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Alterations in the Metabolism of Oestrogens During Treatment with Aminoglutethimide in Breast Cancer Patients
Preliminary Findings

P.E. Lønning, S. Kvinnsland, T. Thorsen and *P.M. Ueland*

Departments of Biochemical Endocrinology, Oncology and Clinical Pharmacology Unit, Department of Pharmacology, University of Bergen, Bergen

Summary

In this small study, the effect of aminoglutethimide on the disposition of oestrogens in women with advanced breast cancer was investigated using bolus injections of 4-[^{14}C]-oestradiol and 6,7-[^{3}H]-oestrone sulphate, alone or in combination.

No alterations in oestrogen disposition were seen after short term (6 hours) aminoglutethimide administration. During long term (3 weeks to 8 months) aminoglutethimide treatment mean 4-[^{14}C]-oestradiol clearance was not changed. ^{14}C-Oestrone sulphate AUC was reduced by 43% at a low dose of aminoglutethimide (125mg twice daily) and by 65% at a high dose (250mg 4 times daily) with hydrocortisone acetate 25mg twice daily. The oestrone sulphate terminal elimination rate constant (λ_z) was concurrently increased (mean of 46 and 79%, respectively, with the 2 dosage regimens).

A possible increase in oestrone sulphate clearance during long term treatment was tested for by injecting 6,7-[^{3}H]-oestrone sulphate. These studies revealed a marked increase (mean 104%) in oestrone sulphate clearance in patients receiving the high dose aminoglutethimide schedule.

Following injection of 4-[^{14}C]-oestradiol plus 6,7-[^{3}H]-oestrone sulphate, the fraction of 4-[^{14}C]-oestradiol metabolised to oestrone sulphate was found to be reduced in all patients (mean 13%). A mean increase of 80% in the urinary excretion of ^{14}C-oestriol was observed after 4-[^{14}C]-oestradiol administration.

Our results, although preliminary, suggest that aminoglutethimide is a potent inducer of aminoglutethimide metabolism, thereby producing a significant reduction in plasma bioavailability of oestrone sulphate. These effects may have a role in the action of aminoglutethimide, a finding which warrants further investigation.

Aminoglutethimide in combination with glucocorticoids was recently introduced as a new endocrine treatment of advanced breast cancer (Santen et al. 1974). Aminoglutethimide is an inhibitor of aromatase and of several other enzymes in the steroidogenic pathways (Santen 1981). Its effect on breast cancer is probably caused by inhibition of oestrogen production. The main prohormone aromatised to oestrogen, androstenedione, is not significantly altered on a conventional aminoglutethimide dosage regimen (Samojlik et al. 1980; Vermeulen et al. 1983). The conversion of adrostenedione to oestrone is inhibited by more than 95% by aminoglutethimide *in vivo* (Santen et al. 1978). Therefore, the effect of aminoglutethimide on breast cancer is thought to be mediated

by its inhibition of the peripheral aromatase system only.

Oestrogens are, to a large extent, metabolised by cytochrome P450-dependent hydroxylations (Breuer et al. 1969). Rifampicin (rifampin), a potent inducer of the mixed function hydroxylases, increases oestradiol metabolism in humans by stimulating catechol oestrogen production (Bolt et al. 1975), and phenytoin reduces oestrone and oestradiol plasma concentrations in patients on oral substitution therapy with oestrogens (Notelovitz et al. 1981). Barbiturates, as well as cyclophosphamide, increase C-6 and/or C-7 hydroxylations of oestrogens in humans (Wenzel & Stahl 1970). Both 16α- and 16β-hydroxylations of testosterone are stimulated by barbiturates in the rat (Conney et al. 1973; Lu et al. 1976; Nakamura & Ueda 1980).

Aminoglutethimide is a potent inducer of several mixed function hydroxylases in the liver endoplasmic reticulum (Lønning et al. 1984a,b; Murray et al. 1979; Santen et al. 1977b). It also increases hepatic hydroxylation of testosterone in the rat when administered for 1 or 2 weeks (Starka et al. 1971). In humans, a decrease in urinary testosterone, as well as its reduced metabolites ethiocholanolone and androsterone, was observed when aminoglutethimide was administered as a test substance to male patients on substitution therapy with testosterone (Horky et al. 1971). This finding may be explained by an increased production of hydroxylated metabolites.

Others have found that the clearance of oestrone measured by a steady-state infusion technique was unaffected by aminoglutethimide treatment (Santen et al. 1978). Because an influence of aminoglutethimide on oestrogen metabolism may have important therapeutic implications, we decided to further investigate its effect on oestrogen metabolism.

We preferred a bolus infusion technique to a steady-state infusion technique to evaluate oestradiol and oestrone sulphate disposition and interconversion for the following reasons:

1. More than 8 hours of infusion may be required to obtain steady-state concentrations of oestrone sulphate following free oestrogen infusions (Longcope 1972; Ruder et al. 1972).

2. By means of a bolus injection technique, oestrone sulphate half-life and distribution volume could be assessed.

Materials and Methods

14 postmenopausal women who were to receive aminoglutethimide as treatment for advanced breast cancer and 2 patients on steady-state aminoglutethimide therapy were studied. Their mean age was 62.5 years (range 44 to 77), and the mean bodyweight was 64.7kg (43 to 82.5). All patients gave their informed consent to participate in the study, which was approved by the local Ethical Committee. All patients were non-smokers. Drugs known to be enzyme inhibitors or inducers were not ingested, except for patient SP who received cimetidine 800mg daily until 4 days before the phase 3 investigation during aminoglutethimide therapy, but not prior to the phase 1 (baseline) injection.

All but 2 patients (WK and RL) had normal livers as assessed by ultrasonography, and serum transaminase concentrations were within normal limits except in patient RL. The patients were clinically euthyroid during the investigation and serum creatinine and BUN were normal.

Protocols (Table I)

4-[^{14}C]-Oestradiol and/or 6,7-[^{3}H]-oestrone sulphate were injected under 3 different conditions, referred to as phases 1 to 3. Administration prior to initiation or after cessation of aminoglutethimide treatment was named phase 1, while injections administered following aminoglutethimide administered as a low dose (125mg 2 times/day) and high dose (250mg 4 times/day with glucocorticoids) drug schedules were named phase 2 and 3, respectively.

Protocol I: 2 patients received an injection of 4-[^{14}C]-oestradiol (phase 1) followed 1 week later by an oral loading dose of aminoglutethimide 1g plus hydrocortisone 50mg, designed to produce plasma concentrations approaching those observed at

Table I. Patients and protocols of treatment[a]

Patient	Protocol	Aminoglutethimide dose	No. of steroid injections	Interval	Steroid injected	Urinalysis
CR	I	1g loading	2	6 hours	[^{14}C] E_2	No
BS	I	1g loading	2	6 hours	[^{14}C] E_2	No
HN	II	250mg qid	2	7 weeks	[^{14}C] E_2	Yes
AB	II	250mg qid	2	5 weeks	[^{14}C] E_2	Yes
LT	II	250mg qid	2	8 weeks	[^{14}C] E_2	Yes
KO	III	125mg bid/250mg qid	3	4/7 weeks	[^{14}C] E_2	Yes
HR	III	125mg bid/250mg qid	3	5/5 weeks	[^{14}C] E_2	Yes
TH	III	125mg bid/250mg qid	3	5/4 weeks	[^{14}C] E_2	Yes
WK	IV	250mg qid	2	5 weeks	[^{3}H] E_1S	No
AK	IV	250mg qid	2	4 weeks	[^{3}H] E_1S	No
RL	V	250mg qid	2	8 weeks	[^{14}C] E_2/[^{3}H] E_1S	No
BV	V	250mg qid	2	6 weeks	[^{14}C] E_2/[^{3}H] E_1S	No
AH	V	250mg qid	2	3 weeks	[^{14}C] E_2/[^{3}H] E_1S	No
KM[b]	V	250mg qid	2	8 months	[^{14}C] E_2/[^{3}H] E_1S	No
SP[b,c]	V	250mg bid	2	7 months	[^{14}C] E_2/[^{3}H] E_1S	No
MH	V	250mg qid	2	3 weeks	[^{14}C] E_2/[^{3}H] E_1S	No

a See text for details.

b First steroid injection given 3 weeks after cessation of aminoglutethimide treatment.

c Note drug dosage.

Abbreviations: IV = intravenous; qid = 4 times daily; bid = twice daily; [^{14}C] E_2 = oestradiol; [^{3}H] E_1S = oestrone sulphate.

steady-state with high-dose protocols. Six hours after the loading dose the patients were given a second 4-[^{14}C]-oestradiol injection (phase 3): after a further 2 hours, oral treatment with aminoglutethimide 250mg 4 times/day and hydrocortisone 50mg twice daily was started.

Protocol II: 3 patients were given an injection of 4-[^{14}C]-oestradiol, followed by treatment with oral aminoglutethimide 250mg 4 times/day (high dose) and hydrocortisone (50mg twice daily for 2 weeks, then 25mg twice daily) for 5 to 8 weeks, at which time the oestradiol injection was repeated (phase 3).

Protocol III: following a phase 1 injection of 4-[^{14}C]-oestradiol, 3 patients were given oral aminoglutethimide 125mg twice daily for 4 to 5 weeks, when they received a second oestradiol injection (phase 2). After a further 4 to 7 weeks' treatment with high dose aminoglutethimide and hydrocortisone, a third oestradiol injection was given (phase 3).

Protocol IV: 2 patients received 2 injections of 6,7-[^{3}H]-oestrone sulphate (phases 1 and 3) separated by 4 to 5 weeks' oral treatment with high dose aminoglutethimide, with hydrocortisone (as above).

Protocol V: 6 patients received 4-[^{14}C]-oestradiol/6,7-[^{3}H]-oestrone sulphate injections. Four patients received a first (phase 1) 4-[^{14}C]-oestradiol/6,7-[^{3}H]-oestrone sulphate injection prior to treatment with high dose aminoglutethimide, and hydrocortisone (see above) for 3 to 8 weeks, at which time the 4-[^{14}C]-oestradiol/6,7 [^{3}H]-oestrone sulphate injection was repeated (phase 3).

Two patients (SP and KM) received their phase 3 injection after aminoglutethimide treatment for 7 and 8 months, and their phase 1 injection 3 weeks after cessation of aminoglutethimide therapy.

Chemicals

All solvents were of analytical or spectrophotometric grade and obtained from Merck AG (Darmstadt, West Germany), except for the diethyl

ether (Den Norske Eterfabrikk, Oslo, Norway). The radiolabelled isotopes of steroids used for the investigations and internal standards were obtained from New England Nuclear Corporation (Dreieich, West Germany), except for 6,9-[^{3}H]-oestriol-16α,β-D-glucuronide (Amersham International, Amersham, UK). Sulphatase (S-9754) was obtained from Sigma Ltd (London, UK) and β-glucuronidase (P. Vulgata) from Baylove Chemicals Ltd (Edinburgh, UK).

^{3}H-Oestradiol and ^{3}H-oestrone were purified on an LH-20 Sephadex® column before use to obtain a purity greater than 97%. ^{3}H-Oestrone sulphate was extracted to remove free steroids, and a purity greater than 97% was measured by chromatography after hydrolysis. ^{3}H-Oestriol glucuronide was purified on a DEHE-Sephadex column eluted with a salt gradient and absorbed on a Sep-Pak C-18 column (Shackleton et al. 1980). The purity of this standard was found to be about 97% using thin layer chromatography (Lisboa & Diczfalucy 1962). 4-[^{14}C]-Oestradiol was more than 98% pure at delivery.

Administration of Labelled Oestradiol and Collection of Blood Samples

Five to 15 μCi 4-[^{14}C]-oestradiol and/or 40 to 70 μCi of 6,7-[^{3}H]-oestrone sulphate, dissolved in ethanol 5% in isotonic saline (10 or 20ml) was administered at 8am after an overnight fast, as a bolus injection over 1 minute. The midpoint of injection was taken as time zero. The patients remained supine for 6 hours after injection: posture has been shown to affect metabolism of steroids with a high extraction ratio, due to alterations in hepatic blood flow (Flood et al. 1973). Thereafter, they were allowed to move freely. Food and other drugs, if necessary, were allowed after 2 hours.

Heparinised blood samples were drawn from an indwelling venous needle in the contralateral arm at the following intervals after isotope injection: 0, 3, 6, 10, 15, 22.5, 30, 45, 60, 90, 120, 150, 180, 210, 240, 300, 360, 480, 600, 720 and 1440 minutes. The number of initial samples were slightly reduced for patients in protocol V because of the larger blood volumes required for parallel analyses.

Aliquots (50μl) of the injected solutions were counted for radioactivity to determine the total dose administered. The blood samples were centrifuged immediately and plasma removed. 500μl of plasma was taken from each sample to determine total radioactivity (protocols I and II). The remainder of the samples was immediately frozen and stored at −20°C until analysis.

Urine from the 6 patients investigated in protocols II and III was collected as individual samples or 24-hour portions for 48 to 72 hours after the isotope injections. One millilitre of saturated ascorbic acid solution was added immediately, and the samples stored at −20°C.

Analysis

Determination of labelled oestrogens in plasma: for protocols I, II and III, ^{14}C-labelled steroids oestrone, oestradiol and oestrone sulphate were separated by a previously reported technique (Myking et al. 1980; Myking & Digranes 1984) with minor modifications. Contamination of oestradiol in the oestrone fraction, and vice versa was less than 3.5%.

Purified fractions were counted in Opti-Fluor® (Packard Corporation) in a Searle Mark III β-spectrometer. Total counts recorded corresponded to a counting error of less than 5%, except for some low values for oestrone and oestradiol which reached standard deviations of between 5 and 10%. All counts were corrected for quench and converted to disintegrations/min by a channel ratio procedure.

Additionally, for protocol IV, 4-[^{14}C]-oestrone (about 800 cpm) was added as an internal standard after sulphatase hydrolysis. Values obtained were corrected for efficiency of hydrolysis, which was 89.1 ± 1.2% (mean ± SD, n = 12) [study in progress]. In protocol V, no internal standards were used. The samples were analysed in duplicate, and counting performed in a LKB Wallac 1219® β-Spectrometer using a channel ratio procedure. Results were corrected for recovery using data obtained from the other experiments (mean recovery oestradiol 91.2%, oestrone sulphate 64.3%).

The results obtained showed a ^{14}C-oestradiol

interassay coefficient of variation (CV) of 4.5%. The CV for ^{14}C-oestrone sulphate and ^{3}H-oestrone sulphate measurements were 5.2 and 8.5%, respectively, and the CV for the ^{14}C-oestrone sulphate/^{3}H-oestrone sulphate ratio of the separate samples was 1.6%.

Determinations of labelled oestrogens in urine: for protocols II and III samples of urine (0.5ml) were analysed for total radioactivity. Samples from the patients in protocol II were treated as follows: using ^{3}H-oestriol-glucuronide as the internal standard, samples (4.5ml) were adsorbed and eluted on Sep-Pak C-18® columns as described by others (Shackleton et al. 1980). It was redissolved in 1ml acetate buffer (0.1 mol/L) pH 4.5, containing β-glucuronidase 1 g/L (2000 IU). After incubation (48 hours at 37°) it was absorbed on a Sep-Pak® column and chromatographed on the LH 20 column eluted with methanol : benzene (10 : 90 and 15 : 85). Only the mid-portion (2ml) of the eluate was collected to avoid interference from other metabolites. The counting error was less than 2%.

Samples from patients in protocol III were analysed in the same way, except that larger urinary samples (13.5ml) were used. These samples were analysed twice, each time in duplicate. Following chromatography, parallel samples from run 2 were pooled and cocrystallised with 100mg non-radioactive oestriol. The samples were recrystallised 4 times from methanol : acetone : acetonitrile. A constant ^{3}H/^{14}C ratio was found in crystals and mother liquor. The intra-assay CV of this analysis was 4.9%.

Determination of aminoglutethimide in plasma: in some patients a single sample was obtained 4 hours after oestradiol injection for aminoglutethimide measurement (protocols I and II) using an HPLC method (Schanche et al. 1984). Otherwise, aminoglutethimide was measured in samples obtained at time zero, 4, 8 and 12 hours following oestrogen injection, and the mean value reported.

Thyroidal hormones and testosterone-binding globulin: were measured in samples obtained in phases 1 and 3 from patients investigated in protocols I, II and V. Analyses were performed using radioimmunoassay and IRMA kits routinely used in this laboratory, with a coefficient of variation of about 5%.

Calculations and Statistics

The pharmacokinetic parameters were calculated using the formulae shown in the Glossary. The intercepts C_1, C_2 and C_3, and the disposition rate constants for oestradiol were calculated by non-linear regression using a Statgraphics® program on the IBM PC 5060®. Only data obtained during the first 6 hours were used, as concentrations after this time were below the limits of assay. The AUC of 6,7-[^{3}H]-oestrone sulphate was measured by the trapezoidal rule, extrapolating to infinity after log-linear least-square regression analysis. All statistical analyses were performed using the Wilcoxin matched pairs signed rank test, and p-values were expressed as 2-tailed.

Results

Aminoglutethimide plasma concentrations in the 2 protocol I patients were 5.6 and 9.8 mg/L 4 hours after the second steroid injection. Corresponding

Glossary of symbols and derivations

$$CL = \frac{D_{IV}}{AUC} \qquad AUC = \frac{C_1}{\lambda_1} + \frac{C_2}{\lambda_2} + \frac{C_3}{\lambda_3}$$

$$V_2 = \frac{D_{IV}}{\lambda_z \cdot AUC} \qquad fm = \frac{CL_{metabolite} \cdot AUC_{metabolite}}{CL_{drug} \cdot AUC_{drug}}$$

$$CL_H = Q \cdot \frac{CL_{int}}{Q + CL_{int}}$$

CL	Total body clearance
D_{IV}	Amount of drug administered as an IV bolus
AUC	Area under the oestradiol and/or oestrone sulphate plasma concentration-time curve
V_z	Apparent volume of distribution during the terminal phase
λ_1, λ_2, λ_3, λ_z	Disposition rate constants
C_1, C_2, C_3	Concentrations obtained by back-extrapolation of the disposition slope to zero
CL_H	Hepatic clearance
CL_{int}	Intrinsic clearance
Q	Hepatic blood flow
f_m	Fraction converted to metabolite (Rowland & Tozer 1980)

concentrations in the protocol II patients were between 5 and 19 mg/L. Steady-state plasma concentrations in patients undergoing protocols III and V were between 1.6 and 1.9 mg/L in phase 2 and between 4.0 and 12.5 mg/L in phase 3.

Kinetics of radiolabelled oestrogens: the oestradiol data were best fitted by a 2-compartment model in 1 patient (AB) and by a 3-compartment model in the remainder.

The pharmacokinetic parameters are detailed in tables II to V: the findings may be summarised as follows. Protocol I shows that, in the patients studied, acute aminoglutethimide administration did not significantly alter the disposition of oestradiol or oestrone sulphate. The clearance of oestradiol was not affected by prolonged administration of aminoglutethimide in protocols II, III or V.

Clearance of oestrone sulphate was significantly increased (mean 104%, $p < 0.01$) following long term treatment in protocols IV and V, which was consistent with a mean 62% increase in λ_z for ^{14}C-oestrone sulphate in protocols II and III.

Table II. Effect of aminoglutethimide on clearance of oestradiol (E_2) measured after intravenous ^{14}C-oestradiol bolus injections before treatment (phase 1) and during low (phase 2) and high (phase 3) dose aminoglutethimide treatment (protocols I, II, III and V)

Patient	Protocol	Phase 1	Phase 2	Phase 3
		E_2 CL (L/h)	E_2 CL (L/h) [Δ%]	E_2 CL (L/h) [Δ%]
CR	I	24.7		27.2 [+10.1]
BS	I	64.1		60.0 [−6.4]
HN	II	24.1		33.1 [+37.3]
AB	II	62.9		67.5 [+7.3]
LT	II	52.2		67.6 [+29.5]
KO	III	59.1	46.9 [−20.6]	34.1 [−42.3]
HR	III	54.9	52.2 [−4.9]	43.7 [−20.4]
TH	III	31.9	26.4 [−17.2]	32.6 [+2.2]
RL	V	38.4		32.2 [−16.1]
BV	V	60.0		56.2 [−6.3]
AH	V	30.4		30.4 [0.0]
KM	V	28.4		28.9 [+1.8]
SP	V	24.9		31.6 [+26.9]
MH	V	35.6		29.6 [−16.9]
Mean (protocols II, III, V)		41.9	41.8 [−14.2]	40.6 [+0.3]
Range		24.1-62.9	26.4-52.2	32.2-67.6

Although only 3 patients were studied with protocol III, which was too few for statistical analysis, the data in table III suggest that there may be a correlation between aminoglutethimide dose and the reduction in oestrone sulphate bioavailability.

The fraction of 4-[^{14}C]-oestradiol metabolised (fm) to oestrone sulphate, measured in the 6 patients in protocol V (table V) was moderately but consistently reduced by aminoglutethimide treatment (mean reduction 13.0%, $p < 0.05$).

Examples of the plasma concentration profiles for individual patients are shown in figures 1 to 3.

Urinary excretion data for total radioactivity and radioactive oestriol for 6 patients are shown in table VI, and a representative curve is shown in figure 4. No consistent alteration in total radioactivity excreted was found. The increase in labelled oestriol excreted, however, was pronounced (mean 80%, $p < 0.05$) over the whole collection period: the same effect was observed following the low and high doses of aminoglutethimide. The oestriol samples crystallised to constant isotope ratio (protocol III) showed a mean purity of 83 ± 7%. No difference in purity between the different phases was recorded.

Other parameters: thyrotrophin levels were slightly increased in all patients by long term aminoglutethimide treatment but, except for one patient (RL), remained within the normal range. Plasma levels of testosterone-binding globulin, thyroxine, triiodothyronine, free thyroxine and thyroxine-binding globulin were all within normal limits (protocols II, III and V), except for a slightly elevated free thyroxine level in one patient (AH) during phase 1. Albumin values were normal in all patients.

Discussion

The aminoglutethimide concentrations measured in plasma in this study were consistent with earlier findings (3 to 22 mg/L) during long term treatment (250mg 4 times/day) [Lønning et al. 1985] for all patients in phase 3. Therefore, the dif-

Table III. Effect of aminoglutethimide on pharmacokinetic parameters of oestrone sulphate (E_1S) obtained after intravenous ^{14}C-oestradiol bolus injections before treatment and during short and long term aminoglutethimide treatment (protocols I, II and III)

Patient	Protocol	Phase 1		Phase 2		Phase 3	
		E_1S AUC (h/L)	E_1S λ_z (h^{-1})	E_1S AUC (h/L) [Δ%]	E_1S λ_z (h^{-1}) [Δ%]	E_1S AUC (h/L) [Δ%]	E_1S λ_z (h^{-1}) [Δ%]
CR	I	0.044	0.424			0.048 [+9]	0.446 [+5]
BS	I	0.203	0.083			0.190 [+6]	0.101 [+22]
HN	II	0.307	0.078			0.051 [−83]	0.133 [+71]
AB	II	0.135	0.192			0.036 [−73]	0.271 [+41]
LT	II	0.097	0.105			0.038 [−61]	0.134 [+27]
KO	III	0.147	0.093	0.094 [−36]	0.128 [+38]	0.058 [−61]	0.255 [+73]
HR	III	0.153	0.104	0.080 [−48]	0.127 [+22]	0.076 [−50]	0.135 [+30]
TH	III	0.166	0.074	0.089 [−46]	0.131 [+77]	0.057 [−66]	0.172 [+132]
Mean (protocols II, III)		0.168	0.108	0.088 [−43]	0.129 [+46]	0.053 [−66]	0.183 [+62]

Abbreviations: E_1S AUC = area under the oestrone sulphate curve; E_1S λ_z = oestrone sulphate terminal elimination rate constant.

Table IV. Effect of aminoglutethimide on pharmacokinetic parameters of oestrone sulphate obtained after intravenous 6,7-[^3H]-oestrone sulphate bolus injections before and during long term aminoglutethimide treatment (protocols IV and V)

Patient	Protocol	Phase 1			Phase 3		
		CL (L/h)	V_z (L)	$t_{½}$ (h)	CL (L/h) [Δ%]	V_z (L)	$t_{½}$ (h)
WK	IV	4.5	40.8	6.9	10.8 [+140]	41.1	2.7
ÅK	IV	5.5	61.0	7.7	12.4 [+125]	63.8	3.6
RL	V	3.8	33.4	6.0	7.6 [+100]	23.6	2.2
BV	V	6.3	58.3	5.5	14.5 [+130]	53.3	2.5
AH	V	8.2	58.3	5.0	15.2 [+85]	61.5	2.8
KM	V	4.3	43.5	7.0	9.4 [+119]	50.5	3.7
SP	V	6.6	31.3	3.3	7.9 [+20]	31.0	2.7
MH	V	3.2	46.0	9.9	7.0 [+119]	40.2	4.0
Mean		5.3	46.6	6.4	10.6 [+105]	45.6	3.0

Abbreviations: CL = oestrone sulphate total clearance; V_z = oestrone sulphate apparent volume of distribution during the terminal (λ_z) phase; $t_{½}$ = oestrone sulphate terminal half-life.

ference in oestrogen disposition between short and long term aminoglutethimide treatment is probably not related to a subtherapeutic plasma aminoglutethimide concentration following short term dosage.

The ^{14}C-isotope was selected for study to avoid *in vivo* ^3H dissociation. Because of the low specific activity of the ^{14}C-isotope, the dose of labelled steroids administered resulted in rather unphysiological initial plasma concentrations. This, however, does not affect clearances (Hembree et al. 1969). Unfortunately, ^{14}C-labelled oestrone sulphate is not commercially available, and therefore a ^3H-labelled compound was used.

Age does not significantly alter oestrone metabolism (Zumoff et al. 1968). Oestrone sulphate

Table V. Fraction of 4-[^{14}C]-oestradiol metabolised to oestrone sulphate (fm) investigated by 4-[^{14}C]-oestradiol/6,7-[^{3}H]-oestrone sulphate double isotope injection technique (protocol V)

Patient	Phase 1 fm_1[a]	Phase 3 fm_3[a]	fm ratio fm_3/fm_1
RL	0.89	0.72	0.81
BV	0.99	0.87	0.88
AH	0.79	0.67	0.84
KM	0.91	0.85	0.93
SP	0.82	0.76	0.93
MH	0.79	0.66	0.84
Mean	0.87	0.76	0.87
Range	0.79-0.99	0.67-0.87	0.81-0.93

a fm is the fraction of oestradiol metabolised to oestrone sulphate, subscripts $_1$ and $_3$ refer to parameters obtained in phase 1 and 3, respectively.

clearance and distribution volume, and clearance of oestradiol were found to correspond with results published by others (Longcope 1972; Longcope & Williams 1974; Ruder et al. 1972).

Our oestradiol data were best fitted by a 3-compartment model, as reported by Longcope and Williams (1974). However, the fraction of oestradiol that was metabolised to oestrone sulphate (protocol V) was higher than that reported by Longcope (1972), who measured this parameter in young men using a steady-state infusion technique.

Mean oestradiol clearance was not affected by aminoglutethimide treatment (table II), but in some patients there was a substantial (− 42 to + 37%) difference in the clearance obtained in the different phases. As discussed elsewhere, oestradiol clearance would be expected to be more dependent on hepatic blood flow than on hepatic metabolic capacity. Three patients (KO, HR and MH) in whom oestradiol clearance during aminoglutethimide treatment was found to be reduced by 16 to 42% all had progression of disease during treatment.

Seven of the 8 patients receiving 6,7-[^{3}H]-oestrone sulphate injections showed an 85% to 140% increase in oestrone sulphate clearance during aminoglutethimide treatment. The increase in the other patient (SP) was only 20% (table IV). This may have been related to her ingestion of cimetidine, a known enzyme inhibitor (Roberts et al. 1981), 800mg daily, for part of the study. Although the cimetidine was withdrawn 4 days before the second oestrone injection, a carryover effect on oestrone metabolism cannot be ruled out. It should also be noted that this patient was taking a lower dose of aminoglutethimide (250mg twice daily) during the study because of gastric side effects. Further she received her phase I injection 3 weeks after cessation of aminoglutethimide therapy. No effect of aminoglutethimide on oestrone clearance occurred in our

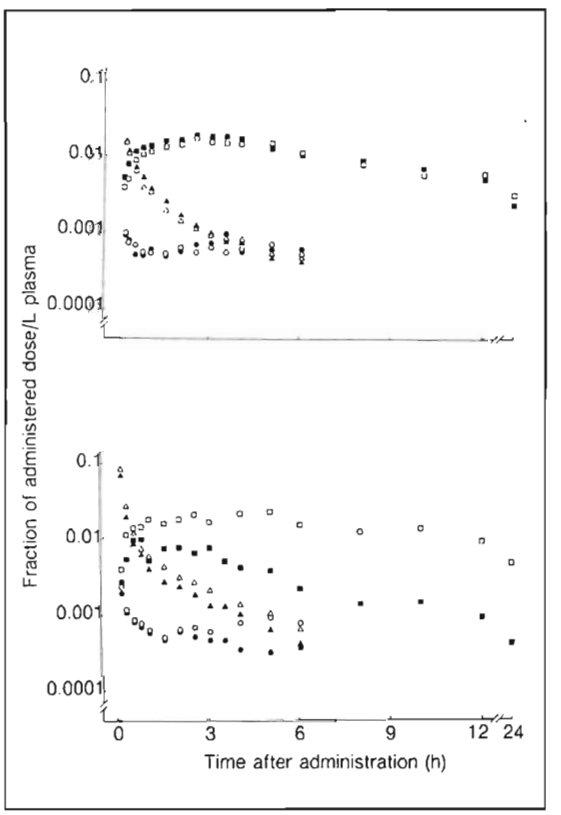

Fig. 1. Concentration of radioactivity expressed as a fraction of administered dose/L following intravenous 4-[^{14}C]-oestradiol in single patients investigated before and after receiving (BS; upper) a single oral bolus dose or (HN; lower) 6 weeks' oral treatment with aminoglutethimide. Open symbols represent concentrations before, and closed symbols represent concentrations after, treatment with aminoglutethimide. Key: △▲ = oestradiol; ○● = oestrone; □■ = oestrone sulphate.

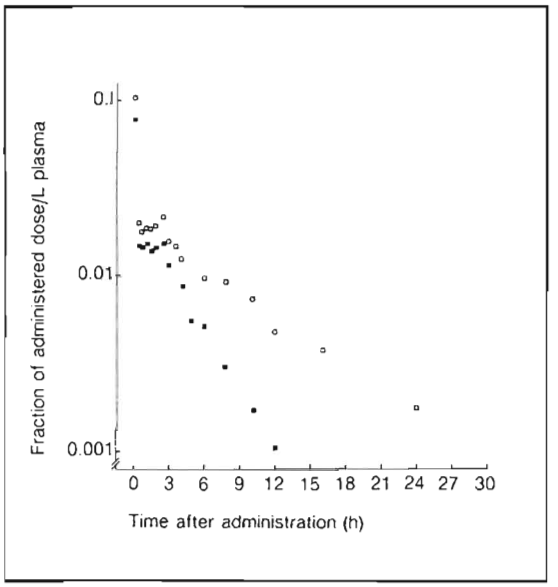

Fig. 2. Plasma concentration curves for 6,7-[^{3}H]-oestrone sulphate expressed as fraction of administered dose/L before (□) and after (■) long term aminoglutethimide treatment in patient WK (protocol IV).

other patient (KM) who had also stopped treatment 3 weeks before her phase 1 injection. However, it is possible that some residual effect remained in patient SP.

The ratio of glucuronides to sulphates in urine is about 9 : 1 (Gurpide 1978). As ^{3}H-labelled oestriol glucuronide was available as an internal standard, we elected to use glucuronidase rather than glucuronidase plus sulphate hydrolysis: this may result in slightly underestimated values. In contrast, the urine samples obtained from the 3 patients in protocol II were not crystallised to a constant isotope ratio, which may result in slight overestimation of these values.

The amounts of urinary oestriol found before the start of aminoglutethimide treatment were consistent with previous reports (Hellman et al. 1967; Lahita et al. 1979). Only about 50 to 70% of an administered dose of ^{14}C-radioactivity is recovered in the urine during the first 72 hours after a ^{14}C-oestrone or ^{14}C-oestradiol bolus injection (Fishman et al. 1966; Zumoff et al. 1968). We obtained similar data for urinary excretion of radioactivity (table VI).

Pharmacokinetic models: this study focused on possible alterations in the disposition of 'highly extracted' substances such as oestradiol. If pharmacokinetic parameters such as clearance or half-life of oestradiol are the only investigations performed, alterations in metabolic pathways may pass undetected. It was for this reason that we included measurements of both plasma and urinary metabolites.

The 4-[^{14}C]-oestradiol injection studies showed a marked decrease in the ^{14}C-oestrone sulphate AUC, which could reflect either increased clearance or a reduced production rate for oestrone sulphate. The 6,7-[^{3}H]-oestrone sulphate injections showed a significant (104%) increase in oestrone sulphate clearance (protocol IV, table IV).

The double isotope injection technique was developed to investigate a possible reduction in the fraction of oestradiol metabolised (fm) to oestrone

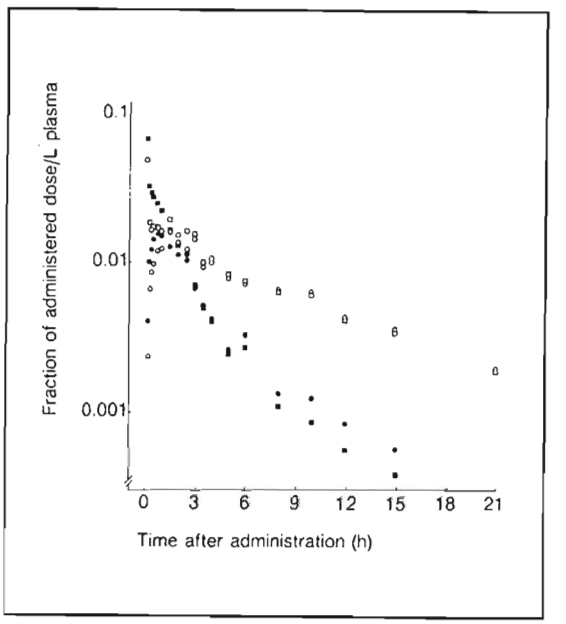

Fig. 3. Plasma concentrations of 4-[^{14}C]-oestrone sulphate (○●) and 6,7-[^{3}H]-oestrone sulphate (□■) expressed as a fraction of administered dose/L before (○□) and after (●■) long term aminoglutethimide treatment in patient RL (protocol V).

Table VI. Effect of long term aminoglutethimide treatment on urinary excretion of ^{14}C-oestriol and total radioactivity during 72 hours following injection as percentage of 4-[^{14}C]-oestradiol administered (protocols II and III)

Patient	Protocol	Phase 1		Phase 2		Phase 3	
		[^{14}C] E_3	total (^{14}C)	[^{14}C] E_3	total (^{14}C)	[^{14}C] E_3	total (^{14}C)
HN	II	8.1	38.6[a]			14.5	32.6[a]
AB	II	17.2	63.9[b]			29.6	73.7[b]
LT	II	8.3	45.7[a]			15.7	50.5[a]
KO	III	13.4	60.3[b]	26.3	55.1[b]	27.8	44.2[b]
HR	III	16.3	65.9[b]	26.8	67.1[b]	28.7	68.4[b]
TH	III	8.5	48.4[a]	13.5	47.5[a]	13.1	34.5[a]
Mean							
a		8.3	44.2			14.4	39.2
b		15.6	63.4	26.6	61.1	28.7	62.1

a 48 hours urinary collection.
b 72 hours urinary collection.

sulphate during aminoglutethimide treatment. The fraction metabolised (Rowland & Tozer 1980) is identical to the transfer constant ([ρ]) defined by Gurpide et al. (1963), and can be used regardless of whether part of the oestrone sulphate produced is converted back to oestradiol. Our investigation in protocol V showed that fm was moderately (mean 13%) but consistently reduced following aminoglutethimide treatment.

Interpretation of data: oestrogens are metabolised by similar pathways following conversion to oestrone. Several urinary oestrogen metabolites are produced in the liver by microsomal enzyme hydroxylations, with or without reductions. The major metabolite, oestriol, is produced by hydroxylation of oestrone in the 16α-position, followed by reduction: it is readily measured using our technique. Changes in other major hydroxylation pathways should produce alterations in the oestriol production rate, as these reactions are considered substrate competitive (Bolt 1979). Although the number of patients in our study was small, a number of hypotheses may be formulated.

Firstly, the increased clearance of oestrone sulphate suggest that its metabolism is accelerated by aminoglutethimide. The oestrone sulphate peaks in figure 1 (lower) may be related to enterohepatic circulation of steroids. This process may be marked, as about 50% of total radioactivity can be recovered from bile after intravenous pulse injections of oestrone or oestradiol, whereas less than 10% is normally recovered in faeces (Sandberg & Slaunwhite 1957). Thus, our second hypothesis is that the increased urinary excretion of 4-[^{14}C]-oestriol indicates an increased production rate, as oestriol is quantitatively reabsorbed from the gut and eliminated by only urinary excretion (Støa & Skulstad 1972).

Thirdly, oestradiol, in contrast to oestrone sulphate is a 'highly extracted' substance. Its total blood clearance exceeds splanchnic blood flow (Longcope et al. 1968). From the equation for hepatic clearance (see glossary; Wilkinson & Shand 1975), hepatic oestradiol clearance is more dependent on hepatic blood flow than on metabolic capacity. Accordingly, induction of oestradiol metabolic pathways with significant alterations in metabolite profiles might occur without alterations in total oestradiol clearance (Rowland & Tozer 1980). However, increased conversion of oestrone to oestriol will result in a reduction of intracellular oestrone available for metabolism to oestrone sulphate. Therefore, our findings are consistent with induction of hepatic microsomal enzymes responsible for oestrogen metabolism following exposure to aminoglutethimide.

In the 3 protocol III patients investigated in phases 2 and 3, the AUC of oestrone sulphate was reduced more by the high dose (250mg 4 times daily) than the low dose (125mg 4 times daily) aminoglutethimide regimen. Similarly, aminoglutethimide has previously been shown to induce a dose-dependent increase in warfarin clearance (Lønning et al. 1986).

We did not find an increase in the total urinary radioactivity following long term aminoglutethimide administration in protocols II and III. This was unexpected in view of the increased oestriol excretion. However, Fishman et al. (1966) suggested that a considerable amount of 'missing' radioactivity can be accounted for by unknown 16α-metabolites which are not excreted in the urine. Thus, increased 16α-hydroxylase activity, together with increased production of other metabolites modified in the 16α-position may explain our findings.

Other possible explanations should be considered. Thyroxine has been shown to influence the production of oestriol relative to catechol oestrogens (Fishman et al. 1965), and aminoglutethimide is known to be a mild goitrogenic drug (Santen et al. 1977a). However, the absence of evidence of abnormalities in the patients assessed for thyroid function, makes this explanation unlikely. The low binding of aminoglutethimide to plasma proteins [mean 24% (Lønning et al. 1985)] makes displacement of oestrone sulphate from albumin by aminoglutethimide very unlikely.

Aminoglutethimide could inhibit the conversion of oestrone to oestrone sulphate. This mechanism may also produce an increased amount of oestrone available as a substrate both for 16α-hydroxylase and for other hydroxylases. As this would result in increased urinary excretion of all metabolites, but no increase in the amount of urinary labelled oestriol relative to total radioactivity inhibition of oestrone sulphurylation may not explain our findings.

In theory, oestradiol could be metabolised by an alternative pathway via oestradiol sulphate to oestrone sulphate. Oestradiol sulphate plasma concentrations, however, have been reported to be only about 3 to 7% of oestrone sulphate concentrations (Myking et al. 1980). Thus a significant contribution of this pathway can only be postulated if oestradiol sulphate is very rapidly removed from the circulation or metabolised. Stimulation of intracellular oestrone hydroxylation by aminoglutethimide would also decrease the amount of oestradiol available for metabolism by this alternative route.

Corticosteroids at pharmacological doses have been reported to not influence oestriol production rates in humans (Lahita et al. 1981). In contrast, Longcope et al. (1981) reported that clearance of oestradiol was increased in rhesus monkeys following short term administration of pharmacological doses of dexamethasone. Our study does not suggest an effect of glucocorticoid administration on the metabolism of oestrogens for the following reasons. Firstly, glucocorticoids were also administered to the patients investigated in protocol I, who showed no change in their oestrogen disposition after short term aminoglutethimide treatment. Secondly, cortisol plasma concentrations will remain within the normal range in most patients receiving

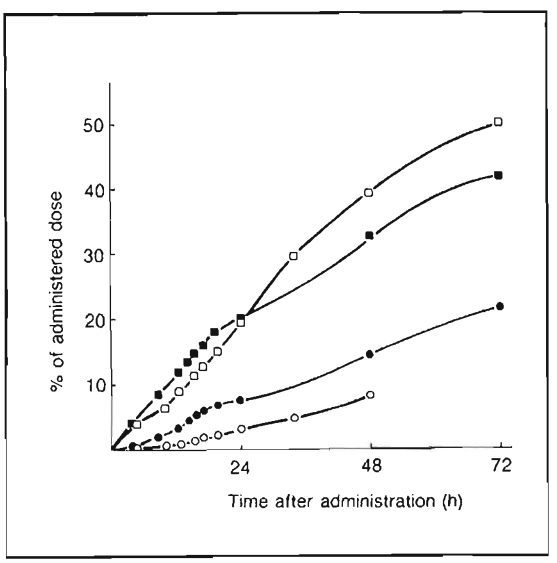

Fig. 4. Cumulative urinary excretion of total radioactivity (□■) and ^{14}C-oestriol (○●) after 4-[^{14}C]-oestradiol injections in patient HN (protocol II) before (open symbols) and after (closed symbols) 6 weeks' treatment with aminoglutethimide.

hydrocortisone acetate 25mg twice daily during long term aminoglutethimide treatment, as aminoglutethimide imposes an inhibitory effect on endogenous cortisol synthesis in the adrenal cortex (Lønning et al. 1985). Thirdly, the 3 patients investigated in protocol III received no glucocorticoids when investigated in phase 2.

Therapeutic Implications

Aminoglutethimide is assumed to exert its therapeutic effect by an inhibition of the peripheral aromatisation of androgens to oestrogens (Santen et al. 1978). This hypothesis does not explain the following observations:

1. Testololactone is a known inhibitor of the peripheral aromatase system, producing a 90% inhibition *in vivo* (Barone et al. 1979), with a significant reduction in oestrone plasma concentration (Judd et al. 1982). Treatment with testololactone, however, results in a low response rate (<15%) in postmenopausal breast cancer patients (Volk et al. 1974). This contrasts with a mean response rate of 32% in more than 900 postmenopausal patients receiving the conventional aminoglutethimide drug schedule (Santen 1981).

2. Published data on aminoglutethimide treatment of premenopausal women with advanced breast cancer reveal an objective tumour regression in 11 of 46 patients reported (Aboul-Enein et al. 1983; Harris et al. 1982; Santen et al. 1980; Wander et al. 1986). This response rate contrasts with the fact that aminoglutethimide has only a minor influence on the ovarian aromatase enzyme system (Santen et al. 1980), and therefore affects the oestrogen production rate minimally in premenopausal women.

In this preliminary study, we have demonstrated a pronounced effect on aminoglutethimide on oestrogen metabolism. An increased metabolism and a decreased production rate of oestrone sulphate might contribute to a reduced plasma bioavailability of this steroid.

Plasma oestrone sulphate concentrations have been reported in 1 study to be significantly decreased during aminoglutethimide therapy (Samojlik et al. 1982). This finding may be due to the inhibitory action of aminoglutethimide on the peripheria aromatase enzyme. Our results suggest additional mechanisms may be involved. The well documented clinical effect of aminoglutethimide on breast cancer may be related to its effects on oestrogen production (aromatase inhibition) and on oestrogen metabolism caused by enzyme-inducing properties of the drug. Aminoglutethimide-promoted enzyme induction is thought to be dose-dependent, and this might indicate that different aminoglutethimide drug schedules may have different effects on the progression of advanced breast cancer. However, further research is required to confirm these findings.

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Authors' address: Dr *P.E. Lønning*, Department of Oncology, University of Bergen, N-5021 Haukeland (Norway).