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Decreased Serum Concentrations of Tamoxifen and Its Metabolites Induced by Aminoglutethimide[1]

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ABSTRACT

The antiestrogen tamoxifen and the aromatase inhibitor aminoglutethimide show similar response rates when used in the endocrine management of advanced breast cancer. However, numerous clinical trials have demonstrated no increase in response rate from treatment with the drug combination of tamoxifen plus aminoglutethimide. We investigated the possibility of a pharmacokinetic interaction between these two drugs in six menopausal woman with breast cancer. All patients were investigated under three different conditions (termed phases A, B, and C). The steady state kinetics of tamoxifen were determined when administered alone (phase A) and after coadministration of aminoglutethimide for 6 weeks (phase B). In phase B, the pharmacokinetics for aminoglutethimide were determined and compared with these parameters after a tamoxifen washout of 6 weeks (phase C). The serum concentration of tamoxifen and most of its metabolites {[*trans*-1(4-β-hydroxy-ethoxyphenyl)-1,2-diphenylbut-1-ene], 4-hydroxytamoxifen, 4-hydroxy-*N*-desmethyltamoxifen, *N*-desmethyltamoxifen, and *N*-desdimethyltamoxifen} were markedly reduced following aminoglutethimide administration, corresponding to an increase in tamoxifen clearance from 189–608 ml/min. The amount of most metabolites in serum increased relative to the amount of parent tamoxifen. These data are consistent with induction of tamoxifen metabolism during aminoglutethimide exposure. We found no effect of tamoxifen on aminoglutethimide pharmacokinetics or acetylation. We conclude that this aminoglutethimide-tamoxifen interaction should be taken into account when evaluating the clinical effect of this drug combination relative to monotherapy.

INTRODUCTION

The growth of human breast cancer is supported by endogenous estrogens (1, 2). Tamoxifen and aminoglutethimide are drugs currently used in the endocrine management of breast cancer, and they probably act by suppressing the growth-stimulating effect of estrogens (2–4).

Tamoxifen [*trans*-1-(4-β-dimethylaminoethoxyphenyl)-1,2-diphenylbut-1-ene] is a nonsteroidal antiestrogen which is effective against breast cancer in both pre- and postmenopausal women. It is assumed to exert its main effects by blocking the action of estrogens at the receptor site (4). Tamoxifen undergoes extensive hepatic metabolism, and in man metabolites formed by *N*-demethylation are the main circulating species. Significant amounts of hydroxylated metabolites, including the primary alcohol, 4-hydroxytamoxifen, (4) and 4-hydroxy-*N*-desmethyltamoxifen (5) have also been demonstrated in serum. This may be important since some hydroxylated metabolites have higher affinity *in vitro* toward the estrogen receptor than the parent drug, tamoxifen (6–9). Thus, biotransformation of tamoxifen may be an important determinant of drug action. Known metabolites of tamoxifen formed through demethylation and hydroxylation are depicted in Fig. 1.

Aminoglutethimide inhibits the enzyme aromatase, which converts androgens to estrogens in peripheral fat tissue (3). This conversion is the main estrogen source in postmenopausal women. In addition, aminoglutethimide may reduce the concentration of plasma estrogens by enhancement of estrogen metabolism (10, 11). Aminoglutethimide causes response rates in postmenopausal breast cancer patients similar to those of tamoxifen, but because of more frequent side effects aminoglutethimide is generally used after tamoxifen as a second line endocrine treatment (12).

Combination therapy with tamoxifen plus aminoglutethimide should afford both estrogen receptor blockade and reduced plasma estrogen levels, and because of different targeting of these drugs the combination is expected to be more effective than monotherapy. This possibility is supported by studies on human breast carcinoma transplanted into nude mice (13), but the results from clinical trials have been disappointing (14–19) since they all show that the response to tamoxifen is not augmented by adding aminoglutethimide (Table 1).

The reason why the response rate is not increased with combination therapy has not been evaluated. A pharmacokinetic interaction should be considered, especially because aminoglutethimide is a potent inducer of certain hepatic mixed function oxidases and enhances the metabolism of several drugs and steroids (10, 20, 21). In addition, tamoxifen might influence the disposition of aminoglutethimide. Tamoxifen is a potent inhibitor of some mixed function oxidases *in vitro* (22) and may inhibit its own metabolism (23–25) as well as the metabolism of other drugs (26–28).

In the present paper we describe the effect of aminoglutethimide on the disposition of tamoxifen in patients receiving steady state tamoxifen treatment. We also report that tamoxifen does not affect aminoglutethimide disposition. The investigation was motivated by the large number of clinical studies of the combination therapy (Table 1) and also by preliminary findings suggesting that aminoglutethimide alters serum levels of tamoxifen and its metabolites.[3]

MATERIALS AND METHODS

Patients. All patients gave their informed consent to participate in the study. Six postmenopausal women were enrolled. All of them had advanced breast cancer relapsing during tamoxifen therapy and were, therefore, transferred to an aminoglutethimide regimen. Patient characteristics are given in Table 2. All patients had normal liver and renal function tests. One patient (K. N.) did not enter the final part of the study (phase C) because of rapidly progressing disease.

Chemicals. Tamoxifen, metabolite B, and metabolite X were obtained from Pharmachemie B.V. (Haarlem, Holland) and metabolites Y, BX, and Z were gifts from Imperial Chemical Industries, PLC, Pharmaceuticals Division (Macclesfield, United Kingdom). Aminoglutethimide and *N*-acetylaminoglutethimide were gifts from Ciba-Geigy (Basel, Switzerland).

Study Protocol. The study protocol was approved by the regional ethical committee.

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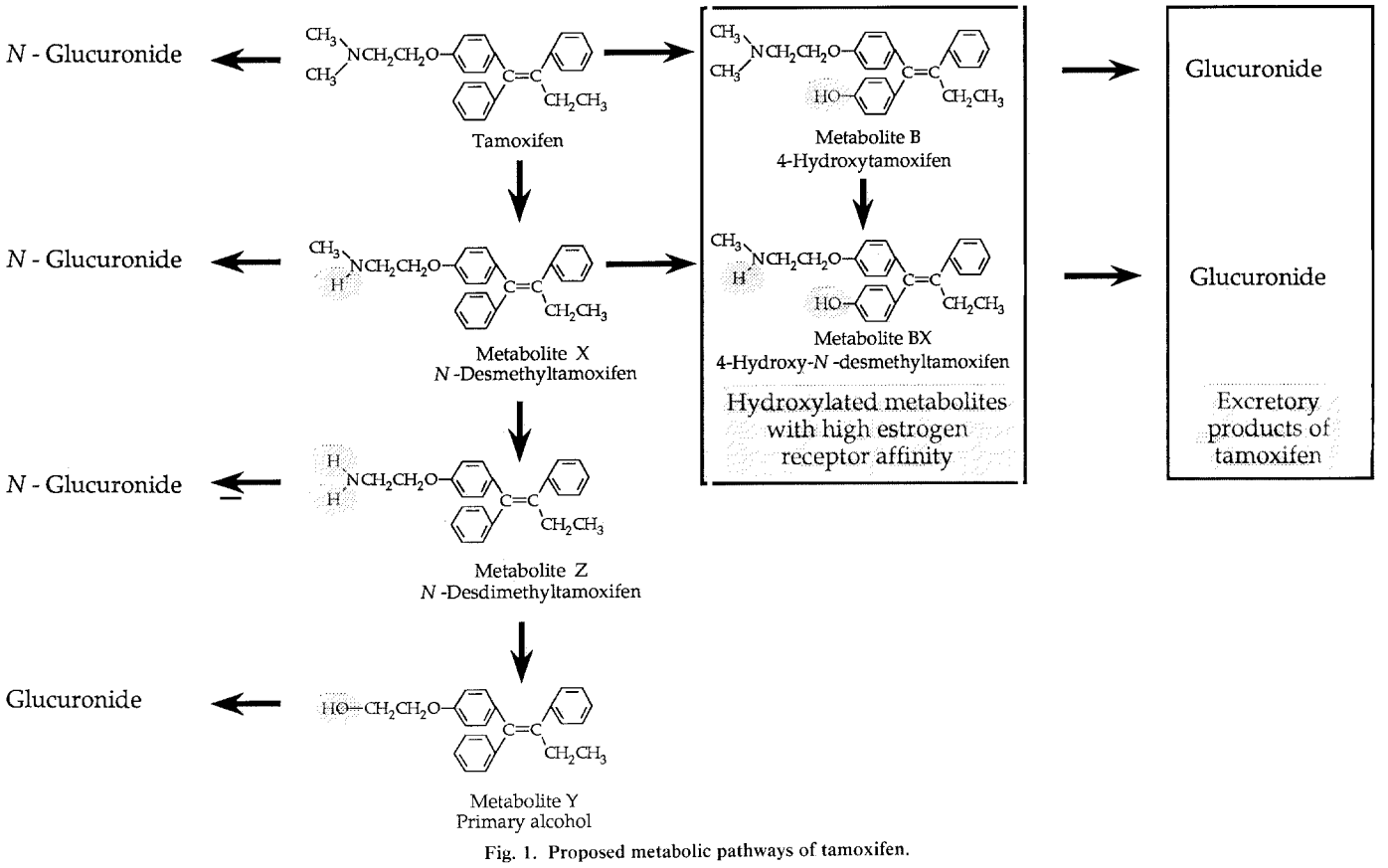

Fig. 1. Proposed metabolic pathways of tamoxifen.

Table 1 *Trials comparing tamoxifen monotherapy with the combination of tamoxifen and aminoglutethimide in breast cancer patients*

Drug[a]	Dose (mg)	Response rate CR + PR n^a	%	Ref.
TAM	10 b.i.d.	18/60	30	14
TAM AG H	10 b.i.d. 250 q.i.d. 20 b.i.d.	23/62	37	
TAM	10 b.i.d.	3/9	33	15
TAM AG H	10 b.i.d. 250 q.i.d. 20 b.i.d.	4/11	36	
TAM	10 b.i.d.	5/26	19	16
TAM AG H	10 b.i.d. 250 q.i.d. 10 b.i.d.	6/26	23	
TAM	10 b.i.d.	21/49	43	17
TAM AG H	10 b.i.d. 250 q.i.d. 10 + 10 + 20	25/51	49	
TAM	10 t.i.d.	32/94	34	18
TAM AG H	10 t.i.d. 250 q.i.d. 20 t.i.d.	24/83	29	
TAM	20 b.i.d.	18/34	53	19
TAM AG H	20 b.i.d. 250 q.i.d. 10 + 10 + 20	11/29	38	

[a] CR, complete response; PR, partial response; *n*, number of patients; TAM, tamoxifen; AG, aminoglutethimide; H, hydrocortisone.

Table 2 *Patient characteristics and drug treatment*

		Treatment		
		Tamoxifen[a]		
Patient	Age (yr)	Duration of treatment before entrance (mo)	Dose (mg)	Aminoglutethimide[b] dose (mg)
A. K.	66	66	30 q.d.	250 q.i.d.
I. L.	60	30	30 q.d.	250 t.i.d.
M. H.	60	41	20 q.d.	250 q.i.d.
B. H.	62	6	30 q.d.	250 q.i.d.
K. N.	47	31	30 t.i.d.	250 q.i.d.
M. F.	60	18	80 q.d.	250 q.i.d.

[a] Phases A and B.
[b] Phases B and C.

Tamoxifen and aminoglutethimide pharmacokinetics were evaluated under three different conditions, termed phases A, B, and C. Drug doses are given in Table 2.

Phase A refers to chronic (>6 months) treatment with tamoxifen given as a single agent. Tamoxifen kinetics and serum levels of its metabolites were determined. For the last 3 days prior to sampling, tamoxifen was given daily at 8 a.m. to all patients after overnight fasting except patient K. N. who received 30 mg t.i.d.[4] at strict 8-h intervals. On the day of investigation, tamoxifen was given at 8 a.m. Blood samples were drawn 0, 0.5, 1, 1.5, 2, 3, 4, 6, 9, 12, 15, and 24 h after the last dose.

Phase B is after treatment with the combination of tamoxifen plus aminoglutethimide and cortisone acetate at fixed doses for 6 weeks. Each patient received the same dose of tamoxifen as during phase A. Aminoglutethimide (250 mg q.i.d) was given with cortisone acetate (50 mg b.i.d for 2 weeks; thereafter 25 mg b.i.d.) as recommended (29). Cortisone acetate is combined with aminoglutethimide treatment because aminoglutethimide blocks the adrenal steroid synthesis (20). During the last 3 days before sampling, tamoxifen was given as in phase A. Aminoglutethimide and cortisone acetate were given at strict 6- and 12-h intervals, respectively. On the day of blood sampling, all drugs were given at 8 a.m. after overnight fasting. Then, cortisone acetate was given after 12 h, and tamoxifen was given after 24 h, but aminoglutethimide was withheld for 48 h. The sampling schedule was as described for phase A with additional samples obtained at 36 and 48 h to allow for determinations of aminoglutethimide half-life.

Phase C is 6 weeks after cessation of tamoxifen therapy. During this period the patients were treated with aminoglutethimide and cortisone acetate only. The kinetics of aminoglutethimide were determined as in phase B.

Blood samples were obtained by venous puncture. Each sample was allowed to clot for 30–60 min prior to centrifugation. Serum was removed and stored at −20°C until analysis. To eliminate between-day variations in the analysis, all samples from each patient were analyzed in the same run.

Determination of Tamoxifen and Its Metabolites. We used a modification of a high performance liquid chromatography assay described previously (30). The method and the modifications are as follows. Samples of 250 μl of serum deproteinized with acetonitrile were post-column on column concentrated on a small precolumn (0.21 x 3 cm), packed with 5 μm ODS material. The analytes were then directed into an analytical ODS Hypersil column (0.21 x 10 cm) by elution and column switching. The mobile phases and other details have been described previously (5, 30). Tamoxifen and its metabolites were post-column converted to fluorophors by UV illumination while passing through a quartz tube and then monitored by fluorescence detection (30).

The within-day precision (CV) of the assay for tamoxifen and its metabolites Y, B, X, and Z were 0.6–5.6% for serum levels between 10 and 800 ng/ml. Because our standard for metabolite BX is a mixture of the *cis* and *trans* isomers (5), the CV was not determined for this metabolite.

Determination of Aminoglutethimide and *N*-Acetylaminoglutethimide. Serum was deproteinized using a mixture of acetonitrile and perchloric acid. The samples were chromatographed on a 3-μm ODS Hypersil column, which was eluted isocratically as described previously (31). The absorbance was routinely recorded at 242 nm.

The CVs for aminoglutethimide and *N*-acetylaminoglutethimide at a concentration of 0.5 μg/ml are 3.9 and 2.6%, respectively.

Identification of Metabolite BX by LC/MS. For patient K. N., all serum samples from phase A and all samples from phase B were pooled in separate tubes. Ten ml from each pool was extracted with 10 volumes of hexane/butanol (98/2, v/v). The supernatant was evaporated in plastic beakers at 55°C under nitrogen, redissolved in 1 ml 50% acetonitrile, and centrifuged. The supernatant was transferred to sample vials, capped, and analyzed. The analytical column was connected to a LC/MS thermospray system (model 201; Vestec, Houston, TX). Before entering the thermospray, the effluent from the column was mixed with 0.1 M ammonium acetate, delivered at a rate of 0.3 ml/min via a zero dead volume T-connector. The flow rate of the HPLC system was 0.7 ml/min.

Pharmacokinetic Calculations. The area under drug concentration-time curve during steady state corresponding to one dose interval was calculated, using the trapezoidal rule (32). Clearance was calculated by the formula:

$$Cl = \frac{F \cdot D}{AUC_{\tau}^{ss}} \quad \text{(A)}$$

where F is the fraction of the dose (D) absorbed, and AUC_{τ}^{SS} is the area under the concentration-time curve corresponding to one dosing interval during steady state treatment (33). For aminoglutethimide, F is close to 1 (34). For tamoxifen, the value for F is unknown in humans, but F is close to 1 in animals (35). Because there is indirect evidence of good absorption in man (24), we assumed an F value equal to 1 in all patients under all conditions investigated (phases A and B).

The fraction of drug converted to the metabolite (f_m) is given by the equation (32):

$$f_m = \frac{AUC_{met} \cdot Cl_{met}}{AUC_{drug} \cdot Cl_{drug}} \quad \text{(B)}$$

where AUC_{met} and AUC_{drug} are the area under the serum concentration-time curve for the metabolite and drug, respectively. Cl_{met} is the clearance for metabolite, and Cl_{drug} is the clearance for the parent drug.

Rearrangement of equation B gives:

$$\frac{AUC_{met}}{AUC_{drug}} = f_m \cdot \frac{Cl_{drug}}{Cl_{met}} \quad \text{(C)}$$

A formula expressing the relationship between AUC_{met} and f_m and Cl_{met} was obtained by combining equations A and B:

$$AUC_{met} = f_m \cdot \frac{F \cdot D}{Cl_{met}} \quad \text{(D)}$$

Statistical Methods. The Wilcoxon signed rank test for paired data was used to compare the tamoxifen pharmacokinetic parameters obtained in phases A and B and aminoglutethimide parameters in phases B and C. P values were always expressed as two tailed.

RESULTS

Effect of Aminoglutethimide on Tamoxifen Kinetics and Metabolism. We compared the steady state pharmacokinetics and serum metabolite concentrations of tamoxifen given as a single

[4] The abbreviations used are: t.i.d., 3 times/day; q.i.d., 4 times/day; b.i.d, 2 times/day; q.d., 1 time/day; CV, coefficient of variation; ODS, octadecylsilane; metabolite Y, [*trans*-1(4-β-hydroxyethoxyphenyl)-1,2-diphenylbut-1-ene]; metabolite B, 4-hydroxytamoxifen; metabolite BX, 4-hydroxy-*N*-desmethyltamoxifen; metabolite X, *N*-desmethyltamoxifen; metabolite Z, *N*-desdimethyltamoxifen; LC/MS, liquid chromatography/mass spectrometry; HPLC, high performance liquid chromatography; CL, total body clearance; AUC, area under the concentration-time curve; C_{max}, maximum concentration during one dosing interval; C_{min}, minimum concentration during one dosing interval; M, the molecular ion; m/z, the mass to charge ratio.

Table 3 *Effect of aminoglutethimide treatment on tamoxifen pharmacokinetics*

		Tamoxifen			Metabolite Y			Metabolite B			Metabolite BX			Metabolite X			Metabolite Z		
Patient	Aminoglutethimide[a]	C_{max}[b]	C_{min}[c]	AUC[d]	C_{max}	C_{min}	AUC	C_{max}	C_{min}	AUC	C_{max}	C_{min}	AUC	C_{max}	C_{min}	AUC	C_{max}	C_{min}	AUC
A. K.	−	113	66	1761	24	13	314	4	3	62	11	6	192	207	141	3804	23	12	370
	+	50	23	767	19	7	271	3	1	41	9	2	117	121	78	2061	18	8	271
	−/+	2.3	2.9	2.3	1.3	1.9	1.2	1.3	3.0	1.5	1.2	3.0	1.6	1.7	1.8	1.8	1.3	1.5	1.4
I. L.	−	160	93	2647	18.1	6	194	10	4	129	8	3	119	264	170	4545	40	20	630
	+	65	24	770	13	4	119	6	2	63	0	0	0	95	64	1909	15	5	238
	−/+	2.5	3.9	3.4	1.4	1.5	1.6	1.7	2.0	2.0				2.8	2.7	2.4	2.7	4.0	2.6
M. H.	−	229	104	2929	8	1	42	18	10	312	55	15	500	308	135	3793	32	12	613
	+	81	36	1052	7	1	53	14	10	248	0	0	0	117	77	2078	13	7	230
	−/+	2.8	2.9	2.8	1.1	1.0	0.8	1.3	1.0	1.3				2.6	1.8	1.8	2.5	1.7	2.7
B. H.	−	433	212	7775	37	16	478	2	0	11	62	33	1166	379	268	7578	62	38	1204
	+	124	44	1494	35	6	315	0	0	0	3	0	2	160	86	2432	33	9	387
	−/+	3.5	4.8	5.2	1.1	2.7	1.5				20.7		583	2.4	3.1	3.1	1.9	4.2	3.1
K. N.[e]	−	356	279	7515	159	112	3208	21	12	409	64	45	1302	1100	860	23277	231	164	4627
	+	93	63	1792	83	54	1531	5	4	94	9	0	150	333	240	6731	100	62	1849
	−/+	3.8	4.4	4.2	1.9	2.1	2.1	4.2	3.0	4.4	7.1		8.7	3.3	3.6	3.5	2.3	2.6	2.5
M. F.	−	323	143	4728	160	61	2171	3	0	35	74	13	770	647	424	12588	109	63	1832
	+	162	31	1558	75	22	845	3	0	39	0	0	0	361	178	5484	78	28	1096
	−/+	2.0	4.6	3.0	2.1	2.8	2.6	1.0		0.9				1.8	2.4	2.3	1.4	2.3	1.7
Mean	−	269	150	4559	68	35	1068	10	5	160	46	19	675	484	333	9264	83	52	1546
Mean	+	96	37	1239	39	16	522	5	3	81	4	0.3	45	198	121	3449	43	20	679
	−/+	2.8	4.1	3.7	1.7	2.2	2.0	2.0	1.7	2.0	11.5	63.3	15.0	2.4	2.8	2.7	1.9	2.6	2.3
Significance[f]	(P) − *vs.* +			0.032			0.063			0.063			0.032			0.032			0.032

[a] −, without aminoglutethimide treatment; +, during aminoglutethimide treatment; −/+, ratio.
[b] ng/ml.
[c] ng/ml.
[d] $ng \cdot h \cdot ml^{-1}$.
[e] Patient K. N. used tamoxifen three times daily. *AUC* in this patient is estimated during 8 h and normalized to 24 h.
[f] Wilcoxon signed rank test for paired data.

agent (phase A) with these parameters in the same patients when they were given the drug combination of tamoxifen plus aminoglutethimide for 6 weeks (phase B).

Fig. 2 shows the steady state serum profiles for tamoxifen and its metabolites in patient M. H. during one dosing interval in the absence and presence of aminoglutethimide. In agreement with earlier results (36), the serum levels of tamoxifen and metabolites Y, BX, X, and Z reached a maximum concentration (C_{max}) about 2 h after drug intake (data not shown). The difference between C_{max} and the lowest level during one dosing interval (C_{min}) was reduced for tamoxifen and its metabolites during aminoglutethimide treatment (Fig. 2 and Table 3). Notably, the concentrations of metabolite BX were reduced to near the detection limit during the combination therapy. The results for all 6 patients are summarized in Table 3.

The marked reduction in the amount of metabolite BX in serum during aminoglutethimide treatment (Table 3), was confirmed by mass spectrometry analysis. The LC/MS traces for the $(M + 1)^+$ ion show that this metabolite nearly disappeared in serum during aminoglutethimide treatment (Fig. 3).

Aminoglutethimide caused a significant decrease in *AUC* ($P = 0.032$) for tamoxifen (mean reduction, 73%; range, 80–56%), corresponding to a mean increase in tamoxifen clearance of 222% (Table 4). *AUC* for most metabolites was reduced (mean reduction, about 50%) (Table 3).

The ratio AUC_{met}/AUC_{drug} increased 35–80% during aminoglutethimide treatment for all metabolites, except metabolite BX (Table 5).

Aminoglutethimide Pharmacokinetics and Acetylation. The pharmacokinetics of aminoglutethimide and its metabolite, *N*-acetylaminoglutethimide, were determined in patients receiving chronic treatment with the drug combination of tamoxifen plus aminoglutethimide (phase B) and after tamoxifen was withdrawn for 6 weeks (phase C). In phase C neither tamoxifen nor its metabolites were detected in patient sera, with the exception of metabolite X which was found in low concentrations (<1 ng/ml) in sera from three patients (A. K., M. H., and M. F.). The

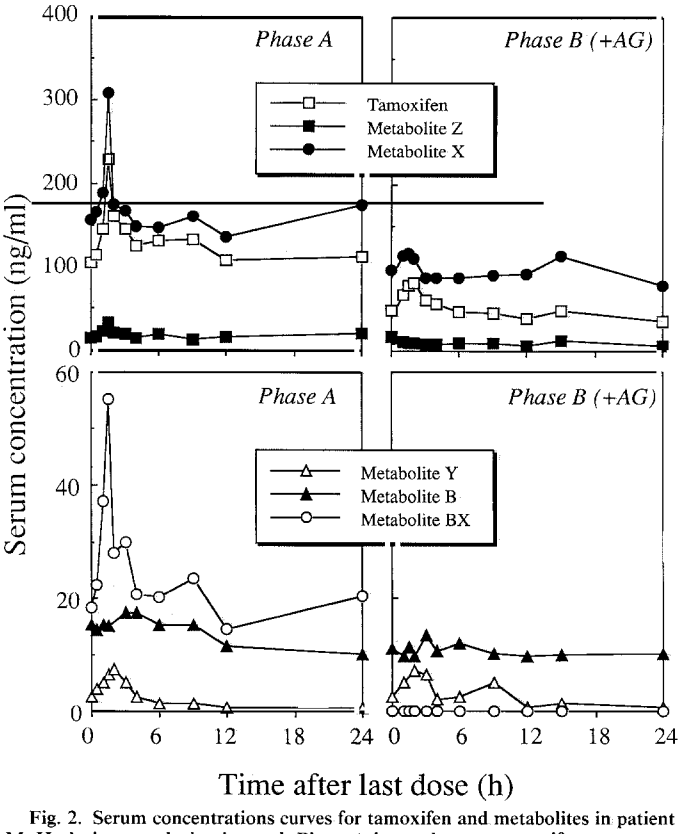

Fig. 2. Serum concentrations curves for tamoxifen and metabolites in patient M. H. during one dosing interval. Phase A is steady state tamoxifen treatment. Phase B is after 6 weeks of combination therapy with tamoxifen and aminoglutethimide. The tamoxifen dose was 30 mg once daily in both phases.

results from a single patient (B. H.) are shown in Fig. 4. Data from all patients are summarized in Tables 4 and 6.

Tamoxifen did not affect the pharmacokinetics of aminoglutethimide or its conversion to *N*-acetylaminoglutethimide (Fig. 4 and Tables 4 and 6).

DISCUSSION

This study demonstrates a pronounced reduction in the serum concentrations of tamoxifen and most of its serum metabolites during aminoglutethimide treatment (Table 3). Several explanations should be considered. Aminoglutethimide may decrease the serum concentration of tamoxifen and its metabolites by reducing the absorption of tamoxifen, reducing tamoxifen protein binding, or by enhancement of tamoxifen metabolism.

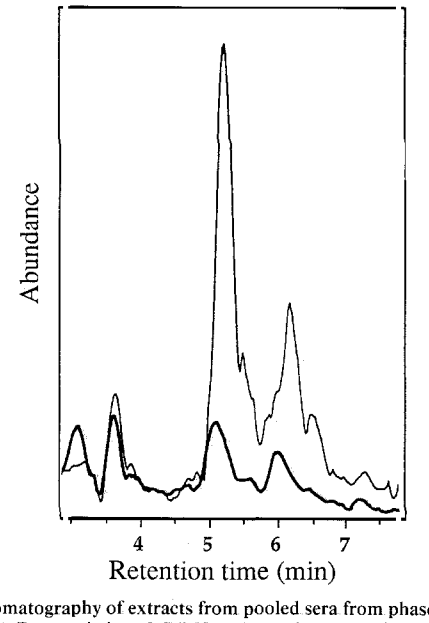

Fig. 3. Chromatography of extracts from pooled sera from phase A and phase B (patient K. N.). Reversed phase LC/MS and sample preparation were performed as described in the text. *Top trace*, selected ion-monitoring trace for the $(M + 1)^+$ ion for metabolite BX (374 m/z) from phase A (tamoxifen as single drug); *bottom trace*, phase B (tamoxifen combined with aminoglutethimide). The second peak eluting after 6 min is due to interference from the tamoxifen peak (372 m/z).

Aminoglutethimide is not known to influence the growth of intestinal bacteria or drug uptake. Thus, there are no data to suggest that aminoglutethimide may impair tamoxifen absorption.

Tamoxifen is highly (>98%) bound by protein in serum (30) and alterations in protein binding may affect the metabolism and distribution of this drug. Because aminoglutethimide is only moderately protein bound (about 25%) (34), it is unlikely that aminoglutethimide can displace tamoxifen from its binding sites.

Our data show that aminoglutethimide reduces the serum level and enhances the elimination of tamoxifen, corresponding to an increase in tamoxifen clearance from 189–608 ml/min (Table 4). This effect from aminoglutethimide is probably due to induction of tamoxifen metabolism, because there is ample evidence that aminoglutethimide may stimulate metabolic processes important in tamoxifen biotransformation.

Tamoxifen is metabolized by hydroxylations and demethylations followed by glucuronidation of the different metabolites as well as of tamoxifen itself (Fig. 1) (4, 35, 37). Aminoglutethimide is an efficient inducer of cytochrome P450 mixed function oxidases (10, 20, 21, 38, 39), and it shows similarities with phenobarbital in this respect (40). Treatment of rats with

Table 4 *Interaction between aminoglutethimide and tamoxifen*

Patient	Clearance of tamoxifen (ml/min)		Clearance of aminoglutethimide (ml/min)	
	−AG[a]	+AG[b]	−TAM[c]	+TAM[d]
A. K.	284	652	105	84
I. L.	189	649	113	87
M. H.	114	317	175	235
B. H.	64	335	111	107
K. N.[e]	200	837		
M. F.	282	856	71	86
Mean	189	608	115	120
Significance (*P*)[f]	0.032		>0.20	

[a] No aminoglutethimide, phase A.
[b] Aminoglutethimide treatment, phase B.
[c] No tamoxifen, phase C.
[d] Tamoxifen treatment, phase B.
[e] K. N. did not enter the final part of the study because of rapidly progressing disease.
[f] Wilcoxon signed rank test for paired data.

Table 5 *Effect of steady state aminoglutethimide treatment on the amount of tamoxifen metabolites relative to parent drug in serum*

Patient	Aminoglutethimide	AUC_τ^{ss}[a] for metabolite/AUC_τ^{ss} for tamoxifen				
		Y	B	BX	X	Z
A. K.	−	0.18	0.04	0.11	2.16	0.21
	+	0.35	0.05	0.15	2.69	0.35
I. L.	−	0.07	0.05	0.04	1.72	0.24
	+	0.15	0.08	<0.001[b]	2.48	0.31
M. H.	−	0.01	0.11	0.17	1.29	0.21
	+	0.05	0.24	<0.001[b]	1.98	0.22
B. H.	−	0.06	0.001	0.15	0.97	0.15
	+	0.21	0.00	0.001	1.63	0.26
K. N.	−	0.43	0.05	0.17	3.10	0.62
	+	0.85	0.05	0.08	3.76	1.03
M. F.	−	0.46	0.01	0.16	2.66	0.39
	+	0.54	0.03	<0.001[b]	3.52	0.70
Mean	−	0.20	0.04	0.13	1.98	0.30
	+	0.36	0.07	0.04	2.68	0.47
P − *vs.* +[c]		0.032	>0.10	0.032	0.032	0.032

[a] AUC_τ^{ss}, *AUC* in steady state during one dosing interval.
[b] BX not detectable during aminoglutethimide therapy.
[c] Wilcoxon signed rank test for paired data.

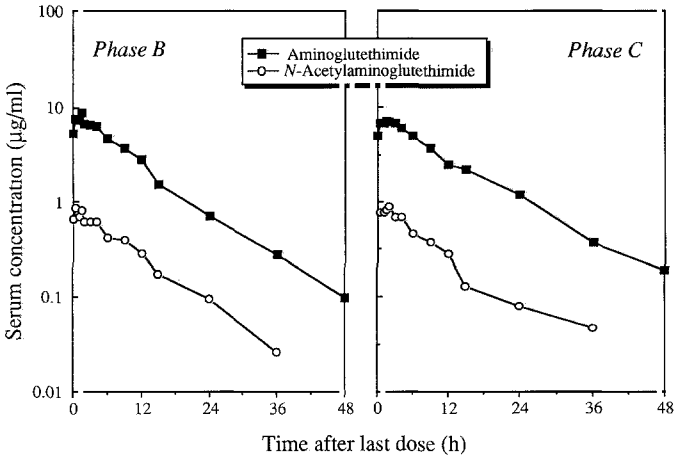

Fig. 4. Serum concentration curves for aminoglutethimide and *N*-acetylaminoglutethimide in patient B. H. Phase B is after 6 weeks of combination therapy with tamoxifen and aminoglutethimide. Phase C is during administration of aminoglutethimide as a single agent 6 weeks after cessation of tamoxifen treatment. In both phases the kinetics of aminoglutethimide were recorded during a period of 48 h of withdrawal of this drug.

barbiturate increases demethylation of tamoxifen in liver microsomes *in vitro* (41).

Induction of glucuronidation has been reported in man after treatment with other well-known enzyme inducers such as phenytoin, phenobarbital, and rifampicin (42), and recently rat liver glucuronidation was found to be enhanced by aminoglutethimide (43). The two hydroxylated metabolites, B and BX, are excreted in bile (5). Their biliary excretion as glucuronides may significantly contribute to their total clearance, and induction of glucuronidation of hydroxylated tamoxifen metabolites by aminoglutethimide may decrease serum levels of these species.

N-Glucuronidation of tertiary amines has been demonstrated only in higher primates (42), suggesting a metabolic pathway for tamoxifen in man, not existing in most experimental animals. Stimulation of tamoxifen *N*-glucuronidation (Fig. 1) by aminoglutethimide would enhance the metabolic clearance of the drug but cannot explain the altered ratio between *AUC* for a metabolite relative to that of the parent drug.

The observation that *AUC* for tamoxifen metabolites is reduced (Tables 3 and 5) also agrees with the idea that aminoglutethimide affects tamoxifen metabolism. Our study does not allow delineation of the kinetics behind the reduction in metabolite *AUC*. According to equation D, AUC_{met} depends on the fraction of tamoxifen converted into the metabolite as well as on the metabolite clearance. These (f_m and Cl_{met}) are parameters not accounted for by the present study design. However, reduction of *AUC* for tamoxifen metabolites may be due to reduced f_m or increased Cl_{met}. Reduction in f_m may result if aminoglutethimide stimulates the formation of metabolites not detected by our HPLC system, which was optimized for the analysis of triphenylethylenes present in human serum during monotherapy (30). Increased metabolite clearance may occur following enhancement of metabolic glucuronidation.

Our patients were given cortisone acetate as a glucocorticoid substitution during aminoglutethimide treatment. There is evidence that corticosteroids may affect the metabolism of some drugs (40, 44). We do not consider cortisone acetate responsible for the observed alteration in tamoxifen metabolism for two reasons. First, aminoglutethimide is an inhibitor of adrenal cortisol synthesis, and the cortisone acetate substitution does not increase plasma cortisol above physiological levels (34). Second, aminoglutethimide is an enzyme inducer also in the absence of glucocorticoid substitution (39).

The effect of aminoglutethimide on tamoxifen metabolism has important implications. Obviously, lowering the serum concentration of tamoxifen and its active metabolites reduces their effects. In addition, aminoglutethimide increases the relative amount in serum of most metabolites compared with the parent drug (Table 5). This also suggests that the tamoxifen-aminoglutethimide interaction is due to increased metabolism *and not decreased gastrointestinal absorption (see above).* An increased ratio AUC_{met}/AUC_{drug} is observed for the hydroxylated metabolite B, whereas the ratio decreases for metabolite BX, another hydroxylated metabolite. These metabolites have considerably higher affinity for the estrogen receptor than tamoxifen itself (6–9). It has recently been demonstrated that the inhibition of growth of the estrogen receptor-positive MCF-7 cells in the presence of tamoxifen and metabolites Y, B, X, and Z parallels the relative affinity of these agents for the estrogen receptor (45). Effects of higher doses of tamoxifen given in combination with aminoglutethimide may therefore be influenced by the altered metabolite profile of tamoxifen.

Tamoxifen is a weak estrogen agonist and strong antagonist, and tamoxifen metabolites may also have agonistic or antagonistic properties (46). Thus, alterations in tamoxifen metabolism induced by aminoglutethimide may increase the amount of estrogen agonists at the expense of estrogen antagonists. Such a metabolic effect would counteract the biological effect of aminoglutethimide thought to be mediated by estrogen depletion, and a decreased additive effect of the drug combination of tamoxifen and aminoglutethimide would ensue. This could explain the negative results from the clinical trials of this drug combination.

Our results also show major variations in ratios of tamoxifen to its metabolites in the absence of aminoglutethimide therapy (Table 3). *This raises the question that breast cancer patients* who respond to tamoxifen therapy may have a tamoxifen metabolism different from that of the nonresponders.

There are occasional reports that tamoxifen interacts with other drugs (26–28). We observed no effect of tamoxifen ad-

Table 6 *Effect of tamoxifen treatment on aminoglutethimide pharmacokinetics and acetylation*

Tamoxifen[a]		Aminoglutethimide			*N*-Acetylaminoglutethimide
		AUC_τ^{ss}[b] (μg·h·ml^{-1})	$T_{½}$ (h)	V_Z[c] (liter)	AUC_τ^{ss} (μg·h·ml^{-1})
+	Mean[d]	40.5	7.4	76.1	4.2
	SD	13.5	1.4	42.1	2.4
−	Mean	39.3	7.2	75.8	3.8
	SD	12.4	1.8	38.7	1.6

[a] +, during tamoxifen treatment (phase B); −, without tamoxifen (phase C).
[b] AUC in steady state during one dosing interval.
[c] Pharmacokinetic volume of distribution during terminal phase.
[d] $n = 5$.

ministration on the disposition of aminoglutethimide (Tables 4 and 6).

In conclusion, the present report demonstrates that aminoglutethimide markedly reduces serum concentrations of tamoxifen and its metabolites, probably by inducing tamoxifen metabolism. Our findings suggest that clinical trials performed on tamoxifen plus aminoglutethimide combined therapy (14–19) may be biased by low tamoxifen serum levels and change in its metabolite profile. This may explain why combination therapy did not result in significantly higher response rates than tamoxifen monotherapy (Table 1). Future clinical trials of the combination therapy should therefore include serum concentration monitoring and tamoxifen doses should possibly be increased to compensate for decreased bioavailability of tamoxifen and its metabolites.

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