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Influence of Tamoxifen on Sex Hormones, Gonadotrophins and Sex Hormone Binding Globulin in Postmenopausal Breast Cancer Patients

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Estrone sulphate (E_1S) may be an important estrogen source in breast cancers, particularly in postmenopausal women. Recent studies have shown that tamoxifen inhibits the uptake and metabolism of E_1S to estradiol (E_2) in cell cultures. To evaluate a possible influence of tamoxifen on E_1S disposition *in vivo*, we measured plasma levels of E_1S together with unconjugated estrogens (E_1 and E_2), androgens (T, A, DHEA and DHEAS), SHBG, FSH and LH in 32 postmenopausal breast cancer patients before and during tamoxifen treatment. In a subgroup of 10 patients, we measured 24 h urinary excretion of estrogen metabolites to evaluate the influence of tamoxifen treatment on estrogen metabolism and total estrogen production. Tamoxifen increased plasma levels of E_1S (mean increase of 18.1%, $P < 0.05$) and the ratio of E_1S/E_1 (mean increase of 25.7%, $P < 0.01$) and E_1S/E_2 (mean increase of 34.7%, $P < 0.0005$). No significant change in plasma E_1 was seen, but plasma E_2 was reduced (mean reduction of 12.1%, $P < 0.005$). The fall in plasma E_2 was probably secondary to a fall in plasma T (mean reduction of 11.9%, $P < 0.05$) due to a reduced ovarian excretion of this androgen. The mechanism may be a reduced gonadotrophin stimulation of the ovary, as plasma FSH and LH fell by mean values of 45.5 and 48.1%, respectively ($P < 0.0001$ for both). The increase in plasma E_1S was accompanied by a reduced ratio of $2OHE_1/E_1$ in urine (mean reduction of 38.2%, $P < 0.025$) indicating reduced 2-hydroxylation. Possible mechanisms for these alterations are discussed.

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INTRODUCTION

Tamoxifen is the contemporary endocrine treatment modality most widely used in breast cancer. The drug is thought to act by blocking the estrogen receptor (ER) in the tumour cell, but alternative mechanisms of action have been proposed [1].

Evidence suggests that tamoxifen also influences steroid disposition. It interacts with ovarian estrogen synthesis and elevates plasma estrogen levels in premenopausal women [2, 3], but studies on plasma and urinary estrogens in postmenopausal patients treated with tamoxifen have provided conflicting results [4–8].

The estrogen conjugate, estrone sulphate (E_1S), seems to play a role as an estrogen source for tumour cells in postmenopausal women [9]. Plasma concentrations of E_1S are about one order of magnitude higher than plasma E_1 in postmenopausal women, and tumour cells contain the enzymes needed to convert E_1S into the biologically active E_2 [10].

Tamoxifen may influence plasma E_1S by several mechanisms. It inhibits the uptake and metabolism of E_1S in tumour cells *in vitro* [11, 12]. In addition, animal studies have shown tamoxifen to inhibit mixed function oxidases [13], and previous studies from our group have shown that drugs which affect estrogen metabolizing enzymes may influence plasma E_1S/E_1 ratio [14].

The aim of this study was to evaluate a possible

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influence of tamoxifen on plasma E_1S *in vivo*. We measured plasma levels of E_1S and the two major unconjugated estrogens, E_1 and E_2 in postmenopausal women before and during tamoxifen treatment. To further address possible mechanisms involved plasma levels of the two major estrogen precursors, testosterone (T) and androstenedione (A) were measured together with SHBG and the gonadotrophins. We also measured the ratio of plasma DHEAS to DHEA to evaluate any possible change in the ratio of sulphated to unconjugated hormones.

In a subgroup of 10 patients 24 h urinary excretion of the main estrogen metabolites was measured to assess any impact on estrogen metabolic pathways and total estrogen production. We also measured plasma levels of tamoxifen and its major metabolites to be able to correlate possible changes in hormone levels to drug concentrations.

PATIENTS AND METHODS

Thirty-two postmenopausal women who were to receive tamoxifen treatment for advanced breast cancer gave their verbal informed consent to participate in the study. Their median age was 64 yr (range 49–79 yr). None of the patients received any other forms of endocrine treatment or drugs known to influence drug or hormone disposition. The tamoxifen drug schedule was 30 mg o.d. in all patients.

Blood samples were obtained before commencing tamoxifen treatment and at different occasions following 3–12 months on treatment (median 6 months). Each patient had samples obtained on 1–5 occasions during therapy (median 2 occasions). Heparinized blood samples were obtained by venipuncture between 8 a.m. and 10 a.m. after an overnight fast. Plasma was separated by centrifugation, and stored at −20°C until analysis.

Ten of the patients had 24 h urine collected before commencing on tamoxifen and at one occasion during treatment. Urine was collected and stored as described elsewhere [15].

Plasma E_1, E_1S and E_2 was measured as described elsewhere [16, 17]. The sensitivity limits of the assays were 2.1 pM, 6.3 pM and 25.9 pM for E_2, E_1 and E_1S, respectively. The within assay Cv was less than 5% for E_2 and E_1 and 6% for E_1S. Plasma sex hormones and SHBG were determined by commercial radioimmunoassay (DHEA, DHEAS, A and T) and IRMA (SHBG, LH and FSH) kits (Orion Diagnostica, Diagnostic Products Corporation and Diagnostic System Laboratories). The intra-assay coefficient of variation was below 7% for all analyses.

Urine estrogen metabolites were determined using a GC–MS method as previously described [18–20], total urinary excretion was given as amount excreted/24 h corrected for possible losses by the creatinine clearance factor as given elsewhere [15]. Tamoxifen and its major metabolites were measured by HPLC as described earlier [21, 22]. The intra-assay Cv was $<5\%$ for tamoxifen and all metabolites measured. When plasma samples were obtained on more than one occasion during treatment, the mean value was used for statistical comparison.

Plasma hormone levels were tested for normal distribution using Q-Q plots [23] on raw values and values obtained after appropriate linear transformations. Considering urinary oestrogen metabolites, the number of patients (10) was too small to analyse for normal distribution. These values were tested for goodness to fit to a normal distribution by including values of breast cancer patients not receiving any hormone treatment from other protocols to achieve a number of about 25. All plasma and urinary values were found well fitted to a log normal distribution. Accordingly, values obtained before and during treatment were compared using a Students paired t-test on logarithmic transformed data. All P-values were expressed as 2-tailed.

Simple Pearson's correlations were calculated using the SYSTAT program on a Macintosh II computer. Factors found to correlate significantly in univariate tests were then taken into a general linear interactive multivariate model manually exploring different alternatives and testing for colinearity.

RESULTS

Plasma estrogens, androgens, LH, FSH and SHBG before and during tamoxifen treatment are given in Table 1. Plasma E_2 was slightly suppressed (mean suppression of 12.1% $P < 0.005$). Contrary, plasma E_1S was slightly elevated (mean increase of 18.2%, $P < 0.05$), and the plasma E_1S/E_1 and E_1S/E_2 ratios were increased by mean values of 25.7% ($P < 0.01$) and 34.7% ($P < 0.0005$), respectively. Plasma E_1 tended to decrease (non-significant), but there was a small increase in the plasma E_1/E_2 ratio of 7.7% ($P < 0.05$).

Total T was moderately suppressed (mean suppression of 11.9%, $P < 0.05$), but the plasma T/SHBG ratio fell by a mean value of 46.7% ($P < 0.0001$). While a slight reduction in plasma levels of the other androgens (DHEA, DHEAS and A) was observed, none of these alterations were of statistical significance.

Tamoxifen treatment caused no change in total body aromatization evaluated from the E_1/A and E_2/T ratios.

Plasma levels of FSH and LH were supressed by 45.5 and 48.1%, respectively ($P < 0.0001$ for both), while plasma SHBG increased by a mean value of 65.2% ($P < 0.0001$).

Urinary estrogens before and during tamoxifen treatment are given in Table 2. Tamoxifen treatment had no influence on the total amount of 24 h urinary estrogen metabolites excreted (mean values before and during treatment of 32.6 nmol/24 h and 34.1 nmol/24 h, respectively). Although there was no significant change in the excretion of any of the estrogen metabolites

Table 1. Plasma hormone and SHBG levels before and during treatment with tamoxifen and % change of each hormone; geometrical mean values (with 95% confidence limits of the mean)

Compounds	Before treatment	On tamoxifen	Change (%)	*P*-value
Compounds				
E_2	18.9 pM (15.1–23.7)	16.6 pM (13.3–20.8)	−12.1 (−19.2 − 4.3)	0.004
E_1	74.4 pM (64.3–86.1)	70.9 pM (62.1–79.7)	−6.9 (−12.7 + 1.1)	0.093
E_1S	424.5 pM (331.3–544.0)	501.7 pM (396.2–635.2)	+18.2 (+1.7 + 37.3)	0.031
DHEA	8.46 nM (6.26–11.44)	7.22 nM (5.29–9.86)	−14.6 (−31.0 + 5.5)	0.14
DHEAS	2.11 μM (1.61–2.77)	1.99 μM (1.45–2.72)	−6.0 (−19.6 + 10.0)	0.42
A	1.57 nM (1.25–1.96)	1.51 nM (1.16–1.96)	−3.9 (−16.1 + 10.0)	0.55
T	1.09 nM (0.83–1.44)	0.96 nM (0.77–1.20)	−11.9 (−22.2 − 0.3)	0.046
SHBG	49.0 nM (40.0–59.9)	81.0 nM (67.6–96.9)	+65.2 (+46.7 + 86.1)	0.0001
FSH	61.7 IU/l (53.8–70.7)	33.6 IU/l (26.4–42.9)	−45.5 (−54.0 − 35.3)	0.0001
LH	31.8 IU/l (26.2–38.4)	16.5 IU/l (12.8–21.2)	−48.1 (−59.4 − 33.7)	0.0001
Ratios				
E_1/E_2	3.88 (3.41–4.42)	4.18 (3.65–4.79)	+7.7 (+0.9 + 14.9)	0.026
E_1S/E_2	22.3 (18.2–27.3)	30.0 (24.5–36.7)	+34.7 (+16.5 + 55.6)	0.0002
E_1S/E_1	5.70 (4.67–6.95)	7.17 (6.03–8.53)	+25.7 (+6.5 + 48.4)	0.0084
DS/D	249.8 (210.3–297.0)	275.3 (209.5–361.7)	+10.2 (−15.2 − 43.1)	0.46
T/SHBG	0.022 (0.015–0.033)	0.012 (0.009–0.016)	−46.7 (−55.3 − 36.4)	0.0001
E_1/A	0.047 (0.039–0.058)	0.046 (0.036–0.058)	−2.2 (−14.8 + 12.3)	0.75
E_2/T	0.017 (0.014–0.022)	0.017 (0.015–0.021)	−0.0 (−13.9 + 15.8)	0.98

Abbreviations: E_1, estrone; E_2, estradiol; E_1S, estrone sulphate; DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosterone sulphate; DS/D, dehydroepiandrosterone sulphate/dehydroepiandrosterone; A, androstenedione; T, testosterone; SHBG, sex hormone binding globulin.

measured (E_1, E_2, E_3, $2OHE_1$, $2OHE_2$, $2MeOE_1$, $16\alpha OHE_1$, $16\beta OHE_1$, $16ketoE_2$ or $4OHE_1$), the ratio $2OHE_1/E_1$ was reduced by a mean of 38.2% (Table 2, $P = 0.025$). The ratio between total 2-hydroxylated E_1 metabolites ($2OHE_1 + 2MeOE_1$) and 16α-hydroxylated E_1 metabolites was non-significantly reduced by a mean value of 37.1%, while the ratio between total amount of 2-hydroxylated metabolites from E_1 and E_2 ($2OHE_1 + 2MeOE_1$ and $2OHE_2$) and total amount of 16-oxygenated metabolites ($16\alpha OHE_1$, E_3, $16ketoE_2$ and $16\beta OHE_1$) was non-significantly reduced by a mean value of 34.4% (Table 2).

Univariate analysis revealed a weak correlation between the increase in the ratios of E_1S/E_1 and E_1S/E_2 and the reduction in the urinary excretion of $2MeOE_1$ ($P < 0.05$), and non-significant correlations between the increase in plasma E_1S and the reduction in urinary excretion of $2\text{-}OHE_1$ or $2MeOE_1$ ($0.05 < P < 0.10$) and between the increase in the ratios of E_1S/E_1 and E_1S/E_2 on the one side and the reduction in the urinary excretion of $2OHE_1$ ($0.05 < P < 0.10$) on the other side.

Plasma levels of tamoxifen and its major metabolites are given in Table 3.

Table 2. Excretion of oestrogens in urine expressed as nmol/24 h and some metabolite ratios of relevance before and on treatment with tamoxifen; geometrical mean values (with 95% confidence limits of the mean)

	Before treatment	On tamoxifen	Change (%)	*P*-value
$2OHE_1$	5.59 (3.61–8.66)	4.01 (2.56–6.27)	−28.4 (−52.5 + 8.0)	0.099
$2OHE_2$	1.51 (0.79–2.87)	1.46 (0.76–2.82)	−3.0 (−34.8 + 44.3)	0.87
$4OHE_1$	0.61 (0.38–1.99)	0.56 (0.36–0.85)	−9.4 (−49.5 + 62.4)	0.71
$2MeOE_1$	1.94 (1.23–3.08)	1.41 (0.86–2.30)	−27.4 (−56.5 + 21.4)	0.19
E_1	5.79 (3.77–8.89)	6.72 (5.25–8.59)	+16.0 (−15.8 + 59.8)	0.32
E_2	0.93 (0.47–1.86)	1.09 (0.72–1.64)	+16.9 (−19.4 + 69.6)	0.37
E_3	8.61 (4.87–15.21)	9.85 (6.40–15.15)	+14.3 (−33.4 + 96.6)	0.59
$16\alpha OHE_1$	2.70 (1.37–5.32)	3.10 (1.48–6.48)	+14.8 (−36.4 + 107.3)	0.61
$16\beta OHE_1$	1.54 (0.75–3.16)	1.81 (1.19–2.76)	+17.6 (−37.3 + 120.3)	0.57
$16ketoE_2$	1.28 (0.64–2.55)	1.70 (1.11–2.59)	+32.8 (−13.9 + 105.0)	0.17
Tot uE	33.9 (22.2–51.7)	35.8 (27.2–47.1)	+5.8 (−23.4 + 45.9)	0.70
$2OHE_1/E_1$	0.97 (0.65–1.45)	0.60 (0.45–0.79)	−38.2 (−58.6 − 7.8)	0.024
Met ratio 1	0.65 (0.37–1.16)	0.41 (0.20–0.83)	−37.1 (−68.2 + 24.6)	0.16
Met ratio 2	0.65 (0.40–1.04)	0.43 (0.22–0.82)	−34.4 (−65.3 + 24.0)	0.17

Abbreviations: $2OHE_1$, 2-hydroxyestrone; $2OHE_2$, 2-hydroxyestradiol; $4OHE_1$, 4-hydroxyestrone; $2MeE_1$, 2-methoxyestrone; E_1, estrone; E_2, estradiol; E_3, estriol; 16α-hydroxyestrone; $16\beta OHE_1$, 16β-hydroxyestrone; Tot uE, total urinary estrogens; Met ratio 1, $(2OHE_1 + 2MeOE_1)/(16\alpha OHE_1 + E_3)$; Met ratio 2, $(2OHE_1 + 2MeOE_1 + 2OHE_2)/(16\alpha OHE_1 + E_3 + 16ketoE_2 + 16\beta OHE_1)$.

Table 3. Plasma levels of tamoxifen and its major metabolites during steady state treatment. Geometrical mean values (with 95% confidence limits of the individual observations)

Compound	Concentration (ng/ml)
Tamoxifen	125.3 (63.8–246.1)
Desmethyltamoxifen	218.5 (102.3–466.7)
4-Hydroxytamoxifen	2.5 (0.5–11.6)
Desmethyl-4-hydroxytamoxifen	21.6 (5.9–79.7)
Desdimethyl-tamoxifen	32.9 (10.8–100.5)
Metabolite Y	25.8 (9.3–71.9)

None of the alterations in plasma hormones correlated to plasma levels of tamoxifen or any of its major metabolites (data not shown). On the other hand, univariate analysis revealed the change in the urinary 2-OHE_1/E_1 ratio to correlate positively to plasma levels of desmethyltamoxifen ($P = 0.005$) and desdimethyltamoxifen (metabolite Z, $P = 0.034$); patients having the highest plasma levels of tamoxifen metabolites experienced the smallest reduction in the urinary 2-OHE_1/E_1 ratio (data not shown). Multivariate analysis showed change in the urinary 2-OHE_1/E_1 ratio to correlate positively to plasma levels of desmethyltamoxifen ($P = 0.005$).

DISCUSSION

Previous studies on plasma estrogens in postmenopausal patients treated with tamoxifen have provided conflicting results [4–8]. This may be due to minor alterations in mean plasma estrogen levels and interindividual variations. Except for a small study evaluating "total estrone" [7] no previous study has looked at plasma E_1S levels in relation to tamoxifen treatment. A significant observation in our study was the increase in plasma E_1S (absolute levels and the ratio of E_1S to unconjugated E_1 and E_2) due to tamoxifen treatment.

E_1S may be an important estrogen source to the breast cancer cells. These contain enzymes (sulphatase and dehydrogenase) that convert E_1S into the biologically active E_2 [9, 10]. Circulating E_1S is thought to arise from sulphatation of plasma E_1 and E_2, as there is no evidence of any direct glandular secretion of this estrogen conjugate. The plasma concentration of E_1S is about 10-fold higher than that of E_1 and 20–40-fold higher than that of E_2 in postmenopausal women [24].

The possibility exists that tamoxifen may interact with the cellular uptake or metabolism of E_1S. Conflicting evidence from *in vitro* studies indicates that tamoxifen inhibits the cellular uptake of E_1S or inhibits the conversion of E_1S to E_2 in tumour cells [10–12, 25–28]. Thus, while a minor increase in plasma E_1S as well as a minor decrease in plasma E_2 itself may be of little biological importance, if this indicates a reduced tissue uptake or reduced conversion of E_1S to E_2 in the tissue, it may be consistent with a reduced supply of E_2 to the tumour cell *in vivo*.

An alternative explanation is that the increase in plasma E_1S could be secondary to alterations in estrogen metabolism. Tamoxifen has been found to inhibit certain microsomal mixed function oxidases [13], but to enhance 16α-hydroxylation of E_2 in animals [29]. Levin *et al.* reported that tamoxifen enhances the urinary excretion of "total oestrogen glucuronides" and the excretion of E_1 and E_2 but reduces the excretion of E_3 [6]. However, they measured urinary estrogens with radioimmunological methods which lack the specificity necessary for urine hormone measurement. The major estrogen metabolizing pathways are 2- and 16α-hydroxylations to yield 2-OHE_1 and E_3 as the main metabolites. These pathways are competitive, in as much as an increase in the activity of one pathway results in a reduced activity of the other [30]. The ratios between these metabolites are influenced by endogenous as well as exogenous compounds [31–33]. In the present study we found a non-significant reduction in the excretion of the catechol estrogen metabolites, a slight increase in the excretion of the other estrogen metabolites, but no change in total urinary estrogen excretion. We observed however a moderate, but significant reduction in the ratio of urinary 2OHE_1 to E_1 and a non-significant reduction in the ratio of total 2-hydroxylated to 16α-hydroxylated urinary estrogens, indicating a reduced 2-hydroxylation of E_1 (Table 2). We have previously shown that alterations in estrogen metabolizing enzymes may influence on the E_1S/E_1 ratio [14]. It has been suggested that E_1S may be a better substrate for the 2-hydroxylation than E_1 [34]. If tamoxifen (or any of its metabolites) inhibits the 2-hydroxylase, this may be reflected in a decreased ratio of 2-OHE_1/E_1 in the urine but also elevation of plasma E_1S as observed in the present study. We believe this is the mechanism because a reduced excretion of 2-hydroxylated metabolites was seen in combination with a slight increase in the excretion of 16α-hydroxylated metabolites. Interestingly, this is the reverse of the effect of indol-3-carbinol on estrogen metabolism [35].

The finding that the 2-OHE_1/E_1 ratio decreased most in patients having the lowest plasma level of desmethyltamoxifen is not easily explained, but it suggests some influence of tamoxifen on estrogen metabolizing enzymes. One possibility is that tamoxifen and estrogens may compete for the same mixed function oxidases [36].

The decrease in plasma E_2 may be due to the drop in plasma T, as tamoxifen suppressed plasma T but did not influence the aromatization indexes (E_2/T and E_1/A, Table 1). The finding of a modest albeit significant drop in plasma T contrasts the finding of previous studies, who reported that tamoxifen treatment did not influence plasma androgen levels [8, 37]. About 50% of postmenopausal plasma T is of ovarian origin [24], and recent evidence suggests that treatment with luteiniz-

ing hormone-releasing hormone-analogues (LHRH-analogues) suppresses plasma T in postmenopausal women [38]. Therefore, tamoxifen may suppress plasma T and the T/SHBG ratio due to the decrease of gonadotrophins. That tamoxifen suppresses plasma FSH and LH but increases plasma SHBG is in accordance with the findings of others [5, 39, 40].

About 30–40% of plasma E_2 is bound to SHBG, 2–3% is free E_2 and the rest is bound to other plasma proteins, mainly albumin [41]. Results from *in vitro* protein binding studies suggest that an increase of the SHBG binding capacity may reduce the amount of E_2 bound to albumin and other "low affinity binding sites" with only a marginal influence on the free steroid fraction [42]. The influence of such a "shift" in protein binding on E_2 delivery to the tumour cell is unknown. It is therefore not possible to assess the influence of an increase in SHBG concentration on E_2 uptake in the tissue.

In conclusion, we found a moderate but significant suppression in plasma T and E_2 during tamoxifen treatment. Combined with an increase in plasma SHBG this indicates a reduced plasma level of their free fractions. An increase observed in the plasma E_1S/E_1 and the E_1S/E_2 ratios is suggested to be due to an influence of tamoxifen on estrogen metabolism.

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