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The influence of a graded dose schedule of aminoglutethimide on the disposition of the optical enantiomers of warfarin in patients with breast cancer

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Summary. The pharmacokinetics of the optical enantiomers of warfarin (*R*-warfarin and *S*-warfarin) were investigated in patients treated for breast cancer with aminoglutethimide (AG). The patients received 125 mg AG b.i.d. (i.e., low-dosage regimen); 250 mg AG q.i.d. together with cortisone acetate (i.e. high-dosage regimen); or an escalating dose schedule was followed (i.e. low-dosage regimen followed by high-dosage regimen). The pharmacokinetics for *R*-warfarin and *S*-warfarin were determined before initiation of AG treatment and again after 2, 4, or 8 weeks of continuous AG treatment. The plasma clearance for both enantiomers showed a moderate increase (mean 41.2%) in patients receiving the low AG dose, whereas in patients treated according to the high-dosage regimen a marked increase (mean 90.8%) was observed. There was a corresponding reduction in warfarin half-life, and no alteration in distribution volume. These effects on the warfarin pharmacokinetics appeared after 14 days of AG treatment, and after this time point there was no further increase in warfarin clearance. Notably, the effect of AG on warfarin kinetics was the same for both enantiomeric forms of warfarin. These data show that there is a dose-response relationship between AG dose and induction of warfarin metabolism.

Introduction

Aminoglutethimide (AG) inhibits the growth of hormone-dependent metastatic breast carcinoma, and is currently used in the palliative treatment of advanced breast cancer in postmenopausal women [21]. The therapeutic efficiency has been attributed to the fact that AG interferes with estrogen biosynthesis. The main target of AG is probably aromatase, the enzyme system responsible for the conversion of androgens to estrogens. This pathway is the major source of estrogens in postmenopausal women [22].

The conventional dosage regimen for AG is 250 mg q.i.d., combined with hydrocortisone [8]. This drug schedule is referred to as a "high-dose schedule" in this study. In vivo studies have shown that a lower dose AG drug schedule (125 mg b.i.d.) exerts a pronounced inhibitory effect on the aromatase system [3], and low-dose AG without hydrocortisone has been reported to produce a decrease in plasma estrogens similar to that obtained with conventional high-dose therapy [4, 26]. This regimen is referred to as a "low-dose schedule" in this study. Low-dose treatment with AG is now undergoing clinical trials as an alternative treatment for breast cancer [5, 14, 24].

AG given as high-dose treatment enhances the metabolism of several drugs [10, 12, 13, 21], suggesting that AG is an effective inducer of the mixed function oxidase system of the liver. Induction of drug metabolism in liver may form the basis for interactions between AG and other drugs given to patients with breast cancer. We have previously reported on the clinical implications of enhancement of warfarin metabolism by high-dose AG treatment [11]. Whether low-dose AG treatment has a similar effect on drug metabolism is an open question.

The anticoagulant drug, warfarin, has an asymmetric carbon atom and exists in two isomeric forms, *R*- and *S*-warfarin. The clinical form is a racemate, but S-warfarin is more effective as an anticoagulant than the *R*-form [6]. One important point is that warfarin undergoes stereoselective hydroxylation in the liver. *S*-Warfarin undergoes faster elimination than the *R*-isomer because of differences in metabolism. Some drug interactions involving warfarin are stereoselective and affect the *S*-enantiomer only [9, 17]. The differential metabolic fate of *R*- and *S*-warfarin has recently been exploited by using the plasma half-life of the R-and *S*-enantiomers as a measure of induction of the hepatic mixed function oxidase system [9, 17].

The aim of the present investigation was to characterize the ability of AG to induce drug metabolism by using *R*- and *S*-warfarin as a clinical probe. The following aspects of AG/warfarin interaction were addressed: (1) dose dependency, (2) time dependency and (3) possible stereoselectivity.

Patients and methods

Patients. Nine women receiving AG as a treatment against metastatic breast cancer were included in this study. Their mean age was 62 years (range 39–74 years), and their mean body weight 71.3 kg (range 53–87.5 kg). Two patients (W. B. and B. S.) were moderate smokers (5–10 ciga-

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rettes per day). Apart from AG, drugs known to affect hepatic drug metabolism were not ingested by the patients participating in the study. All patients had normal platelet counts, thrombotests, bleeding times, and serum transaminase activities. All patients gave informed consent to participation in the study. Ultrasonography of the liver was normal in all nine patients.

AG dose schedules. Patients received either 125 mg AG b.i.d. (low-dosage regimen) or 250 mg AG q.i.d. in combination with 25 mg cortisone-acetate b.i.d. (high-dosage regimen).

Four patients received low-dose AG for the first 4 weeks of treatment and two patients for 8 weeks before initiation of the high-dosage regimen. Two patients received treatment with high-dose AG from the start of drug therapy, and these two patients received 50 mg cortisone acetate b.i.d. for the first 2 weeks, followed by 25 mg b.i.d. [8].

Protocol and blood sampling. The plasma half-life, clearance, and distribution volume of *R*- or *S*-warfarin were determined under the following conditions:

a) Before initiation of treatment with AG. This is referred to as phase 1.

b) After treatment for 2 or 4 weeks. This intermediary period of treatment is referred to as phase 2. The patients received either low- or high-dose treatment.

c) After treatment with AG for a total time of 8 weeks. This is defined as phase 3. Some patients received low-dose treatment for the first 4 weeks, followed by high-dose treatment for the next 4 weeks, whereas others were treated with the same dose (high or low) throughout the period.

In one patient (A. F.) AG therapy had to be discontinued after less than 8 weeks, because of rapid progression of the disease state.

Each patient was given the same warfarin enantiomer throughout the study. The drugs were dissolved in water, and given as a single oral dose of either 0.20 mg *S*-warfarin/kg body weight or 0.33 mg *R*-warfarin/kg body weight. The dose of *S*-warfarin was smaller than that of the less potent anticoagulant, *R*-warfarin, to avoid an untoward anticoagulant effect. The dose was always administered at 8 a. m. after an overnight fast. Food or other drugs (AG or hydrocortisone) were allowed 2 h after warfarin ingestion.

Blood samples for warfarin determination were taken at 0, 2, 4, 6, 9, 12, 24, 36, 48, 72, 96 and 120 h after intake of the *R*-enantiomer. The same protocol was used for the *S*-enantiomer, except that the last blood sample was collected after 96 h. In some patients additional blood samples were collected, for example when venepunture was done for other reasons or if routine sample(s) were accidentally lost.

Blood samples for AG determinations were collected 0, 6, 9, and 12 h after administration of warfarin, and the mean AG concentration value calculated.

All blood samples were allowed to coagulate for 30 min to 1 h. Then serum was prepared by centrifugation, and serum samples stored in darkness at −20 °C until analysis.

Chemicals and drugs. Acetonitril (HPLC-grade) was purchased from Rathburn Chemicals, Ltd, Peeblesshire, Scotland. ODS hypersil 3-μm microparticle medium for reversed-phase chromatography was from Shandon Southern Products Ltd, Cheshire, UK., and Pelliguard LC 18 (40 μm) from Supelco, Inc., Bellefonte, Pa USA The analytical columns were slurry-packed with hypersil at 9000 psi, using a Shandon column packer.

The optical isomers of warfarin were kindly supplied by Chemoswed Co., Malmø, Sweden. The purity of the enantiomers was evaluated by optical rotation ([alfa] of +90.8° for the *R*-isomer and −88.4° for the *S*-isomer). AG was a gift from Ciba-Geigy, Basel, Switzerland. Both *R*- and S-warfarin and AG were pure according to HPLC analysis (see below).

Drug analyses. Warfarin (*R*- and *S*-enantiomers) was analyzed by an HPLC assay described previously [25]. Briefly, samples of deproteinized serum were subjected to chromatography on a reversed-phase (Hypersil) column eluted with 23% acetonitril in 100 m*M* ammonium formate, pH 3.5. The flow rate was 2 ml/min. The absorbance was routinely recorded at 305 nm using a photodiode array detector. The retention time was 2.6 min for both isomers. The identity of the warfarin peaks was confirmed by recording the spectra at the up and down slope and at the top of the peak. All samples were analyzed in duplicate.

AG and its major plasma metabolite, *N*-acetyl-AG, were analyzed as described recently [23]. Serum was deproteinized by a mixture of acetonitril and perchloric acid. The chromatographic system was the same as that used for warfarin analysis, except that the acetonitril concentration of the mobile phase was reduced to 11% and the absorbance was routinely recorded at 242 nm.

Pharmacokinetic calculations. These were performed as recommended by The American College of Clinical Pharmacology [1].

Clearance (CL) for warfarin was calculated according to the formula

$$CL = \frac{f\,D}{AUC},$$

where f = fraction of the dose absorbed, D = dose of drug, and AUC the area under the serum concentration curve from time zero to infinity. The value for f is regarded as 1 since warfarin is almost completely absorbed after oral administration and there is no detectable first-pass metabolism [16, 18]. AUC was determined by the trapezoidal rule from time zero to the time corresponding to the last significant value for serum warfarin. The residual area was added and calculated by extrapolation to infinity after log-linear least square regression analysis of the terminal part of the curve.

The apparent volume of distribution (V_z) was calculated by equation

$$V_z = \frac{f\,D}{\lambda_z\,AUC},$$

where λ_z is the terminal rate constant. This constant was estimated from the terminal elimination phase using log linear regression analysis.

Statistical analysis. Trends were analyzed using Spearman rank correlation coefficients (R_s).

Results

Warfarin elimination curves and curve fit

The warfarin serum concentration curves obtained after oral administration of the *R*- or *S*-enantiomer to two pa-

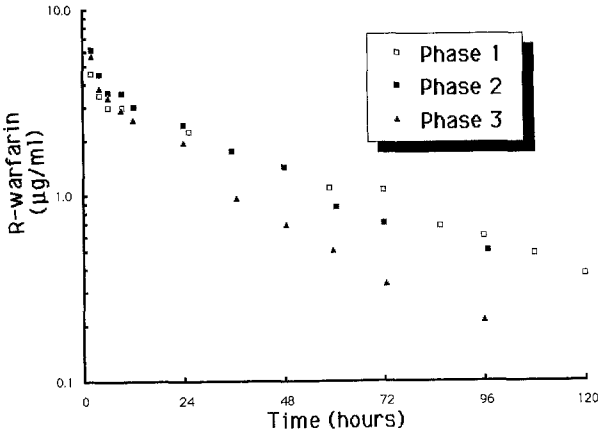

Fig. 1. Serum concentration curves for *R*-warfarin in a patient (M. V.) before start of AG therapy (phase 1), after receiving low-dose AG for 4 weeks (phase 2), and after receiving high-dose AG for the next 4 weeks (phase 3)

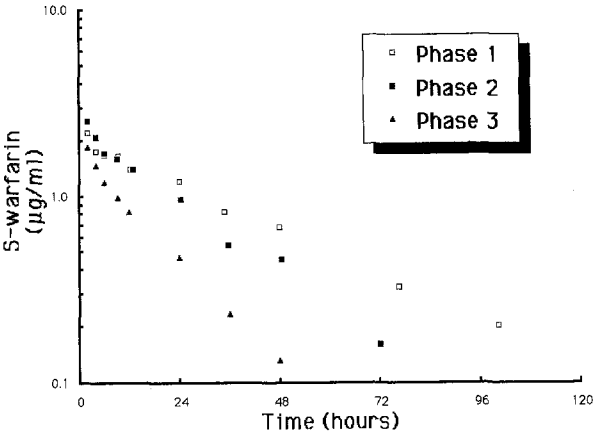

Fig. 2. Serum concentration curves for *S*-warfarin in a patient (W. K.) before start of AG therapy (phase 1), after receiving low-dose AG for 4 weeks (phase 2), and after receiving high-dose AG for the next 4 weeks (phase 3)

Table 1. Pharmacokinetic parameters of *R*-warfarin before and during treatment with AG[a]

Patients	Phase of treatment	Duration of treatment (weeks)	Dosage regimen[b]	AG in serum (µg/ml)	Warfarin				
					Clearance		$t_{1/2}$ (h)	V_Z	
					(l/h)	(ml h^{-1} kg^{-1})		(l)	(ml/kg)
A.F.	1	–	–	–	0.12	2.07	29.5	4.98	88
	2	4	low	0.59	0.14	2.39	25.6	4.98	88
G.J.	1	–	–	–	0.09	1.49	57.4	7.74	123
	2	4	low	2.64	0.19	3.02	27.8	7.61	121
	3	8	low	3.88	0.18	2.83	37.3	9.57	152
D.J.	1	–	–	–	0.14	2.74	42.9	8.94	168
	2	2	high	9.48	0.22	4.05	21.6	6.70	126
	3	8	high	6.39	0.24	4.48	26.4	9.06	171
M.V.	1	–	–	–	0.14	1.77	37.9	7.65	97
	2	4	low	1.67	0.17	2.14	29.9	7.29	92
	3	8	high	5.60	0.25	3.20	21.5	7.87	100
W.B.	1	–	–	–	0.31	4.24	32.4	14.46	198
	2	4	low	0.83	0.37	5.09	25.6	13.73	188
	3	8	high	3.70	0.55	7.51	15.3	12.09	166

[a] *R*-Warfarin was given as a single oral dose before initiation of treatment (phase 1), after treatment for 2 or 4 weeks (phase 2), and after 8 weeks of AG treatment (phase 3)

[b] The patients were trated according to the low- or high-dosage regimen

tients in phases 1, 2 and 3 of AG treatment (see experimental section), are shown in Fig. 1 and 2. The curves were sometimes biphasic (6 out of 26), with an initial short phase followed by an elimination phase, consistent with a two-compartment model. The alfa-phase was often masked by a delayed absorption, giving a monophasic serum concentration curve, and a one-compartment model gave the best curve fit. To treat all data equally, model-independent calculations were chosen throughout this study.

Warfarin kinetics and AG dose

Clearance, half-life and distribution volume were determined for either *R*- or *S*-warfarin in nine patients treated with AG (Tables 1 and 2).

Low-dose AG treatment of patients induced an increase in warfarin clearance after 4 weeks of treatment (phase 2 versus phase 1), which varied markedly from one patient to another (range 15%–103%). There was a corresponding decrease in warfarin half-life in serum, whereas distribution volume was not affected. Notably, the same response was observed for *R*- and for *S*-warfarin. Four patients were treated with low-dose AG for up to 8 weeks (phase 3). In these patients, there was no further increase in clearance of *R*- or *S*-warfarin (Tables 1 and 2).

High-dose AG treatment was given to two patients from the initiation of therapy. After 2 weeks there was a marked increase (57% and 65%) in clearance of both *R*- and *S*-warfarin, with a corresponding reduction in half-life. No further effect was observed when the AG therapy was continued for 8 weeks. Four patients treated with low-dose AG for 4 weeks showed a mean increase in warfarin clearance of about 39%. The high-dose treatment in these patients induced a further increase in clearance, to a mean

Table 2. Pharmacokinetic parameters of *S*-warfarin before and during treatment with AG[a]

Patients	Phase of treatment	Duration of treatment (weeks)	Dosage regimen[b]	AG in serum (μg/ml)	Warfarin				
					Clearance		$t_{1/2}$ (h)	V_Z	
					(l/h)	(ml h^{-1} kg^{-1})		(l)	(ml/kg)
E.E.	1	–	–	–	0.33	3.81	27.8	13.39	153
	2	4	low	2.02	0.38	4.57	20.2	11.07	133
	3	8	low	1.38	0.42	4.81	20.8	12.65	145
K.O.	1	–	–	–	0.51	6.02	17.6	12.85	153
	2	2	high	14.09	0.84	10.06	10.3	12.61	150
	3	8	high	12.58	0.81	9.69	10.3	12.10	144
W.K.	1	–	–	–	0.19	2.74	30.4	8.41	120
	2	4	low	0.78	0.29	4.13	19.7	8.19	117
	3	8	high	9.45	0.49	6.99	14.4	10.17	145
B.S.	1	–	–	–	0.36	4.69	20.7	10.63	140
	2	4	low	0.91	0.59	7.70	11.1	9.34	123
	3	8	high	3.46	0.79	10.35	7.3	8.26	109

[a] *S*-Warfarin was given as a single oral dose before initiation of treatment (phase 1), after treatment for 2 or 4 weeks (phase 2), and after 8 weeks of AG treatment (phase 3)

[b] The patients were treated according to the low- or high-dose regimen

value 107% above that obtained in phase 1. Similar results were obtained for *R*- and *S*-warfarin isomers (Tables 1 and 2).

AG in serum

Mean AG serum concentrations of patients receiving low-dose (0.59–3.88 μmg/ml) or high-dose AG drug schedules (3.46–14.09 μg/ml) were as reported in previous studies [12]. The serum concentrations in patients treated according to the low-dosage regimen for 4 weeks showed no correlation ($R_s = 0$) with the increase in warfarin clearance. Nor did the data obtained for patients treated with high-dose AG at time 8 weeks after initiation of therapy reveal any correlation between AG concentration and increase in warfarin clearance ($R_s = -0.32$). In one patient (G. J.) who received low-dose AG and showed a remarkably high increase in warfarin clearance, the amount of AG in serum equalled that measured in serum from patients receiving high-dose AG (Tables 1 and 2).

Discussion

In the present paper we investigated the ability of AG to induce hepatic drug metabolism, using the warfarin enantiomers as a clinical probe. The serum clearance of warfarin reflects the activity of the warfarin-metabolizing enzyme system, and can therefore serve as an index of the effect of AG treatment on warfarin metabolism. The pharmacokinetic parameters of *R*- and *S*-warfarin were determined after oral administration. This was considered adequate, since both warfarin enantiomers are well absorbed [9, 18] and do not undergo significant first-pass metabolism [16].

The pharmacokinetics of warfarin administered IV have been reported to be best fitted by a two-compartment open model [19]. We observed a monoexponential serum curve in most patients (Figs. 1 and 2). This could be explained by the fact that the distribution phase, which subsides within 6 h [19], is obscured by the peak concentration occurring 3–6 h after PO administration [18].

The values we obtained for clearance, terminal half-life, and distribution volume for *R*- and *S*-warfarin in phase 1 (Tables 1 and 2) are in accordance with those published by others [7].

We have previously demonstrated that treatment with conventional high-dose AG for 4–8 weeks induced a marked increase in the clearance of warfarin racemate [11]. In the present paper it is demonstrated that the increase in warfarin metabolism induced by AG is fully developed within 14 days (Tables 1 and 2). Other studies on induction of drug metabolism in man have shown a similar time dependency [20]. Furthermore, the introduction of the low-dose AG treatment as a therapeutic regimen in the management of breast cancer [24] warranted the investigation of the dose requirement of the inductive effect. We observed that low-dose AG induced a significant increase in warfarin clearance, which was further increased by high-dose treatment (Tables 1 and 2). This shows that within the therapeutic range there is a dose-response relationship for the inductive effect of AG. Several other inducers of the hepatic mixed function oxidase system, like barbiturates and rifampicin, have been shown to produce a dose-dependent induction [2, 15]. We observed no significant relation between the AG serum concentration and increase in warfarin clearance. This may be explained by the large interindividual variations in serum AG level and the limited number of patients studied.

AG induced the metabolism of the two warfarin enantiomers to the same extent (Tables 1 and 2), showing that no stereoselectivity exists. Our data are in accordance with an effect of AG on a common metabolic pathway, e.g. 6-hydroxylation, which is shared by both enantiomers [9]. However, an additional effect on 7-hydroxylase cannot be excluded.

In conclusion, the possible implications of the findings reported are as follows:

1) A moderate induction with low-dose AG suggests that drug interactions should also be expected with this therapeutic regimen.

2) Alterations in drug metabolism might be expected if a patient is transferred to another AG drug schedule.

3) The nonselective induction of metabolism of both warfarin isomers shows that drug interaction between AG and warfarin cannot be avoided by administration of one isomer.

4) Several steroids, including estrogens, are metabolized by hydroxylations catalyzed by the mixed function oxidase system. The possible role of a dose-dependent AG-promoted induction of steroid metabolism in the mechanism of action of this drug, is a subject under study in our laboratory.

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