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Single-Dose and Steady-State Pharmacokinetics of Aminoglutethimide

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Summary

The oral pharmacokinetics of aminoglutethimide were determined in 17 patients receiving the drug therapeutically. The absorption of aminoglutethimide after oral intake was almost complete as judged by recovery of radio-labelled drug in the urine. The plasma half-life of the drug was markedly reduced (mean 43%) during multiple-dose administration as compared with a single dose, but only a moderate increase in total clearance (mean 26.9%) was observed. This finding was consistent with a significant reduction (mean 29.2%) in apparent volume of distribution (Vd) occurring during prolonged treatment.

These alterations in drug distribution could also be demonstrated after a drug-free interval of 96 hours during treatment. The reduction in apparent volume of distribution could not be explained by altered plasma protein binding of aminoglutethimide, as evaluated by equilibrium dialysis experiments.

Aminoglutethimide is an inhibitor of the non-glandular steroid aromatase and several enzymes in the steroidogenic pathways in the adrenal cortex (Santen and Samojlik, 1979). This drug causes reduced oestrogen production in postmenopausal women. In combination with a glucocorticoid (Santen et al., 1974) aminoglutethimide has recently been introduced as an effective treatment of advanced breast cancer.

Aminoglutethimide has been shown to enhance hepatic metabolism of drugs metabolised by the mono-oxygenases of the endoplasmic reticulum (Lønning et al., 1984a,b; Santen et al., 1974), and an increase of its own metabolism has been suggested (Murray et al., 1979). As initial side effects of aminoglutethimide may be related to its metabolism (Coombes et al., 1982) further investigations of the disposition of this drug seemed necessary.

Materials and Methods

Patients

17 patients (16 females and 1 male) receiving aminoglutethimide as endocrine therapy for advanced breast cancer, took part in this study after giving their informed consent. Mean age was 63 years (range 47 to 82) and mean weight was 70kg (range 56.5 to 87.0). The dosage schedule was aminoglutethimide 250mg bid for the first 2 weeks, followed by 250mg qid, except in 3 patients who received 125mg bid (Harris et al., 1983). Cortisone acetate was given to the patients receiving the high-dose treatment only (50mg bid for the first 2 weeks, then decreased to 25mg bid). None of the patients were smokers, or used other drugs known to affect drug metabolism.

During the investigation the patients were on a

standard hospital diet without charcoal broiled food.

Protocol and Blood Sampling

Plasma half-life, clearance and volume of distribution of aminoglutethimide were determined under 3 conditions: phase 1, 2 and 3.

Phase 1 (single-dose phase): Patients not previously exposed to aminoglutethimide received a single tablet (250mg) of aminoglutethimide ('Orimeten', Ciba-Geigy). The drug was given at 8am. Blood samples were drawn at 0,1,2,4,6,9,12,24,36,48 and 72 hours after drug intake.

Phase 2 (multiple-dose therapy): The patients received the same daily dose of aminoglutethimide for at least 5 days prior to the study. The doses were administered at strictly 6-hour (250mg) or 12-hour (500mg) intervals for at least the last 48 hours of this 5-day period. Blood samples were drawn at 2-hour intervals for the first 12 hours. Treatment was then discontinued for 24 to 72 hours (for half-life determination) and blood collected as described above for phase 1.

Phase 3 (single-dose administration after phase 2): Patients received multiple doses according to the regimen described above. Aminoglutethimide, but not cortisone acetate, was then discontinued for 96 hours. After this drug-free interval a single dose was administered, and blood samples were collected (as described for phase 1).

Food and other drugs were not given from 8 hours before to 2 hours after the intake of aminoglutethimide. Blood samples were obtained by venous puncture, and the blood allowed to clot for 30 minutes prior to centrifugation. Serum was stored at −20°C until analysis.

Studies with Radiolabelled Aminoglutethimide

Three patients were studied in phase 2 and one patient in phase 1. [2-^{14}C] aminoglutethimide (5.48 μCi/mg, radiochemical purity 98%) was a gift from Ciba-Geigy Ltd (Basel); 7.5 or 25 μCi of this isotope was given as a single pulse together with the ordinary dose of aminoglutethimide, and 24-hour urine samples were collected for the next 3 days. Total recovery of radioactive drug was determined in the urine. In addition, from the patient given 25 μCi, blood samples were collected at 0, 1, 2, 4, 6, 12, 24, 36, 48 and 72 hours after the radioactive pulse was given. Aminoglutethimide plasma concentrations were measured by high-performance liquid chromatography (HPLC) [Schanche et al., 1984], and the total radioactivity in the plasma was determined by liquid scintillation counting. Three of these blood samples, drawn at 2, 8 and 24 hours, were fractionated by HPLC. The aminoglutethimide peak from 5 parallel HPLC runs were pooled, lyophilised, and the radioactivity associated with aminoglutethimide determined by liquid scintillation counting.

Determination of Protein Binding of Aminoglutethimide by Equilibrium Dialysis

Serum obtained from 6 patients in all 3 different conditions (phase 1, 2 and 3) were included. Samples of 0.5ml were transferred to dialysis bags, all of which were placed in a common compartment, and dialysed against 1 litre of 150 mmol/L phosphate buffer (pH 7.4) supplemented with 4 μg/ml of [^{14}C] aminoglutethimide (5 μCi/mg) for 48 hours at 4°C. Samples of 100μL were taken both from the internal and external volumes, mixed with scintillation fluid (10ml of Scint Hei 4), and the radioactivity determined by liquid scintillation counting. The radioactive material was identified as aminoglutethimide by HPLC. Both the final volumes of the bags and the protein concentration were determined.

Drug Analysis

Aminoglutethimide and its main plasma metabolite, N-acetylaminoglutethimide, were determined by reversed-phase liquid chromatography, by a method described in detail by Schanche et al. (1984). Briefly, the column was eluted isocratically at ambient temperature with 11% acetonitrile in 100 mmol/L ammonium formate (pH 3.5). The

flow rate was 2 ml/min. Most samples were analysed in duplicate. The coefficient of variation of this method at a concentration of 0.5 μg/ml was 3.9% and 2.6% for aminoglutethimide and N-acetylaminoglutethimide respectively, and the detection limit about 0.1 μg/ml for both compounds.

In serum from patient GH who was receiving co-trimoxazole in phase 2, interfering material with an absorption maximum (λmax.) at 264nm co-chromatographed with N-acetylaminoglutethimide (λmax. 247). This material was separated from N-acetylaminoglutethimide by increasing the final acetonitrile concentration to 14%.

Aminoglutethimide concentration was found to be stable in serum samples stored at −20°C for one year. N-acetylaminoglutethimide, on the other hand, showed a variable decomposition to aminoglutethimide (mean about 10% degradation/year).

Pharmacokinetic Calculations

Clearance (CL) after single-dose ingestion was calculated by the formula:

$$CL = \frac{F \cdot D}{AUC} \quad \text{(Eq. 1)},$$

where F is the fraction of administered dose systemically available, D is the dose, and AUC is the area under the plasma concentration-time curve from time zero to infinity. AUC was measured by the trapezoidal rule, adding the residual area calculated by extrapolation of curve to infinity after log linear least square regression analysis. The same equation as shown above for single-dose clearance was also applied in the steady-state situation using the AUC measured during a dose interval (AUC^{ss}_{τ}) and the maintenance dose (DM). Half-life was obtained by terminal log least square curve fitting. The apparent volume of distribution (Vd) was calculated by the equations:

$$Vd = \frac{F \cdot D}{\lambda_z \cdot AUC} \quad \text{(single-dose)} \quad \text{(Eq. 2)}$$

or

$$Vd = \frac{F \cdot DM}{\lambda_z \cdot AUC^{ss}_{\tau}} \quad \text{(steady-state)} \quad \text{(Eq. 3)},$$

where λ_z is the smallest disposition rate constant.

Statistical Methods

Normal distribution of parameters were tested for by Q-Q plots (Johnson and Wichern, 1982) of data, their logarithmic or inverse transformations. Comparison of 3 or more parameters, which were normally distributed, was made by analysis of variance (two-way). Two sets of parameters were compared by the Student paired test, using the Bonferroni correction for multiple comparison for the

Table I. Urinary recovery of radioactivity after oral intake of [^{14}C] aminoglutethimide

Patient	Phase	Percentage recovery			
		day 1	day 2	day 3	total
IU	1	51.2	41.4		92.6
JJ	2	69.4	18.4	2.2	90.0
BG	2	61.3	14.6	3.9	79.8
JK	2	86.2	10.3	1.3	97.8
Mean		67.0	20.7	2.5	90.1

determination of the final p value.

When the Q-Q plots suggested that the values were not normally distributed, multiple comparison was made by a two-way non-parametric analysis of variance (Friedman test). Paired data was analysed by the Wilcoxon matched-pair signed test or the Wilcoxon 2-sample test, using the Bonferroni correction for the determination of final p values.

Significance levels were always expressed as two-tailed. Trends were tested for by using the Spearman rank correlation coefficient (r_s).

Results

Knowledge of the absorption of aminoglutethimide after oral administration was critical for the pharmacokinetic calculations. The fraction of dose systemically available (F) could not be determined from experiments involving intravenous injection of the drug, because a useful vehicle has never been tested in animal experiments and the intravenous route has never been used in humans. However, after oral intake of [^{14}C] aminoglutethimide the isotope was almost completely recovered in the urine (table I). This finding was consistent with an absorption fraction approaching 1.

Examples of typical plasma concentration profiles for aminoglutethimide and N-acetylaminoglutethimide in a patient (JK) tested in the 3 different situations are shown in figures 1, 2 and 3.

Peak plasma concentrations were always reached between 1 and 4 hours. Log linear regression analysis of concentrations *versus* time yielded r values of between 0.88 and 0.99 (mean 0.978). 11 of 40 curves (5 phase 1, 5 phase 2, and 1 phase 3) were

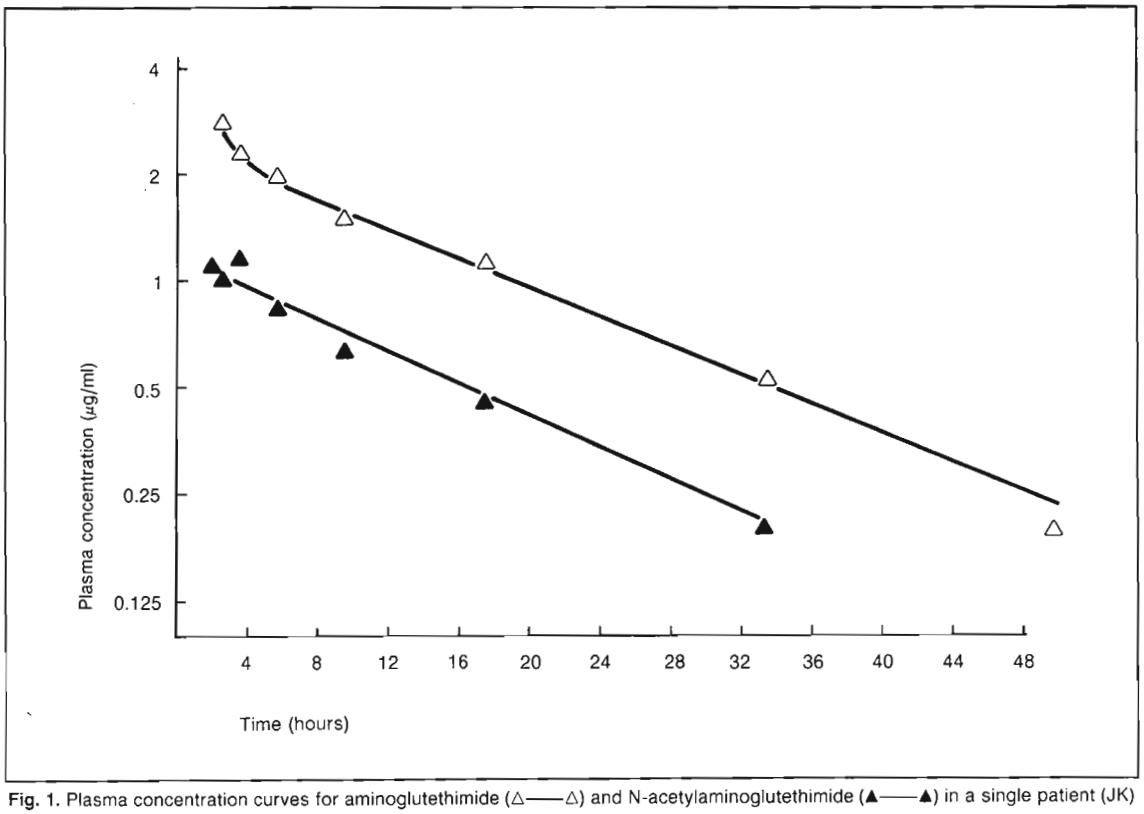

Fig. 1. Plasma concentration curves for aminoglutethimide (△——△) and N-acetylaminoglutethimide (▲——▲) in a single patient (JK) after aminoglutethimide administration in *phase 1*.

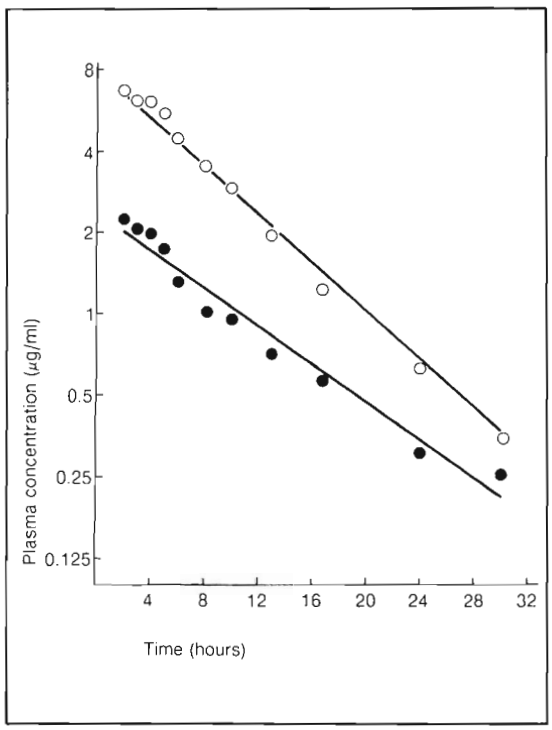

Fig. 2. Plasma concentration curves for aminoglutethimide (○——○) and N-acetylaminoglutethimide (●——●) in a single patient (JK) after aminoglutethimide administration in *phase 2*.

found better fitted by a 2-compartment model (fig. 1). The α-phase was always short-lasting, subsiding within 6 hours. For the other 29 curves a 1-compartment model gave the best curve fit. The highest peak aminoglutethimide concentration was measured in patient JJ during phase 2 (23.1 μg/ml).

Half-lives for N-acetylaminoglutethimide were not significantly different from the half-lives for aminoglutethimide (table III). Concentrations of N-acetylaminoglutethimide were sometimes too low to obtain valid plasma levels after a time period corresponding to 2 half-lives. There was no tendency towards accumulation of this metabolite except in one patient (EK) in whom a small delay in peak concentration together with a slightly longer half-life for N-acetylaminoglutethimide than for the parent compound was seen in phase 2.

The AUC/(N-acetylaminoglutethimide)/AUC (aminoglutethimide) ratio was reduced in all but 2 patients (Wilcoxon $p < 0.001$) during treatment (table III). These 2 patients had the lowest initial ratio. The reduction showed no correlation to the increase in total clearance of aminoglutethimide (r_s between 0.30 and 0.02, depending on whether absolute or relative values were used respectively). However, a significant positive correlation was seen between the initial clearance value and the AUC (N - acetylaminoglutethimide/aminoglutethimide) ratio ($r_s = 0.62$, $p < 0.025$). This ratio, however, was not correlated to the initial half-life ($r_s = 0.22$).

The clearance values, half-lives and distribution volumes in individual patients are shown in table II. When data from all patients are analysed together, a marked (mean 43%) reduction in plasma half-life of the drug was seen between phase 1 and 2 ($p < 0.0001$: Wilcoxon's matched-pair signed rank test). Clearance was significantly increased (mean 26.5%; Student $p < 0.01$ log normal distribution), and volume of distribution (Vd) reduced (mean 30.4%; Student $p < 0.005$, log normal distribution).

In the 6 patients in whom single-dose pharmacokinetics during treatment were studied (phase

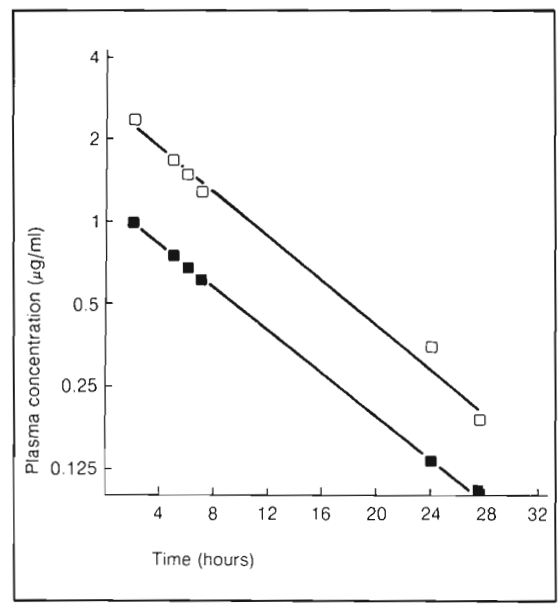

Fig. 3. Plasma concentration curves for aminoglutethimide (□——□) and N-acetylaminoglutethimide (■——■) in a single patient (JK) after aminoglutethimide administration in *phase 3*.

3, table II), analysis of variance showed a significant difference between the distribution volumes in the 3 test situations ($p < 0.005$). A paired Student test yielded significant differences between phases 1 and 3 (mean 23.0% reduction; $p < 0.05$) as well as between phases 1 and 2 (mean 43.8% reduction; $p < 0.025$) but not between phases 2 and 3. Half-lives were also found to be significantly different in the 3 test situations (Friedman, $p < 0.005$). A Wilcoxon 2-sample test gave significant differences in half-lives between phases 1 and 2 ($p < 0.01$), but none of the other possible comparisons yielded significant differences. However, the small number of observations made type II error rather likely. Clearance values obtained in the 3 different phases were not found to be significantly different by analysis of variance.

The elimination curve for [^{14}C] aminoglutethimide was determined in a patient receiving a single dose (25 μCi) of radioactive drug together with a dose of unlabelled drug during treatment at steady-state. The fraction of radioactivity in serum ac-

Table II. Pharmacokinetic parameters of aminoglutethimide given as a single dose (phase 1), at steady-state (phase 2), and as a single dose following multiple-dose therapy (phase 3)

Patient	Phase 1			Phase 2				Phase 3			
	Vd (L)	CL (L/h)	$t_{½}$ (h)	duration of treatment (weeks)	Vd (L)	CL (L/h)	$t_{½}$ (h)	duration of treatment (weeks)	Vd (L)	CL (L/h)	$t_{½}$ (h)
EK	85.6	4.05	14.6	6	73.2	6.55	7.4				
SH	62.3	2.37	18.2	12	52.0	4.53	7.6				
NH	40.5	1.92	14.6	12	26.1	1.97	9.2				
KÅ	50.6	2.95	11.9	12	30.6	3.16	6.7				
LS	41.9	2.45	9.7	9	34.1	2.32	9.1				
MF	71.5	6.08	8.2	12	74.8	7.62	6.8				
EF	114.5	4.47	17.8	12	92.6	4.34	14.8				
IW	45.7	1.65	19.2	1	34.2	2.31	10.3				
GS	59.3	4.74	8.7	12	46.0	6.25	5.1				
ME	97.7	3.51	19.3	4	59.4	5.02	8.2				
GH	58.7	2.74	14.8	1	55.3	3.05	12.6				
JK[a]	102.1	4.96	14.3	24	84.7	9.31	6.3	12	94.4	8.79	7.4
JJ[a]	70.1	2.60	18.7	20	30.9	2.32	9.2	12	53.5	3.26	11.4
EB[b]	119.1	5.49	15.0	1	47.3	5.29	7.1	12	95.0	5.12	12.9
JH[b,c]	88.8	2.93	21.0	1	41.6	2.87	10.0	12	68.9	2.19	21.8
BG[a,c]	125.0	3.52	24.6	16	78.1	5.71	9.5	8	72.3	4.26	11.8
GG[b,c]	57.5	2.93	13.6	1	35.2	2.49	9.8	4	49.0	3.01	11.3
Mean	75.9	3.49	15.5		52.8	4.42	8.9		72.2	4.44	12.8
± SD	26.9	1.24	4.3		20.7	2.09	2.4		17.9	2.16	4.4

a Phase 3 was determined before phase 2.
b Phase 2 was determined before phase 3.
c Aminoglutethimide 125mg bid.
Abbreviations: Vd = apparent volume of distribution; CL = clearance; $t_{½}$ = plasma half-life.

Table III. Pharmacokinetic parameters of N-acetylaminoglutethimide (N-AcAG) after administration of aminoglutethimide (AG) as a single dose (phase 1), at steady-state (phase 2), and as a single dose following multiple-dose therapy (phase 3)

Patient	Phase 1		Phase 2			Phase 3		
	$t_{1/2}$ (h)	AUC ratio (N-AcAG/AG)	duration of treatment (weeks)	$t_{1/2}$ (h)	AUC ratio (N-AcAG/AG)	duration of treatment (weeks)	$t_{1/2}$ (h)	AUC ratio (N-AcAG/AG)
EK	14.2	0.70	6	10.3	0.64			
SH	16.5	0.25	12	8.3	0.16			
NH	14.4	0.12	12	12.6	0.13			
KÅ	11.9	0.13	12	7.9	0.07			
LS	13.6	0.18	9	9.7	0.14			
MF	6.3	0.45	12	4.7	0.18			
EF	17.6	1.12	12	12.6	0.44			
IW	19.8	0.41	1	10.5	0.29			
GS	9.0	0.29	12	7.0	0.09			
ME	17.6	0.81	4	8.5	0.41			
GH	17.4	0.23	1	10.1	0.11			
JK	12.8	0.44	24	8.5	0.37	12	7.6	0.45
JJ	17.1	0.23	20	13.2	0.08	12	13.0	0.16
EB	13.1	0.44	1	10.3	0.44	12	11.0	0.23
JH	20.8	0.31	1	8.9	0.25	12	22.6	0.17
BG	24.1	0.31	16	9.8	0.17	8	11.8	0.18
GG	13.3	0.11	1	9.4	0.10	4	15.8	0.11
Mean	15.3	0.38		9.6	0.24		13.6	0.22
± SD	4.3	0.27		2.1	0.17		5.1	0.12

Abbreviations: AUC = area under the plasma concentration-time curve; $t_{1/2}$ = plasma half-life.

counted for by aminoglutethimide was determined by HPLC at 3 time points, and was found to be 75.6 to 77.1%, and independent of the time elapsed after oral intake of the labelled drug. The elimination curve was constructed for [^{14}C] aminoglutethimide using a factor of 0.765 for the correction for other radioactive material than aminoglutethimide in serum. This elimination curve showed an excellent log linear curve fit ($r = 0.99$) consistent with a 1-compartment model. Furthermore, half-life, time to reach peak concentration and volume of distribution of the labelled aminoglutethimide were the same as those for the unlabelled drug measured concomitantly.

Plasma Protein Binding

The binding of aminoglutethimide to plasma protein was between 13.0 and 32.4% (mean 24.3% ± SD 5.4%) as determined by equilibrium dialysis. No correlation was found between the volume of distribution and protein binding.

Discussion

Absorption of aminoglutethimide after oral administration was evaluated by determination of urinary recovery of radioactivity. This was performed after single-dose administration (phase 1)

and during multiple-dose therapy at steady-state (phase 2) because an increased absorption fraction during treatment would mimic a reduction in volume of distribution. Almost complete absorption was observed under both conditions (table I), which is in agreement with data provided by others (Nicholls, 1982). But possible interindividual variation in absorption should be kept in mind.

A presystemic clearance of drug might occur after oral administration. This might obscure determination of pharmacokinetic parameters. However, a rough estimate of the presystemic clearance of aminoglutethimide can be obtained from knowledge of hepatic blood flow (90 L/h; Ganong, 1973), and aminoglutethimide distribution between blood cells and plasma. The latter parameter has been estimated to be 1.4 to 1.7 (Thompson et al., 1981). Total blood clearance may be obtained from the following relationship: plasma CL/blood CL = blood concentration/plasma concentration (Rowland and Tozer, 1980). Assuming that total clearance equals hepatic clearance, the data (table II) are consistent with a first-pass elimination of 2 to 8%. We used an F value (fraction of administered dose systemically available) of 1 in our calculations, and no correction was made for first-pass metabolism. This may cause an overestimation of the clearance values, especially at high values, because the first-pass elimination fraction, and thereby the F value, are proportional to hepatic clearance. Therefore, the increase in clearance from phase 1 to phases 2 and 3 may be slightly overestimated, while the reduction in volume of distribution under the same conditions may in fact be underestimated.

Thompson et al. (1981) reported that the pharmacokinetics of aminoglutethimide given as a single dose (tablet or solution) were best fitted by a 2-compartment model. They found that peak concentrations were reached within 0.3 to 1.5 hours after drug intake. The α-phase subsided within a few hours. In contrast, we found that the pharmacokinetics of aminoglutethimide in most patients were best explained by a 1-compartment model. This may be related to the fact that our patients showed a somewhat later peak plasma concentration. Delayed absorption may mask a rapid α-phase and thereby a distinct change in curve slope, which is required for a 2-compartment model fit.

N-acetylaminoglutethimide is regarded as an inactive metabolite of aminoglutethimide since it has been shown to have minor or no inhibitory effect on desmolase or on aromatase *in vitro* (Coombes et al., 1980; Foster et al., 1983). The elimination of this metabolite showed the same elimination rate constant as for aminoglutethimide, suggesting that production is the rate-limiting step in the elimination of this metabolite (Rowland and Tozer, 1980). The AUC ratios between N-acetylaminoglutethimide and aminoglutethimide obtained in the present study (table III) were in accordance with results obtained by Adam et al. (1984), but our values showed a larger spread with some especially high values in phase 1. However, AUC ratios above 1 have been reported previously (Coombes et al., 1980, 1982; Stuart-Harris et al., 1985). We observed a marked decrease in the AUC ratio after prolonged treatment in all but 2 patients. During phase 1, these 2 patients had the smallest AUC ratios observed. Our finding of a positive correlation between the initial clearance value of aminoglutethimide and the AUC ratio contrasts with the findings of Adam et al. (1984) who reported a significant negative correlation coefficient.

The AUC ratio of N-acetylaminoglutethimide/aminoglutethimide expresses the relationship between the serum concentrations of aminoglutethimide and N-acetylaminoglutethimide in the different phases. Since no information is available regarding the clearance of N-acetylaminoglutethimide, it is not possible to calculate from our data the fraction of aminoglutethimide metabolised to the N-acetyl metabolite (Rowland and Tozer, 1980). While the first investigations based on unspecific spectrophotometric assays reported 39 to 54% of aminoglutethimide to be eliminated in the urine unchanged and 4 to 25% excreted as N-acetylaminoglutethimide (Douglas and Nicholls, 1965), recent investigations using HPLC methods have reported mean values of aminoglutethimide excreted in the urine unchanged after a single dose to be in the range of 20% during 24 hours (Coombes et al.,

1982) to 10.7% during 48 hours (Adam et al., 1984). Values of N-acetylaminoglutethimide reported in the same investigations were 6.4% and 2.9%, respectively. Urinary excretion of unchanged aminoglutethimide therefore accounts for only a minor part of total aminoglutethimide clearance. Renal clearance of N-acetylaminoglutethimide has been found to be identical to the renal clearance of aminoglutethimide (Adam et al., 1984). If the volumes of distribution of aminoglutethimide and N-acetylaminoglutethimide are of the same magnitude, their identical disposition rate constants (λ) obtained in our study suggest that total N-acetylaminoglutethimide clearance is identical to or higher than the total clearance of aminoglutethimide (Rowland and Tozer, 1980). Therefore, a further metabolism of N-acetylaminoglutethimide cannot be excluded, and an alteration in metabolic conversion of N-acetylaminoglutethimide might contribute to the observed change in the AUC ratios of aminoglutethimide/N-acetylaminoglutethimide.

We found a reduction in mean serum aminoglutethimide half-life from approximately 15 hours during single-dose adminstration (phase 1) to about 9 hours during multiple-dose therapy (phase 2) [table II]. Similar data have been obtained by Murray et al. (1979) and Thompson et al. (1981) using a spectrophotometric assay, and more recently by Adam et al. (1984) using a HPLC technique. Murray et al. and Adam et al. obtained a slightly lower, and Thompson et al. a somewhat higher clearance value after single-dose administration than that obtained in the present study. Murray et al. (1979) reported a pronounced increase (about 100%) in clearance during multiple-dose therapy, whereas only a moderate increase (mean 26.5%) was observed by us (table II). Adam et al. (1984) reported a 36.6% increase in systemic clearance after 1 week of treatment.

Indirect evidence supports the conclusions obtained in this study and by Adam et al. (1984). Recently, several hydroxylated metabolites of aminoglutethimide have been identified in human urine (Foster et al., 1984; Jarman et al., 1983) in addition to the previously reported minor metabolites N-formylaminoglutethimide and nitroglutethimide (Baker et al., 1981). Most of these metabolites seem to be of minor importance quantitatively, except for hydroxyaminoglutethimide (Jarman et al., 1983). The concentration of this metabolite has recently been measured in urine from several patients, showing large interindividual variation (Goss et al., 1985). Unfortunately, the daily urinary output of this metabolite was quantitated in one patient only, accounting for between 9 and 25% of the aminoglutethimide dose, administered as multiple-dose therapy. As urinary excretion of this metabolite increased significantly during the first weeks of aminoglutethimide therapy, an induced production of this metabolite has been suggested to be responsible for the increase in aminoglutethimide clearance (Goss et al., 1985; Jarman et al., 1983). If this metabolite is excreted by the urinary pathway only, an increased metabolic conversion from aminoglutethimide towards hydroxyaminoglutethimide, in the order of 25% of the aminoglutethimide dose as observed in the patient by Goss et al. (1985), could correspond to an increase in total aminoglutethimide clearance of less than 35%, assuming that other metabolic pathways remain unchanged. Furthermore, the findings of Stuart-Harris et al. (1985) of a linear relationship between the dose of aminoglutethimide and the measured plasma aminoglutethimide concentration suggest that only minor alterations in aminoglutethimide clearance occur after one month on a dose regimen of 62.5mg bid. On the other hand, we found aminoglutethimide induction of warfarin metabolism to be dose-dependent, with a significant increase in warfarin clearance when the aminoglutethimide dose was increased from 125mg bid to 250mg qid (unpublished results). Therefore, the results obtained by Stuart-Harris et al. (1985) also suggest that the autoinduction of aminoglutethimide metabolism is of small magnitude.

Total urinary output of aminoglutethimide, N-acetylaminoglutethimide and hydroxyaminoglutethimide accounted for only between 40 and 75% of the aminoglutethimide dose administered at steady-state in the single patient of Goss et al. (1985), and the metabolism of aminoglutethimide is still incompletely understood.

The volume of distribution after single-dose administration reported by Thompson et al. (1981) as well as the value reported by Adam et al. (1984) equalled that obtained by us in phase 1. In the study of Adam et al. (1984) the volume of distribution was measured after one week of treatment, and a non-significant mean reduction of about 8.5% was observed.

A marked reduction in the volume of distribution of aminoglutethimide after multiple-dose therapy was demonstrated in the present study. This reduction was significant both when comparing volumes of distribution in phase 1 with values obtained at steady-state (phase 2) and after intake of a single-dose following multiple-dose administration (phase 3). This unexpected finding does not seem to be an artefact related to a different experimental design or different equations used for the assessment of volume of distribution. The determination of the volume of distribution of aminoglutethimide after a single oral dose or multiple doses at steady-state is not dependent on the selection of model. The equations for the calculations of Vd (see equations 2 and 3) are model independent, and the disposition rate constants (λ) were estimated from the final slope of the curves (Chiou, 1982). The volume of the central compartment (V_c) obtained after intravenous bolus injections has been shown to depend on sampling frequency (Gupta et al., 1979). However, the volume of distribution during the terminal phase is independent of sample protocols as long as the time interval is sufficient to obtain the smallest disposition rate constant (λ_z).

A decrease in volume of distribution during treatment would result in reduction in half-life of the drug, since half-life, in contrast to clearance, is a distribution volume-dependent parameter (Gibaldi and Koup, 1981). Thus, the marked reduction in half-life observed in the present study could to a large extent be attributed to a decrease in the volume of distribution, and only partly to induction of drug metabolism.

In general, a reduction in volume of distribution must be related to alteration of either a central or peripheral compartment. We found no changes in plasma protein binding of aminoglutethimide during treatment. Therefore, other explanations should be considered, e.g. a change in tissue binding of the drug (Gibaldi and Koup, 1981). Saturable tight binding of aminoglutethimide to peripheral tissue(s) may occur. In this case, the slow release of aminoglutethimide from these sites should give rise to a terminal elimination characterised by a long half-life. This was definitely not observed when multiple-dose administration was discontinued. However, we cannot exclude the possibility that a very slow release of aminoglutethimide from peripheral compartments might occur resulting in indetectable plasma concentrations of the drug.

Infusions of hydrocortisone has been reported to influence the metabolism of antipyrine (Breckenridge et al., 1973) and phenylbutazone (Aarbakke et al., 1977). However, cortisol plasma values measured during treatment in 10 patients participating in the present study were all within the normal range except during the initial fortnight where cortisone acetate was administered at double doses. Therefore, there is no obvious reason to assume alteration in disposition of aminoglutethimide induced by cortisol levels.

Enterohepatic circulation of aminoglutethimide may affect the apparent volume of distribution. A substantial enterohepatic circulation of aminoglutethimide has been demonstrated in the rat (Egger et al., 1982), whereas we found only a limited excretion of radioactivity (2.6 and 7.2% of the administered dose) into the bile of 2 patients with bile drainage (unpublished results). Nevertheless, the possibility of enterohepatic circulation of aminoglutethimide in humans deserves further attention.

Only 2 patients participating in this study developed transient fever and skin rash as side effects (KÅ and MF). No special features regarding the disposition of either aminoglutethimide or its N-acetylated metabolite in these 2 patients was observed (table II). The patient (GG) receiving treatment with aminoglutethimide 250mg qid later developed a serious granulocytopenia. Other side effects were limited to an initial moderate lethargy;

no other side effects were seen during continuous treatment.

Therapeutic Implications

Our data suggest that autoinduction of aminoglutethimide metabolism is not the only mechanism whereby the half-life of the drug is decreased after multiple-dose treatment. Reduction in the volume of distribution of aminoglutethimide during long term treatment may contribute significantly to the overall disposition of this drug. It has been claimed that the side effects during initiation of aminoglutethimide treatment may be related to insufficient drug metabolism (Murray et al., 1979). Our findings suggest that the reduction of side effects seen by modification of the conventional dosage schedule for the first 2 weeks (i.e. aminoglutethimide at half dosage, double dose of the glucocorticoid) [Kvinnsland et al., 1984; Murray et al., 1979] probably results from the increased glucocorticoid administration.

A decrease in the volume of distribution, as observed in the present study (table II), would be expected to produce larger variations in plasma drug concentration during a dose interval and higher peak concentrations. However, the mean plasma concentration should remain unchanged since this parameter depends only on clearance.

During multiple-dose therapy we observed no side effects except for a single case of granulocytopenia (Kvinnsland et al., 1984). Initial side effects, e.g. lethargy, occurred at much lower aminoglutethimide plasma concentrations than those seen during multiple-dose therapy.

Our data therefore suggest that induction of metabolism of aminoglutethimide is of moderate magnitude and minor importance in most patients. We recommend a full dose schedule of aminoglutethimide from the start of therapy.

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