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The effect of acetylator phenotype on the disposition of aminoglutethimide

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1 Aminoglutethimide (AG) 500 mg was administered orally to four normal volunteers and eight patients undergoing treatment for metastatic breast cancer. In each subject the acetylator phenotype was established from the monoacetyldapsone (MADDS)/dapsone (DDS) ratio.

2 Acetylaminoglutethimide (acetylAG) rapidly appeared in the plasma and its disposition paralleled that of AG.

3 A close relationship ($P < 0.01$) was observed between the acetyl AG/AG and MADDS/DDS ratio suggesting that AG may undergo polymorphic acetylation like DDS.

4 AG half-life was 19.5 ± 7.7 h in seven fast acetylators of DDS and 12.6 ± 2.3 h in five slow acetylators and its apparent metabolic clearance was significantly ($P < 0.01$) related to the acetylAG/AG ratio.

5 Over 48 h the fast acetylators excreted 7.7 ± 4.4% of the administered AG dose in the urine as unchanged AG as compared to 12.4 ± 2.8% in slow acetylators. A much smaller fraction of the dose was excreted as acetylAG: 3.6 ± 1.5% by fast and 1.9 ± 1.0% by slow acetylators respectively.

6 After 7 days treatment with AG at an accepted clinical dose regimen to the eight patients there were significant reductions in the half-lives of AG ($P < 0.01$) and acetylAG ($P < 0.01$) and a trend ($0.1 > P > 0.05$) towards reduction of the acetylAG/AG ratio which became significant ($P < 0.05$) if the one patient on a known enzyme inducer was omitted. The mean apparent volume of distribution was not significantly ($P > 0.1$) altered but the mean apparent systemic clearance of AG was increased ($P < 0.05$). These changes are attributed to auto-induction of oxidative enzymes involved in AG metabolism.

Keywords aminoglutethimide acetylation polymorphism pharmacokinetics enzyme induction

Introduction

Aminoglutethimide, AG, [3-(4-aminophenyl)-3-ethyl-piperidine-2,6-dione] is being used increasingly for the palliative endocrine therapy of metastatic breast carcinoma in post-menopausal women (Santen *et al.*, 1982a). It inhibits both adrenal steroid synthesis (Cash *et al.*, 1967; Fishman *et al.*, 1967) and the peripheral aromatisation of adrenal androgens (Santen *et al.*, 1982b). When given with replacement hydrocortisone, AG blocks oestrogen synthesis in the adrenal cortex, in extraglandular peripheral tissues containing aromatase and in the breast carcinoma itself.

Whilst much is now known of its biochemical

effects, details of its metabolism remain obscure. Older studies (Douglas & Nicholls, 1972) have indicated that *N*-acetylation to acetamidoglutethimide (acetylAG) is a major (4-25%) pathway and more recent studies suggested that this acetylation is polymorphic (Coombes *et al.*, 1982). Other metabolites that have been demonstrated are *N*-formyl-aminoglutethimide, nitroglutethimide and *N*-hydroxylaminoglutethimide (Baker *et al.*, 1981). However, little information exists relating the acetylation phenotype to the pharmacokinetics of aminoglutethimide (AG) and acetamidoglutethimide (acetylAG). Furthermore, repeated doses of AG result in autoinduction (Murray *et al.*, 1979) and the influence of this process upon the pharmacokinetics of the drug has not been studied.

In this paper we report studies relating the pharmacokinetics of AG to acetylator phenotype and autoinduction.

Methods

Subjects

After approval by the Ethics Committee of Guy's Hospital, four healthy volunteers agreed to participate in the study. Eight patients with progressive metastatic breast carcinoma after prior endocrine therapy (ovarian ablation or tamoxifen) in the Breast Unit, Guy's Hospital, who were about to start treatment with aminoglutethimide gave informed consent to participate in the study. Details of these subjects appear in Table 1. All the normal volunteers and patients had normal haematological indices and clinical chemistry except for patient PM who had hypercalcaemia.

Acetylator phenotyping

The acetylator phenotype was determined according to the criteria of Gelber *et al.* (1971) using the ratio of monoacetyldapsone (MADDS) to dapsone (DDS) concentrations in plasma at 2–4 h following oral administration of 100 mg DDS. Plasma DDS and MADDS concentrations were assayed by a modification of the high performance liquid chromatographic method of Carr *et al.* (1978).

Pharmacokinetic study of aminoglutethimide

AG 500 mg was administered in a gelatin capsule; this was the first treatment dose in the patients with breast cancer. All subjects were given this dose except patient MF (Table 1) who received 375 mg also in a gelatin capsule due to her low body weight of 42 kg. Each received the AG capsule with 200 ml water after an overnight fast. No alcohol or smoking was allowed during the pharmacokinetic sampling. It was thought unethical to stop other therapies which the patients was receiving (Table 1). None of these drugs was polymorphically acetylated nor were they known microsomal enzyme inducers (except in the case of patient MF who was receiving chronic treatment with phenytoin). Food was given 4 h after drug administration. Venous blood samples were taken before dosing and at 0.5, 1, 1.5, 2, 3, 4, 5, 6, 9, 12, 15, 24, 30 and 48 h in the volunteers and at 2, 4, 6, 9, 12, 15, 24, 30 and 48 h in the patients. Blood was taken into lithium heparin tubes via an indwelling cannula fitted with a heparin lock. The plasma was immediately separated and stored at −20°C pending analysis. Two total urine collections were made over 0–24 h and 24–48 h in each subject. The volumes were noted and aliquots were stored at −20°C.

After completion of this sampling schedule, each patient but not the normal subjects, recieved AG 250 mg 12 hourly as Orimeten (Ciba-Geigy) tablets plus hydrocortisone 20 mg 12 hourly. After 1 week of therapy AG was stopped for a 48 h 'washout' period and the plasma and urine samples taken as described above following the same single dose of AG 500 mg in a gelatin capsule. During this second phase, replacement therapy with hydrocortisone was continued.

Drug analysis

AG and acetylAG were simultaneously estimated in plasma and urine by high performance reverse phase liquid chromatography. In brief, 0.5 ml plasma or urine was mixed with 15 μl of a solution of 100 μg/ml phenacetin in methanol (internal standard) and 0.5 ml pH 6 acetate buffer and then extracted with 3 ml dichloromethane for 10 min. After centrifugation the organic phase was transferred to a clean dry tube and evaporated to dryness in a stream of dry nitrogen at 40°C. The residue was taken up in 250 μl of solvent system (methanol/water v/v 42:58) and 50 μl injected into the chromatograph. The solvent was delivered at 1 ml/min to a 25 cm × 4.6 mm μ Bondapak C18 column of 10 μm particle size (Waters Associates,

Table 1 Details of subjects studied

Acetylator phenotype	*Subject*	*Sex*	*Age (years)*	*Weight (kg)*	*Height (cm)*	*Initial AG dose (mg)*	*Concurrent drug therapy*
Fast	DB	F	71	58.7	155	500	None
	MF	F	65	41.7	163	375	D, Pa, Ph
	*PM	F	41	68.0	165	500	D, Pa, Pd, Pr, Di
	EH	F	73	54.5	150	500	None
	MT	F	26	74.0	150	500	Normal subject
	AM	M	31	65.0	168	500	Normal subject
	HE	F	71	62.5	150	500	A, H, M, I
Slow	PJ	F	74	56.1	150	500	None
	PP	M	29	92.0	180	500	Normal subject
	EdH	F	40	44.0	173	500	T
	ML	F	42	68.1	173	500	Do, F, C, K
	SA	M	25	65.0	169	500	Normal subject

Key to concurrent drugs:
A = amiloride; C = cyclopenthiazide; D = dextropropoxyphene; Di = diamorphine; Do = dothiepin; F = flurbiprofen; H = hydrochlorthiazide; I = ibuprofen; K = potassium chloride; M = methyldopa; Pa = paracetamol; Pd = prednisolone; Ph = phenytoin (200 mg/day); Pr = propranolol; T = thyroxine.
* Patient hypercalcaemic

Waltham, MA, USA). AG and acetylAG were detected by UV absorption at 245 nm.

Peaks present in the h.p.l.c. effluent were identified by their retention time and co-chromatography with authentic AG and acetyl-AG. No other metabolites of AG were available to us and it is possible that these are unresolved by this assay. The *N*-formyl derivative may have similar physico-chemical properties to acetylAG but this is a minor metabolite accounting for approximately 0.5% of an oral dose of AG (Baker *et al.*, 1981; Coombes *et al.*, 1982). The interassay coefficients of variation ($n = 6$) for AG in plasma ranged from 14.3% at 1 μg/ml to 6.9% at 8 μg/ml and in urine from 3.7% at 10 μg/ml to 1.9% at 160 μg/ml. For acetylAG the interassay coefficients of variation were in plasma 8.3% at 0.5 μg/ml and 4.8% at 4 μg/ml and in urine 4.5% at 5 μg/ml and 0.8% at 80 μg/ml.

Reagents

All solvents were Analar grade and were used as purchased from BDH Chemicals Ltd (Poole, Dorset).

We are grateful to Dr M. Jarman (Royal Marsden Hospital, Sutton) and Professor P. J. Nicholls (Welsh School of Pharmacy, Cardiff) for kind gifts of acetylAG and to Dr V. A. John of Ciba-Geigy Ltd (Horsham, Sussex) for the gift of pure AG.

Data analysis

The terminal elimination rate constant (λ_z) was determined by least squares fitting of ln (plasma concentration) on time from which $t_{½} = 0.693/\lambda_z$. Areas under the plasma concentration *vs* time curve (AUC) were estimated by the linear trapezoidal rule with appropriate extrapolation to infinite time. No intravenous form of AG is available and there is presently no formal estimate of the bioavailability fraction (F) of this drug available. However, on the basis of the elimination of radioactivity following oral administration of radio-labelled AG, Dalrymple & Nicholls (1984) have suggested that the bioavailability of the drug from solution is essentially complete and that this is likely also to apply to solid formulations of the drug. Apparent systemic clearances (CL) and apparent volumes of distribution (V) can be determined assuming

$$F = 1 \text{ from } CL = \frac{\text{Dose}}{\text{AUC}} \text{ and } V = \frac{CL}{\lambda_z}.$$

Renal AG clearance was found from

$$\frac{\text{Amount excreted in urine 0 to 48 h}}{[\text{AUC}]_0^{48}}$$

and apparent metabolic clearance obtained by subtraction from apparent systemic clearance.

(To convert concentration to μmol/l, multiply by 4.268 for AG and 3.619 for acetylAG.)

The distribution of the variates considered in this investigation are unknown. Therefore non-parametric statistical methods were used throughout: Spearman rank correlation, Mann-Whitney U test for two independent samples, and Wilcoxon matched pairs signed ranks test for two related samples. Regression lines were fitted by the non-parametric method of Brown and Mood (Daniel, 1978).

Results

Absorption of AG was rapid following oral administration (Figures 1 and 2) although the sampling schedule adopted did not allow determination of absorption rate constants. AcetylAG was rapidly generated from AG and its disposition profile paralleled that of AG closely.

Table 2 shows the acetylator phenotype as determined by the MADDS/DDS ratio and the corresponding acetylAG/AG ratio determined from the ratio of $[AUC]_0^\infty$ for each compound. Because of the parallelism of disposition similar ratios were obtained from the individual plasma concentrations of AG and acetylAG at any time point. A close relationship ($r_s = 0.87$; $P < 0.01$) exists between the MADDS/DDS and acetylAG/AG ratios. In the absence of a larger population study it is not possible to assert that a bimodal distribution of AG acetylation exists although this close relationship suggests this may be so.

The pharmacokinetic parameters of AG and acetylAG following a single dose of AG are summarised in Table 3. The mean half-life of AG in the group of fast DDS acetylators studied was 19.5 h as compared with 12.6 h in the slow acetylators ($P < 0.05$; excluding MF as she was probably fully induced with phenytoin). The relationship of half-life with the AG acetylation ratio was significant ($r_s = 0.724$; $P < 0.01$). There were no significant differences in the apparent volume of distribution between these two groups or in the maximum plasma AG concentration attained (overall mean 8.5 ± 1.9 (s.d.) μg ml^{-1}) or time of maximum plasma concentration (overall mean 1.1 ± 0.6 h) between the two groups. The apparent metabolic clearance was therefore also related to the

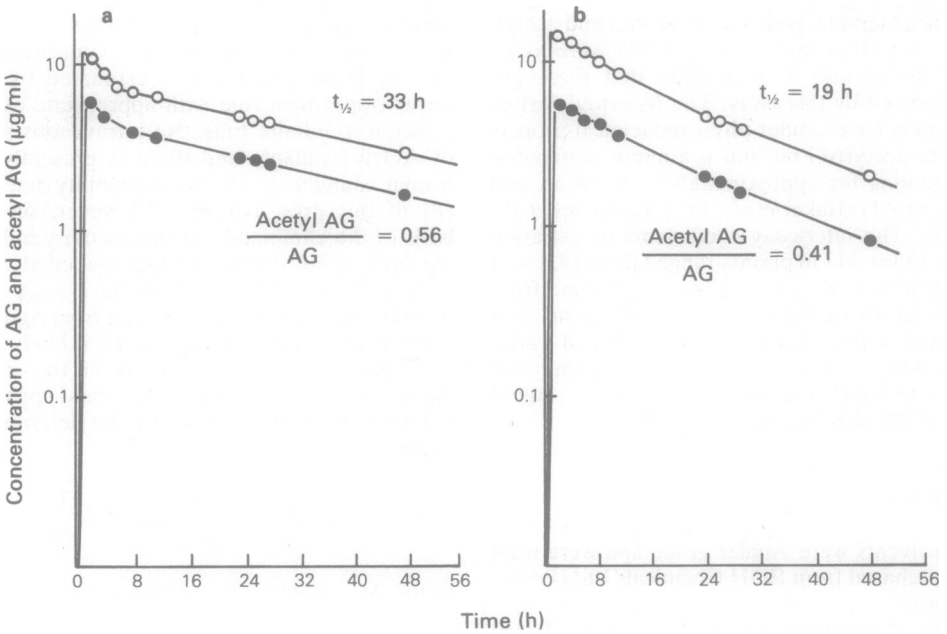

Figure 1 Plasma concentrations of aminoglutethimide (AG: ○) and acetylaminoglutethimide (AcetylAG: ●) in a patient (DB) of rapid acetylator phenotype after administration of a single dose of 500 mg AG at the first dose (a, before induction) and after 1 week's therapy with AG 250 mg, 12 hourly and a 48 h 'washout' period (b, after induction). The ratio of the areas under the plasma concentration, time curves (acetylAG/AG) are shown for each study.

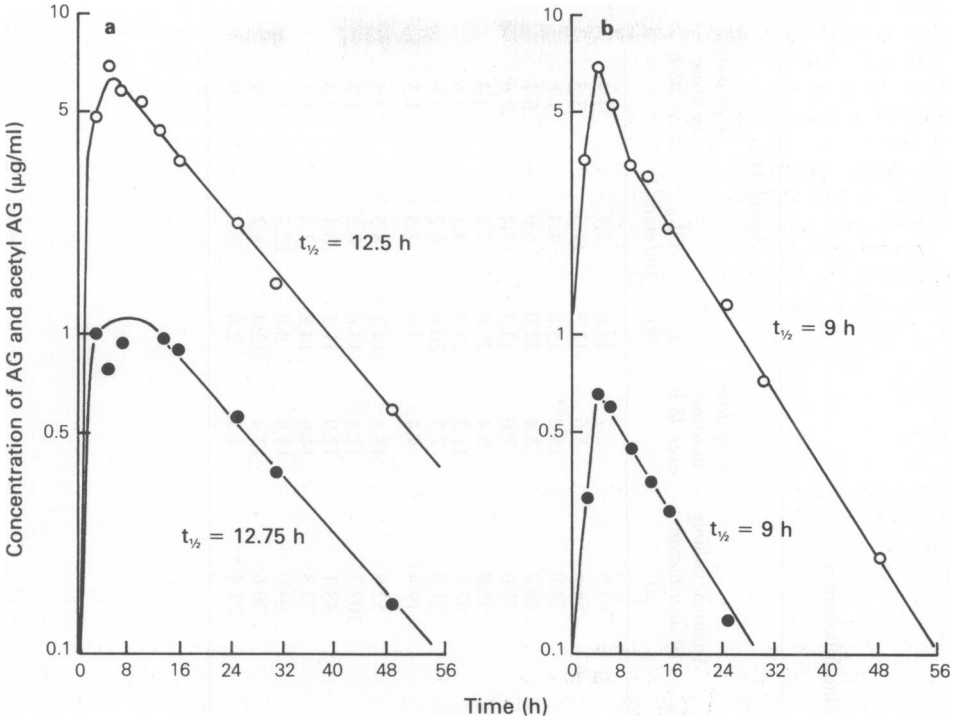

Figure 2 Plasma concentration for aminoglutethimide (AG: ○) and acetyl aminoglutethimide (AcetylAG: ●) in a patient (ML) of slow acetylator phenotype after administration of a single dose of 500 mg AG at the first dose (a, before induction) and after 1 week's therapy with AG 250 mg, 12 hourly and a 48 h 'washout' period (b, after induction).

AG acetylation ratio ($r_s = -0.715$; $P < 0.01$). Thus lower acetylation ratios ('slow acetylation') were associated with higher clearances and shorter half-lives. A similar result was obtained using plasma AUC determined over the 48 h sampling period, rather than extrapolation to infinite time. This indicates the relatively small contribution of the infinite correction term to overall AUC.

The percentage of the AG dose excreted in the urine as AG and acetylAG over 48 h after a single dose of AG in fast and slow DDS

Table 2 Acetylation phenotype and plasma AUC of aminoglutethimide and its acetyl metabolite

Acetylator phenotype	*Subject*	*MADDS / DDS ratio*	*AG $[AUC]_0^\infty$ (μg ml^{-1} h)*	*Acetyl AG $[AUC]_0^\infty$ (μg ml^{-1} h)*	*Acetyl AG $[AUC]_0^\infty$ / AG $[AUC]_0^\infty$*
Fast	DB	1.47	383.8	223	0.58
	MF	0.50	112.3	25.5	0.23
	PM	0.50	140.1	34.3	0.25
	EH	1.08	230.0	126.3	0.55
	MT	0.41	122.5	31.4	0.26
	AM	0.41	174.3	61.5	0.41
	HE	0.64	171.8	68.4	0.40
Slow	PJ	0.33	200.5	38.0	0.19
	PP	0.22	92.2	13.9	0.15
	EdH	0.29	183.8	24.3	0.13
	ML	0.14	129.4	30.4	0.22
	SA	0.12	91.7	10.8	0.12

Table 3 Pharmacokinetic parameters for aminoglutethimide and its acetyl metabolite following a single dose (500 mg) of aminoglutethimide

		Aminoglutethimide						*Acetyl-AG*		
Acetylator phenotype	*Subject*	$t_{½}$ *(h)*	*Apparent systemic clearance CL (ml min^{-1})*	*Renal clearance CL_R (ml min^{-1})*	*Apparent metabolic clearance CL_M (ml min^{-1})*	*Apparent volume of distribution (l)*	*% of dose in urine over 48 h*	$t_{½}$ *(h)*	*CL_R (ml min^{-1})*	*% of dose in urine over 48 h*
Fast	DB	33.2	21.7	0.9	20.8	63.6	2.7	33.8	2.7	4.4
	MF*	11.5	55.7	4.4	51.3	54.6	5.6	11.3	11.5	3.3
	PM**	14.4	59.5	19.9	39.6	74.0	15.0**	15.8	4.4	1.6
	EH	19.2	27.8	1.5	26.3	46.2	3.4	22.0	6.0	6.1
	MT	12.1	68.0	5.1	62.9	71.0	7.0	13.3	7.4	2.6
	AM	23.1	47.8	5.1	42.7	95.6	8.4	25.0	7.6	4.4
	HE	23.3	48.5	7.4	41.1	97.7	11.7	23.3	4.1	2.6
	Mean	19.5	47.0	6.3	40.7	71.8	7.7	20.6	6.2	3.6
	s.d.	7.7	16.8	6.4	14.2	19.4	4.4	7.8	2.9	1.5
Slow	PJ	14.6	41.6	6.3	35.3	52.5	13.6	14.1	8.5	3.5
	PP	14.0	90.3	10.8	79.5	109.7	11.1	13.6	8.3	1.3
	EdH	12.3	45.3	6.3	39.0	48.1	12.9	11.3	8.1	2.3
	ML	13.2	64.4	10.3	54.1	73.8	14.6	14.8	3.4	1.1
	SA	8.8	90.9	14.9	76.0	69.0	14.8	8.0	12.7	1.5
	Mean	12.6	66.5	9.7	56.8	70.6	13.4	12.4	8,2	1.9
	s.d.	2.3	23.6	3.6	20.4	24.4	1.5	2.8	3.3	1.0

* MF received only 375 mg AG

** PM was on forced diuresis for hypercalcaemia.

acetylators is shown in Figure 3. Slow DDS acetylators excreted significantly ($P < 0.05$) less acetylAG than fast acetylators. In absolute terms, however, the excretion of this metabolite was low. A greater quantity of AG was excreted in the urine over 48 h and this was significantly ($P < 0.05$) higher in slow DDS acetylators. These differences became less obvious after 1 week's treatment with AG (Table 5).

The effect of a 1 week treatment with AG was studied in eight patients and the resulting changes in pharmacokinetic parameters are shown in Tables 4 and 5. In six patients the AG acetylation ratio was decreased (Table 4 cf. Table 2), in one it was unchanged and in one (MF) there was an increased ratio. Overall, this trend towards reduction was not significant ($0.1 > P > 0.05$). If patient MF was omitted from analysis the change becomes significant ($P < 0.05$). It will be recalled that this patient had received phenytoin for over 1 year and thus might be expected to be maximally induced: it will be noted from Table 3 that she had the shortest AG half-life amongst the fast DDS acetylators. This patient, however, shared in the significantly ($P < 0.05$) increased mean apparent metabolic clearance of AG following AG treatment for 1 week. There were significant ($P < 0.01$) reductions in the mean half-lives of both AG and acetylAG and these were accompanied by increased apparent systemic clearances in all patients except PM (Table 5 cf. Table 3). Following induction, the relationship between the AG acetylation ratio and half-life remained. There were significant relationships between this ratio and both half-life ($r_s = 0.90$; $P < 0.01$) and apparent metabolic clearance ($r_s = -0.74$; $P < 0.05$). No significant ($P > 0.1$) change occurred in mean apparent volume of distribution after AG treatment.

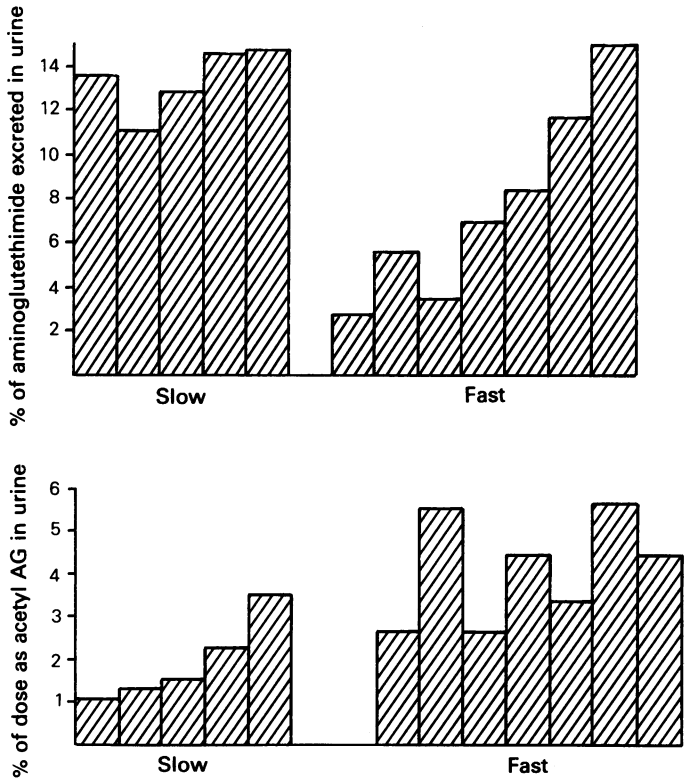

Figure 3 Fraction of the administered dose of aminoglutethimide excreted in the urine as unchanged aminoglutethimide and its acetyl metabolite over 48 h following a single dose of aminoglutethimide.

Table 4 Plasma AUC of aminoglutethimide and its acetyl metabolite in patients for a single dose of 500 mg aminoglutethimide following a week of treatment with 250 mg AG 12 hourly and a 48 h washout period

Acetylator phenotype	*Subject*	*AG* $[AUC]_0^\infty$ ($\mu g\ ml^{-1}\ h$)	*Acetyl AG* $[AUC]_0^\infty$ ($\mu g\ ml^{-1}\ h$)	*Acetyl AG* $[AUC]_0^\infty$ / *AG* $[AUC]_0^\infty$
Fast	DB	298.4	122.1	0.41
	MF*	97.5	28.7	0.30
	PM	165.6	31.6	0.19
	EH	202.1	77.4	0.38
	HE	139.6	42.6	0.31
Slow	PJ	80.3	14.9	0.19
	EdH	148.2	15.9	0.10
	ML	88.2	9.5	0.11

* MF received only 375 mg AG

Discussion

Two previous studies (Murray *et al.*, 1979; Thompson *et al.*, 1981) have investigated the pharmacokinetics of AG using a non-specific spectrophotometric assay. Despite reservations concerning their analytical methods, the pharmacokinetic parameters for AG found in these studies are comparable with those using the high performance liquid chromatographic procedure presented here. The half-lives (for six subjects in both studies) were 13.3 ± 2.7 h (Murray *et al.*, 1979) and 12.5 ± 1.6 h (Thompson *et al.*, 1981). The total body clearances were more variable, however, being 43 ± 6 ml min^{-1} (Murray *et al.*, 1979) and 86.2 ± 5.6 ml min^{-1} (Thompson *et al.*, 1981). In neither of these studies was distinction made between acetylator phenotypes. The apparent volume of distribution in the β-phase for the two compartment open pharmacokinetic model which was fitted to the data of Thompson *et al.* (1981) was 93.3 ± 10.4 l which is similar to that in our study based upon simpler pharmacokinetic assumptions.

Although preliminary data relating to plasma acetylAG concentrations have been presented by Coombes *et al.* (1980), no definitive study has been made of the pharmacokinetics of this compound in man. The presence of the amine function in AG confers upon the molecule its ability to inhibit cholesterol side chain cleavage and aromatase since this is absent in glutethimide which has no antisteroidogenic activity (Foster *et al.*, 1983). Acetylation at this site might therefore be anticipated to reduce the therapeutic effects of AG. *In vitro* studies on steroid synthesis by adrenal cell monolayer cultures showed that whilst 10 $\mu g\ ml^{-1}$ AG inhibited steroid output by more than 90%, the same concentration of acetylAG reduced it by 42% (Coombes *et al.*, 1980). Foster *et al.* (1983), however, found acetylAG to be devoid of inhibitory activity on the desmolase from bovine adrenal cortex at a concentration of 50 μg/ml and on the aromatase of human placenta at a concentration of 20 μg/ml. Clinical experience shows acute side effects of AG administration: lethargy, orthostatic dizziness, ataxia, fever and rashes to be common (up to 43% of patients) but to resolve on chronic treatment (Santen *et al.*, 1982a) possibly due to enzyme induction (Murray *et al.*, 1979). The sedative and central nervous system depressant actions of AG are most probably related to its structural affinity with glutethimide, barbiturates and thalidomide. No data are available concerning possible central sedative properties of acetylAG in man although it has equal antileptazol activity to AG in mice and is reported to be ten times less sedative in that species (Douglas & Nicholls, 1972). The parallelism of AG and acetylAG elimination rates suggests that formation of this metabolite is rate limiting and the first order rate constant for metabolite formation is either greater than or identical with that for AG elimination.

AG markedly accelerates the metabolism of dexamethasone (after only 2 weeks' treatment, its clearance is increased by almost 300%) but has no effect on cortisol, oestrone, ndrostenedione or medroxyprogesterone acetate clearances (Santen *et al.*, 1982b). This has been attributed to enzyme induction. The present study confirms the observation by Murray *et al.* (1979) of a decreased half-life accompanied by an increased apparent systemic clearance on chronic treatment suggesting autoinduction. These effects were rapid and clearly demonstrable following only 1 week of AG therapy. Studies showed that hepatic enzyme induction by glutethimide in man begins within

Table 5 Pharmacokinetic parameters for aminoglutethimide and its acetyl metabolite in patients following a single dose of 500 mg AG after a week of treatment with 250 mg AG 12 hourly and a 48 h washout period

		Aminoglutethimide						*Acetyl-AG*		
Acetylator phenotype	*Subject*	$t_{½}$ *(h)*	*Apparent systemic clearance CL (ml min^{-1})*	*Renal clearance CL_R (ml min^{-1})*	*% of dose in urine over 48 h*	*Apparent metabolic clearance CL_M (ml min^{-1})*	*Apparent volume of distribution (l)*	$t_{½}$ *(h)*	*Renal clearance CL_R (ml min^{-1})*	*% of dose in urine over 48 h*
Fast	DB	18.8	27.9	3.6	11.9	24.3	45.5	19.6	3.7	5.2
	MF*	11.3	64.1	5.4	8.4	58.7	62.8	10.6	10.0	4.6
	PM	11.3	50.3	9.2	19.2	41.1	49.2	11.4	3.3	1.3
	EH	16.9	41.2	4.5	11.1	36.7	60.3	17.1	6.4	6.2
	HE	13.2	59.9	12.3	18.9	47.6	68.4	13.2	4.5	2.1
	Mean	14.3	48.7	7.0	13.9	41.7	57.2	14.4	5.6	3.9
	s.d.	3.4	14.6	3.7	4.9	12.8	9.6	3.8	2.7	2.1
Slow	PJ	6.6	103.8	8.4	8.1	95.4	59.3	9.4	4.4	0.8
	EdH	10.5	56.2	8.2	15.4	48.0	51.0	10.4	15.5	2.8
	ML	8.7	94.5	18.5	19.1	76.0	71.1	9.2	8.3	9.0
	Mean	8.6	84.8	11.7	14.2	73.1	60.5	9.7	9.4	4.2
	s.d.	2.0	25.2	5.9	5.6	23.8	10.1	0.6	5.6	4.3

* MF received only 375 mg AG

2 days of starting medication (Jackson *et al.*, 1978): it is not certain that AG induction was complete after a single week of treatment. Jarman *et al.* (1983) have identified hydroxylaminoglutethimide as an induced metabolite of AG on chronic dosing in three out of four patients and have speculated that formation of this inactive metabolite is the principal factor responsible for the decreased half-life of AG on chronic therapy. Interestingly they also found that acetylAG was almost absent from the urine after 6 weeks' treatment with AG. In the present study induction was accompanied by unchanged renal clearance of AG and acetylAG but increased metabolic clearance of AG which resulted in a decreased half-life accompanied by reduction in the acetylAG/AG ratio. These changed ratios reflected significant ($P < 0.05$) declines in the AUCs of both AG and acetylAG. The data suggest that induction activates oxidative pathways with higher affinity for AG than *N*-acetyltransferase which reduce both the AG pool and the amount of AG available for acetylation and/or the availability of acetyl Co A for this process. Certainly the small amount of acetylAG eliminated in the urine indicates this to be a relatively minor elimination route of the compound overall. Douglas & Nicholls (1972) found it to be the major urinary metabolite in man accounting for 4–25% of the administered dose after a single dose of AG.

N-acetylation of many drugs shows genetic polymorphism with fast and slow acetylation phenotypes although this process is not invariably polymorphic. There is no evidence that it is inducible. Coombes *et al.* (1982) showed that in subjects acetylator-phenotyped into fast and slow acetylators using sulphadimidine, the slow acetylators excreted more AG (mean 28% in 24 h) of a single dose than did fast acetylators (12%) but the latter excreted more acetylAG (8.8%) than the former (3.9%). These observations have thus been confirmed by the present study. The urinary excretion of AG and acetylAG found in the present study is lower than that reported using non-specific colorimetric assay (Douglas & Nicholls, 1972) which found elimination of 34–50 and 4–25% of the dose as AG and acetylAG respectively in 48 h. However, Coombes *et al.* (1982), using high performance liquid chromatography show a wide range of values, those for AG being 0.6–36.5% in 24 h and for acetylAG 2.7–12% in 24 h in a group of fast and slow acetylators. The relationship between DDS acetylation phenotype and these observations permit the tentative conclusion that AG is a drug which demonstrates polymorphic acetylation in man. We have been unable to confirm (see Figures 1 and 2) the preliminary observation by Coombes *et al.* (1980) that the plasma concentrations of acetylAG are higher than the AG concentrations in rapid acetylators. They only studied nine subjects at three time points, however, and although in general the mean acetylAG/AG ratio is higher in their fast acetylators than in the slow acetylators, its value is variable. We have found this ratio to be altered by induction but it probably remains constant within an individual over a single dose interval.

The concept of fast and slow acetylators may be easily applied to drugs in which acetylation is a major pathway for disposition and for which it determines half-life and clearance, e.g. isoniazid (Weber & Hein, 1979), sulphamethazine (Evans & White, 1964), hydralazine (Reece *et al.*, 1980). For DDS, acetylation is a minor elimination pathway and although the ratio of MADDS to DDS accurately reflects acetylator phenotype, the half-life is similar in fast and slow acetylators (Gelber *et al.*, 1971). In the case of AG, our observations are at first sight paradoxical: fast acetylators, although they have a higher acetylAG/AG ratio have a longer half-life and lower clearance for AG than slow acetylators. This paradox has not been previously noted with other drugs but possibly occurs with phenelzine. Caddy *et al.* (1978), on the basis of urinary excretion, found that the half-life of phenelzine in three slow acetylators was shorter (0.40, 0.58 and 0.72 h) than in two fast acetylators (1.03 and 1.61 h). The vasodilator endralazine was required in slightly (but not significantly) lower dosage to produce a given fall in blood pressure in 34 slow acetylators as compared to 16 fast acetylators (Holmes *et al.*, 1983). Although fast acetylators form higher concentrations of acetylated endralazine in the plasma than slow acetylators, no significant difference in endralazine half-life has been found between acetylator phenotypes (Reece *et al.*, 1982; Meredith *et al.*, 1983). Like AG, endralazine does not have acetylation as a major elimination route.

An explanation of these observations is not presently forthcoming, particularly since the overall metabolic fate of AG in man is not completely known. Some form of product inhibition is a possibility, although this would have to be one of regulation of an oxidative pathway(s) by acetyl metabolites). Coombes *et al.* (1982) showed that slow acetylators excreted more nitroglutethimide in the urine than fast acetylators but since even in the former this constituted only 0.1% of the dose, it is unlikely to make a major contribution to AG disposition. Deacetylation of mono- and diacetyldapsone

has been documented in man (Gelber *et al.*, 1971) and in the case of that drug rapidly achieves equilibrium. Possibly acetylAG acts as a reservoir (akin to protein binding) for drugs eventually eliminated by oxidative mechanisms. The solution clearly awaits further investigations to clarify the metabolism of AG which will require the administration of acetylAG as sole agent.

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