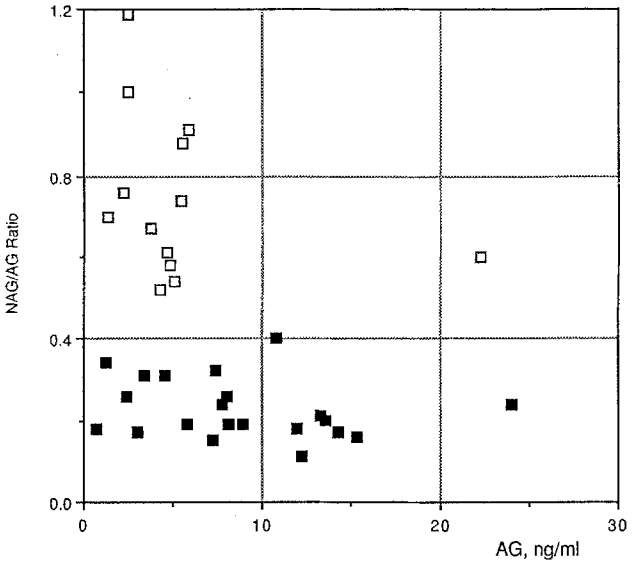

Fig. 2. NAG/AG ratio distribution according to AG levels. □, High acetylators; ■, low acetylators

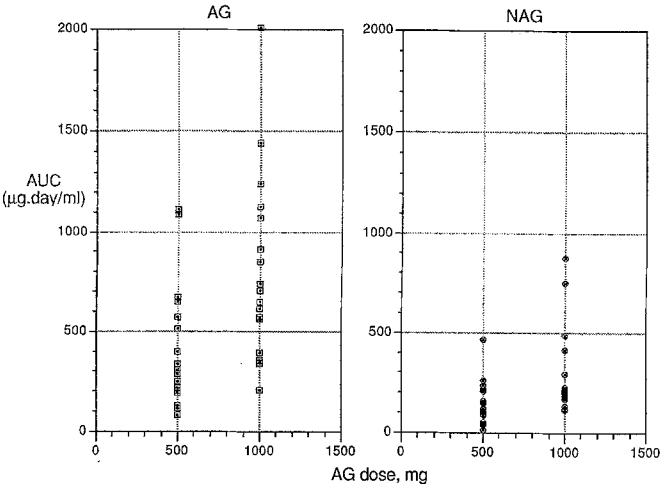

Fig. 3. AUC (μg day ml^{-1}) for (*left panel*) AG and (*right panel*) NAG after treatment with 500 and 1,000 mg AG/day

levels produced by the 500- and 1,000-mg/day treatments (AG, $P = 0.0163$; NAG, $P = 0.035$).

It should be noted that although AG and NAG plasma levels eventually reached a relatively stable plateau after AG doses of 500 mg/day (the intrapatient variation was on average equal to 13% [$AG_{500\ mg}$] and 17% [$NAG_{500\ mg}$]), a progressive, statistically significant decay (Fig. 1) was observed in drug and metabolite plasma levels during the 1,000-mg/day constant-dose therapy (H0, $Conc_{30\ days} = Conc_{90\ days}$; AG, $P = 0.0086$; NAG, $P = 0.015$), possibly indicating auto-induced AG metabolism. This may suggest that a slower dose-escalation schedule should be followed at higher doses so as to avoid a drug-concentration peak during treatment.

NAG was the only metabolite observed in the plasma samples, and its concentration was always lower (or at least the same in two patients) than that observed for the parent drug. Our analytical method can detect the metabolites HAG, FG and NG if they occur at concentrations of >0.05 μg/ml. The ratios between NAG and AG levels ranged from 0.08 to 1.24 (Fig. 2); they were not dependent on the delivered dose of AG ($P = 0.948$).

Although an acetylation test is lacking, it is possible to distinguish between slow and fast acetylator phenotypes by analyzing NAG/AG ratios with statistical K-Means Cluster analysis (KM) [6]. KM partitioned our observations into two subgroups. Cases were iteratively reallocated into the cluster whose center (the mean of the NAG/AG ratios in one cluster) was closest to the case. At the end of the run, each case belonged to the cluster whose center was closest in Euclidean distance to the case. In the present study, we had two clearly defined groups, characterized by two statistically different cluster means of the NAG/AG ratios (slow acetylators: cluster mean, 0.23; fast acetylators: cluster mean, 0.74; $P = 0.0001$; Fig. 2).

Relative plasma bioavailability, expressed in terms of the AUCs (0 to 90-day interval, trapezoidal rule) for the unchanged drug and its main metabolite after 500- and 1,000 mg/days treatments, is shown in Fig. 3. In spite of the great interpatient variation in AUC_{AG} and AUC_{NAG} observed after both treatments, a statistically significant difference was found between the 500 and 1,000 mg/day regimens (AUC_{AG}: mean value, 418.56 vs 828.8 μg/ml^{-1} day, respectively, $P = 0.0064$; AUC_{NAG}: mean 145.56 vs 292.85 μg/ml^{-1} day, $P = 0.0197$).

AG plasma levels and clinical response

Objective response. The objective responses observed in this group of patients after the two treatments are reported in Table 2. Irrespective of the AG dose, a PR was observed in 15 cases (44.1%), NC in 11 patients (32.4%), and PD in 8 cases (23.5%). In our patients, objective response was not influenced by previus endocrine treatment ($P = 0.719$; Table 3). Plasma AG and NAG plateau concentrations measured in responders ranged between 0.6 and 23 μg/ml; the minimal effective plasma concentration is unknown.

Table 2. AG in advanced breast cancer: clinical response after 3-months therapy

Response	500 mg	1,000 mg	Total
PR	9 (53%)	6 (35%)	15/34 (44%)
NC	5 (29%)	6 (35%)	11/34 (32%)
PD	3 (18%)	5 (29%)	8/34 (24%)
Patients (*n*)	17	17	34

Table 3. Clinical response to 3-months' therapy with AG according to prior therapy in patients with advanced breast cancer

Response	Previous treatment			
	None	Hormone therapy	Chemo-therapy	Hormone therapy + chemotherapy
PR	9	2	4	0
NC	1	4	2	4
PD	2	2	1	3
Patients (*n*)	12	8	7	7

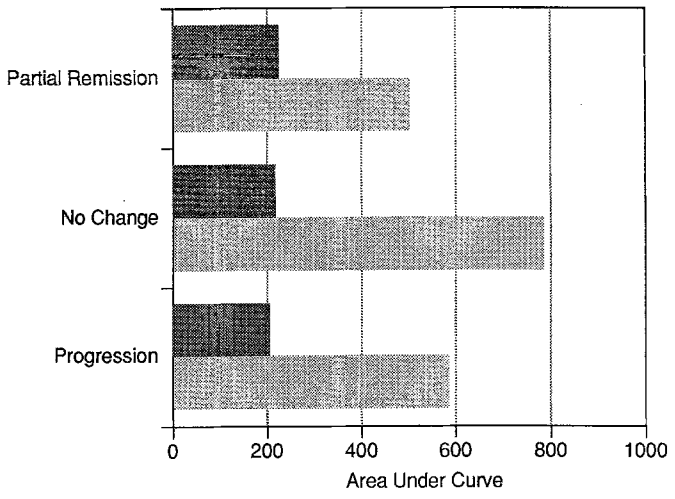

Fig. 4. Plasma bioavailability (AUC, μg day ml^{-1}) of AG (*light shading*) and NAG (*dark shading*) according to objective response

Mean AUC_{AG} and AUC_{NAG}, according to the objective response, are reported in Fig. 4. As can be seen, no clear cutoff point for the response was observed and no differences were noted between the plasma AUC_{AG} and AUC_{NAG} of the three response groups (PR, NC, PD; AG, $P = 0.562$; NAG, $P = 0.693$). Stepwise logistic regression analysis did not indicate correlation between the increase in drug AUC and a better response probability ($P = 0.736$).

The NAG/AG ratio pattern was similar in responding and non-responding patients as well. The efficacy of AG does not seem to be affected by the relative drug bioavailability or drug metabolism. As has recently been reported [5], a low AG dose (500 mg/day) is effective, hence its combination with hydrocortisone acetate (HCA) becomes arguable, particularly in terms of overall clinical response.

Toxicity. Drowsiness, nausea and cutaneous rash were the only side effects observed during AG treatment (Table 4). Demers et al. [4] have reported a relationship between high AG plasma levels and the incidence of side effects. No such relationship was found in the present study. In fact, the drug and metabolite concentrations in patients who experienced side effects were not higher than those in patients who experienced no side effects ($P = 0.875$). We measured AG and NAG concentrations in three patients on the day on which they had a skin rash; drug and metabolite plasma levels were similar to those measured in the other patients. Thus, a different metabolism (NAG/AG ratio) was not an apparent cause of the onset of side effects. Other mechanisms are probably involved in the occurrence of adverse effects.

Table 4. Side-effects metabolism and bioavailability of AG

Symptom	Number of patients	AUC_{AG}	AUC_{NAG}	NAG/AG ratio
Drowsiness	5 (15%)	202.83	52.6	0.26
		1,244.3	204.3	0.34
		913.3	167.5	0.18
		248.7	41.41	0.17
		851.1	749.56	0.88
Skin rash	4 (12%)	248.7	41.4	0.17
		706.5	136.22	0.19
		612.2	115.5	0.19
		217.6	259.9	1.19
Nausea	2 (6%)	913.3	167.5	0.18
		356.73	215.99	0.61
All patients	Mean	614.86	219.2	0.43
	Minimum	82.38	14.58	0.11
	Maximum	2,007.98	870.99	1.19

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