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Clinical pharmacology of aminoglutethimide in patients with metastatic breast cancer

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Summary. The pharmacology of aminoglutethimide (AG) was studied in two subsequent trials without hydrocortisone supplementation. A total of 79 patients with metastatic breast cancer entered the study, and their plasma and urine samples were analyzed by high-performance liquid chromatography (HPLC). Thirty evaluable patients with a median age of 57 years (range, 37–79) were treated with the standard dose of 1000 mg/day, and 37 evaluable patients with a median age of 59 years (range, 35–79) received 500 mg/day. The median follow-up in the two groups was 5 months (range, 1–16) and 4 months (range, 1–21), respectively. After the first oral dose of 500 mg, peak plasma concentrations of AG were observed 1–4 h after administration in 15 patients. The elimination half-life was 10.1 ± 1.7 h (mean ± SD) after initial dosage; it decreased significantly to 6.9 ± 1.2 h after 8 weeks of treatment. The area under the curve of AG concentrations was 92.5 ± 14.2 μg/ml × h. The total clearance rate was 5.5 ± 0.9 l/h and the volume of distribution was 80 ± 11 l. About 23% of the drug was excreted unchanged in the urine. The major metabolite, *N*-acetyl-AG (AAG), had the same half-life as AG. A comparison on day 7 of treatment revealed that doses of 1000 and 500 mg yielded AG plasma concentrations of 9.0 ± 1.2 and 4.5 ± 0.5 μg/ml, respectively. After 1 month of treatment, however, AG plasma levels of 6–7 and 4–5 μg/ml were observed, respectively. A 50% reduction of dose, therefore, resulted in only 30% lower AG levels during continuous treatment. Apparently, the induction of metabolism is of greater importance in standard-dose than in lower dose treatment. The plasma concentrations of AG did not bear a relationship to the clinical response.

Introduction

Aminoglutethimide has been recognized as an effective agent in the hormonal treatment of postmenopausal patients with metastatic breast cancer [12, 15]. By binding to cytochrome P-450 complexes, AG inhibits the cholesterol side-chain cleavage enzyme as well as the C-21, C-11, and C-18 steroid hydroxylases [20]. It was introduced in the treatment of breast cancer as an inhibitor of adrenal steroid production [22]. Most studies have been conducted with hydrocortisone replacement to preclude a decrease in adrenal steroid production [9, 21]. More importantly, AG also inhibits aromatase, an enzyme which converts androstenedione to estrone and testosterone to estradiol [20]. This conversion of androgens to estrogens in peripheral tissues provides the main source of estrogens in postmenopausal women [7]. In vitro studies have revealed that the aromatase enzyme reaction is inhibited at lower concentrations of AG than that needed to inhibit the cholesterol side-chain cleavage [20]. The standard dose recommended to achieve adrenal suppression is 1000 mg AG per day [9, 10, 20–23]. To investigate the more sensitive peripheral aromatization as the major site of action, several investigators have recently reduced the dose [2, 8, 18, 24]. Their results suggest that doses lower than 1000 mg AG daily are effective and better tolerated.

In a study with radioactive AG, the bioavailability of the drug was essentially complete [4]. A spectrophotometric assay [25] and HPLC methods [1, 14] have been used for the measurement of AG in biological fluids. The plasma half-life and clearance rate change during continued treatment suggest that AG induces its own metabolism [17]. Whereas AAG was the major metabolite, others have recently been identified [6, 13]. However, all known metabolites of AG are inactive [5, 6].

We have investigated the clinical pharmacology of AG during two subsequent studies using 1000 and 500 mg AG daily. Hydrocortisone supplementation was not given [12]. The objective was to measure AG and the major metabolite initially and during long-term follow-up. Furthermore, we tried to correlate the plasma concentration of AG with the clinical response.

Materials and methods

Patients. Female patients with progressive metastatic breast cancer were entered in two subsequent phase II studies with AG after giving informed consent. In the first study, the dosage of AG was escalated from 250 mg b.i.d. on days 1 and 2 to 250 mg t.i.d. on day 3, and then to 250 mg q.i.d. on day 4 and thereafter. In the second study, a dosage of 250 mg b.i.d. was administered throughout the

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Abbreviations used: AG, aminoglutethimide; AAG, *N*-acetylaminoglutethimide; G, glutethimide; HPLC, high-performance liquid chromatography; SD, standard deviation; SE, standard error of the mean; AUC, area under the concentration versus time curve

Table 1. Patient characteristics according to dose of AG in two subsequent phase II studies

Patients	AG dose	
	1000 mg/day	500 mg/day
Number entered	36	43
Number evaluable	30	37
Low compliance	3	0
Incomplete blood sampling	3	6
Median age[a]	57 (37–79)	59 (35–79)
Median follow-up[b]	5 (1–16)	4 (1–21)

[a] Years (range)
[b] Months (range)

treatment period. Table 1 outlines the patient characteristics in the two trials. All patients were either postmenopausal or had undergone surgical oophorectomy. Laboratory parameters for renal and hepatic functions were normal. All patients had received other hormonal and/or cytostatic drugs, but the previous therapy was discontinued at least 3 weeks prior to treatment with AG. No concomitant, tumor-specific treatment was allowed except for local irradiation of bone metastases, and the local response to irradiation was excluded from the evaluation of overall response. The response to treatment with AG was assessed according to UICC criteria [11].

Materials. Tablets containing 250 mg AG were supplied by Ciba-Geigy GmbH (Wehr, FRG). Pure standard preparations of AG and AAG were also obtained from Ciba-Geigy. Glutethimide was purchased from Sigma Chemie GmbH (Deisenhofen, FRG). All solvents were HPLC-grade (LiChrosolv; Merck, Darmstadt, FRG).

Samples. Blood samples were obtained daily for 7 days, then weekly for 6 weeks, and thereafter monthly from all patients. Samples were also collected from 15 patients before and at 1, 2, 3, 4, 5, 6, 9, 12, and 24 h after the initial dose of 500 mg AG. Urine samples were taken from these patients for 7 days. Blood was drawn into heparinized glass tubes (Becton-Dickinson, Heidelberg, FRG) and immediately put on ice. Samples were centrifuged at 1500 *g* for 10 min, and the plasma was transferred. All samples were stored at −20° C for a maximum of 1 week before analysis.

Protein binding was measured in fresh plasma obtained from 4 healthy human volunteers. AG and AAG were added to the plasma at concentrations ranging from 0.5 to 50.0 μg/ml, and the samples were ultrafiltered through Centricon YM membranes from Amicon GmbH (Witten, FRG).

Analysis. An isocratic, reversed-phase HPLC method was used to separate and quantitate AG and its metabolite AAG in plasma and urine. G was added as an internal standard. The samples were prepared for HPLC by a clean-up procedure on disposable columns filled with C18 reversed-phase material (Baker Chem., Gross-Gerau, FRG); 1 ml of the sample was applied to the column, washed twice with 1 ml H_2O, and eluted twice with 1 ml dichloromethane. The eluant was evaporated under a stream of nitrogen, and the residue was reconstituted with methanol for HPLC analysis. The chromatograph consisted of an M6000 pump, a U6K manual or WISP cooled automatic injector, a μBondapak C18 column (3.9 × 300 mm, 10 μm particle size), and a data module from Millipore/Waters Chromatography (Eschborn, FRG). The mobile phase was water and methanol 65:35 (v/v) at a flow rate of 2 ml/min. A maximum UV absorbance of AG in the mobile phase was found at 241 nm, and the UV detector (M480 by Millipore/Waters) was set to this wavelength. The results were analyzed according to standard nonlinear kinetics on a DEC-350 computer (Digital Equipment Corp., Munich, FRG). For statistical evaluation, Student's *t*-test ot the Mann-Whitney U-test was performed as indicated.

Results

A representative HPLC chromatogram of plasma obtained 6 h after drug administration is depicted in Fig. 1. The extraction efficiency was 83% for AG and 78% for AAG. The lower limits of detection were 0.1 μg/ml in plasma and 1.0 μg/ml in urine, and the standard curves were linear up to concentrations of 50 μg/ml in plasma and 500 μg/ml in urine. The intra-assay variation coefficients were 3.8% for AG and 4.7% for AAG at 0.1 μg/ml. Inter-assay variation coefficients were determined monthly and were always below 8%. Pretreatment samples and samples not containing an internal standard were analyzed for all patients; neither an interfering peak nor metabolism to G was observed. The concentration of AAG was lower than that of AG in all samples. The mean binding to plasma proteins in vitro was 28% for AG (range, 23%–31%) and 31% for AAG (range, 26%–35%).

The pharmacokinetic curves of AG and AAG in 15 patients after the first oral of 500 mg are shown in Fig. 2. Table 2 gives the pharmacokinetic parameters. Peak plasma concentrations were reached after 1–4 h. Nonlinear regression analysis of concentrations versus time yielded r^2 values of greater than 0.90. The half-life after initial administration was 10.1 ± 1.7 h (Table 2). The half-life was also measured in 8 patients after at least 8 weeks of therapy with 1000 mg AG. After the last dose of 500 mg, the half-life was 6.9 ± 1.2 h (curve not shown). This reduction in elimination half-life was statistically significant (*t*-test, $P < 0.05$). The volume of distribution and total clearance

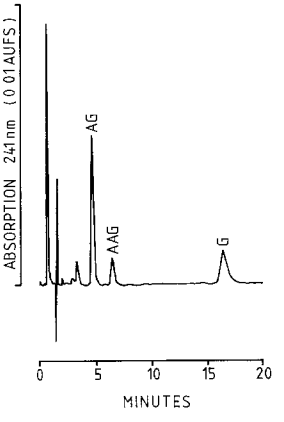

Fig. 1. HPLC chromatogram of a plasma sample 6 h after the first dose of 500 mg AG p.o. (*AUFS*, absorbance units full scale), G was used as internal standard

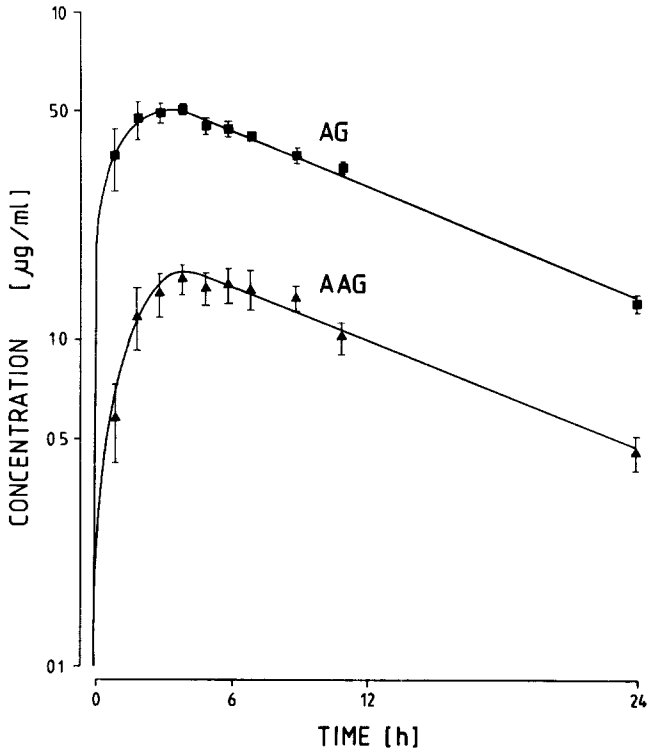

Fig. 2. Pharmacokinetics of AG and AAG in plasma of 15 patients after a single oral dose of 500 mg AG on the first day of treatment (mean ± SEM)

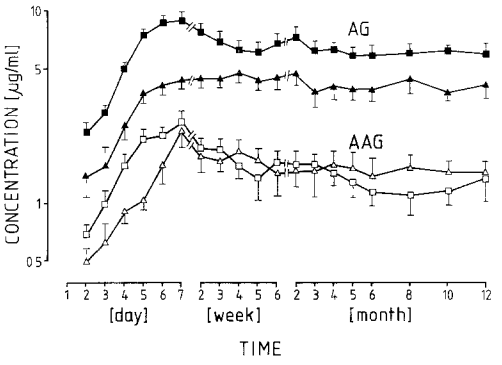

Fig. 3. Plasma concentrations of AG and AAG during long-term follow-up in 30 patients receiving 1000 mg (*squares*) and 37 patients receiving 500 mg (*triangles*) of AG daily p.o. (mean ± SEM)

rate were calculated assuming complete bioavailability, which was reported for the radiolabelled drug [4]. Urinary excretion was determined daily for the initial 7 days of treatment (Table 2). The ratio of AAG to AG showed a large interindividual variation: calculated on the basis of the AUC, it was 32%±16% (mean ± SD) with a variation coefficient of 49%.

The concentrations of AG and AAG during long-term treatment with either 1000 or 500 mg per day are shown in Fig. 3. Seven days after the initiation of treatment, concentrations of 9.0±1.1 and 4.5±0.5 µg/ml were reached with the standard- and low-dose regimens, respectively. For the initial 8 weeks of therapy, the concentrations were also

Table 2. Pharmacokinetic parameters in 15 patients after the first oral dose of 500 mg AG

Compound	C_{max} (µg/ml)	T_{max} (h)	$t_{1/2}$ (h)	AUC (µg/ml per h)	tot Cl (l/h)	V_d (l)	urin excr	
							Day 1	Days 2–7
AG	5.9±0.8[a]	3[b] (1–4)	10.1±1.7[a]	92.5±14.2	5.5±0.9	80±11	21.7±7.3	23.5±5.1
AAG	1.8±0.8	3 (1–6)	10.5±1.7	29.2±13.8	NA	NA	3.0±1.7	2.9±1.7

C_{max}, maximum concentration in plasma; T_{max}, time to reach C_{max}; $t_{1/2}$, elimination half-life; AUC, area under the curve; tot Cl, total clearance rate; V_d, volume of distribution; urin excr, urinary excretion in % of administered dose on day 1 and on days 2–7
NA, not amenable to analysis
[a] Mean ± SD
[b] Median (range)

Table 3. Plasma concentrations of AG in relation to response to treatment

Dose (mg/day)	Response[a] (no.)	AG concentrations in plasma					
		1[b]	2	3	4	6	8
1000	PR (5)	8.7±1.5[c]	7.9±1.4	7.2±1.0	5.9±0.9	6.9±1.5	7.1±1.3
	NC (10)	9.7±2.5	8.7±1.7	8.2±2.1	7.5±1.9	7.8±1.3	7.5±1.2
	PD (15)	8.6±1.5	7.2±0.7	6.2±0.6	5.9±0.6	6.2±0.5	7.7±0.7
500	PR (8)	4.6±0.3	4.5±0.9	4.3±0.5	5.6±0.4	4.9±0.4	5.0±0.8
	NC (14)	5.2±2.0	4.9±0.9	4.3±0.6	5.4±1.2	4.8±1.4	4.2±0.7
	PD (15)	4.6±0.6	4.0±0.7	4.5±0.9	4.0±0.5	4.5±0.7	4.9±0.9

[a] PR, partial remission; NC, no change; PD, progressive disease
[b] Weeks of treatment
[c] Mean ± SEM

evaluated with reference to the clinical outcome. No statistically significant correlation between the plasma levels and tumor response was observed (Table 3).

Discussion

The pharmacokinetic results obtained in this study (Fig. 2, Table 2) compare favorably with data from the literature [17, 20, 25]. The peak plasma concentrations and AUC determined in 15 patients were identical to results for 6 patients reported by Thompson et al. [25]. The elimination half-life of 10 h observed in the present study was shorter than the previously reported value of 13 h [17, 25], but the reduction in half-life to 6.9 h during treatment corresponds to the value of 7.3 h noted in 6 patients by Murray et al. [17]. The volume of distribution and clearance in the present study is in the same range as that determined by Thompson et al. [25]. In earlier investigations employing a less specific spectrophotometric assay, AG was excreted to about 50% [17, 20]. Using the HPLC technique described, 22%–24% and 3.0% of the administered dose were excreted in urine as unchanged AG and AAG, respectively. This is in accordance with data provided by Coombes et al., who also used an HPLC method [3].

AAG is the major metabolite of AG, but it has no inhibitory activity on desmolase and aromatase in vitro [5]. The elimination half-life of this metabolite (Fig. 2) was the same as that of AG, suggesting that production is the rate-limiting step [19]. The AUC ratios of AAG to AG in our study showed a large interindividual variation, which may be due to polymorphic acetylation in humans [3].

Observation of decreases in the half-life of AG during treatment have led other investigators [17, 20] to suggest that this drug induces its own metabolism. To determine the effect on plasma levels, AG concentration was measured during continuous treatment (Fig. 3). With administration of 1000 mg AG daily, a decline in plasma concentration was observed between the 1st and 5th weeks (Fig. 3). This result is interpreted as an effect of induced metabolism. As reported by Santen et al. [23], the side effects of AG on initial treatment with 1000 mg are considerable but usually resolve completely after 4–6 weeks. In another clinical study [12], the relief of side effects coincided with the decline of plasma levels.

No decrease in AG plasma concentration occurred after 1 week of treatment with 500 mg (Fig. 3), which suggests that the lower dose does not lead to an induction of metabolism. A comparison of the two regimens on day 7 shows that doses of 1000 and 500 mg yield AG concentrations of 9.0 and 4.5 μg/ml, respectively. After 1 month, however, plasma levels of 6–7 and 4–5 μg/ml were observed, respectively. The lower dose, therefore, resulted in only a 30% reduction of AG levels during continuous treatment. We conclude that the induction of metabolism is of greater importance in standard-dose than in low-dose therapy.

Concentrations of AAG were not affected by the dose reduction (Fig. 3). The pharmacokinetic properties considered together with this observation suggest that AAG production is limited. Recently, further metabolites have been identified [6, 13]. Jarman et al. reported evidence that hydroxylaminoglutethimide is formed at the expense of AAG [13]. This might have led to the decrease in AAG plasma levels which occurred after 1 week (Fig. 3). Hydroxy-AG was not found in the present study; however, it is reportedly highly unstable [13].

AG plasma concentrations did not bear a relationship to the response to treatment (Table 3). This might indicate that doses lower than 500 mg are active, or that there are stronger determinants for response, such as estrogen receptor (ER) status [15]. Patients in this trial had been assessed for ER status [12], but numbers for ER+ and ER− responders were small and, therefore, not listed in Table 3. The patients in this study did not receive hydrocortisone supplementation, and the results for serum cortisol levels and response to adrenocorticotropin have been published separately [16]. Further studies are needed to elucidate the minimum required dose and the minimum effective plasma concentration of AG.

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