Serum concentrations of tamoxifen and its metabolites increase with age during steady-state treatment

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Abstract  It has been suggested that the concentrations of tamoxifen and its demethylated metabolites increase with age. We measured the serum concentrations of the active tamoxifen metabolites, 4OHtamoxifen (4OHtam), 4-hydroxy-N-desmethyltamoxifen (4OHNĐtam, Endoxifen), tamoxifen and its demethylated metabolites. Their relations to age were examined. One hundred fifty-one estrogen receptor and/or progesterone receptor positive breast cancer patients were included. Their median (range) age was 57 (32–85) years. Due to the long half-life of tamoxifen, only patients treated with tamoxifen for at least 80 days were included in the study in order to insure that the patients had reached steady-state drug levels. Tamoxifen and its metabolites were measured by liquid chromatography-tandem mass spectrometry. Their serum concentrations were related to the age of the patients. To circumvent effects of cytochrome (CYP) 2D6 polymorphisms we also examined these correlations exclusively in homozygous extensive metabolizers. The concentrations of 4OHNĐtam, tamoxifen, NĐtam (N-desmethyltamoxifen), and NDDtam (N-desdimethyltamoxifen) were positively correlated to age (n = 151, \( p = 0.017, 0.045, 0.011, \) and 0.001 respectively). When exclusively studying the CYP2D6 homozygous extensive metabolizers (n = 86) the correlation between 4OHNĐtam and age increased (\( p = 0.008 \)). Up to tenfold inter-patient variation in the serum concentrations was observed. The median (inter-patient range) concentration of 4OHNĐtam in the age groups 30–49, 50–69, and >69 years were 65 (24–89), 116 (25–141), and 159 (26–185) ng/ml, respectively. We conclude that the serum concentrations of 4OHNĐtam, tamoxifen, and its demethylated metabolites increase with age during steady-state tamoxifen treatment. This may represent an additional explanation why studies on the effects of CYP2D6 polymorphisms on outcome in tamoxifen-treated breast cancer patients have been inconsistent. The observed high inter-patient range in serum concentrations is likely due to inter-patient differences in CYP2D6 activity.

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concentrations of tamoxifen and its metabolites, especially in the highest age group, suggest that use of therapeutic monitoring of tamoxifen and its metabolites is warranted.

**Keywords** Tamoxifen · Metabolites · Age · Endoxifen · CYP2D6

**Abbreviations**
- 4OHtam: 4-Hydroxytamoxifen
- 4OHNDtam/endoxifen: 4-Hydroxy-N-desmethyltamoxifen
- tamNox: Tamoxifen-N-oxide
- NDTam: N-desmethyltamoxifen
- NDDtam: N-desdimethyltamoxifen
- SERM: Selective estrogen receptor modulator
- ER: Estrogen receptor

**Introduction**

The selective estrogen receptor modulator (SERM) tamoxifen is used in the treatment of breast cancer patients with estrogen receptor positive (ER+) tumors. It is the gold standard in adjuvant endocrine therapy for premenopausal women and may also be used as a preventive agent in premenopausal as well as postmenopausal women with a high risk of breast cancer.

Tamoxifen acts as an estrogen antagonist or agonist depending on tissue type [1] which may be due to variation of ER co-activators and inhibitors in tissues. The agonistic effects of tamoxifen are associated with favorable effects, such as prevention of bone fractures, but also some rare, serious detrimental effects may occur, including endometrial cancer and venous thromboembolic events. Furthermore, adverse effects like hot flushes and vaginal discharge are often observed [2, 3]. Used advantly a compliance of only 50 % has been observed after 5 years treatment [4–6].

The anticancer effect of tamoxifen is believed to be due to the hydroxylated metabolites, 4-hydroxytamoxifen (4OH-tam), and 4-hydroxy-N-desmethyltamoxifen (4OHNDtam/endoxifen), because of their high affinity for the ER [7, 8], whereas the demethylated metabolites of tamoxifen have been associated with its side effects [9]. As 4OHNDtam is present in blood in concentrations about ten times higher than 4OHtam it is supposed to be the most important metabolite [10]. Multiple enzymes are involved in activation and inactivation of tamoxifen. Some of these enzymes are polymorphic, including CYP2D6, CYP2C19, CYP3A4 sulfotransferase (SULT), UDP-glucuronosyltransferase (UGT) [11–14], and other drugs may modulate their activity [15–18].

Multiple factors including drug interactions [15–18], genetic polymorphisms of tamoxifen metabolizing enzymes [10, 18–20], and seasonal variation [21] influence the pharmacokinetics of tamoxifen. Effects of these factors may explain inconsistent results from clinical studies linking genetic polymorphisms of these enzymes to clinical outcome during tamoxifen treatment [22–25].

In the present study we analysed serum samples from breast cancer patients obtained during steady-state tamoxifen treatment and observed that the serum levels of tamoxifen and its metabolites increase with age. Furthermore, the inter-patient range of serum concentrations was up tenfold and highest in the oldest age group. The results suggest that tamoxifen treatment may be improved by means of therapeutic drug monitoring.

**Materials and methods**

**Study population**

The study protocol and the main results have been described in detail elsewhere [10]. From October 2002 to October 2003, Caucasian women with breast cancer were consecutively recruited from Haukeland University Hospital, St Olav University Hospital and Førde Central Hospital in Norway. All patients were ER and/or PR positive. Written informed consent was obtained from each patient. The protocol was approved by the National Committee for Medical and Health Research Ethics (NEM). Due to the long half-life of tamoxifen, only patients treated with 20 mg tamoxifen adjuvant per day for more than 80 days were included in the study in order to insure that patients had reached steady-state drug levels. Postmenopausal status was defined as FSH levels \( \leq 20 \) IU/L and estradiol (E2) levels \( \leq 70 \) pmol/L, using the automated analyzer Immulite 2000 (Diagnostic Products Corporation, Los Angeles, CA). When only one of these criteria was fulfilled, the patients were defined as perimenopausal.

**Determination of tamoxifen and its metabolites concentrations in serum**

We used a high-pressure liquid chromatography–tandem mass spectrometry system that was developed for the determination of tamoxifen and its metabolites in serum [10, 26]. The assay was modified to improve the sensitivity by changing the API 2000/Qtrap mass spectrometry system from Applied Biosystems (AB MDS Sciex, Concord, Canada) to the API 4000, equipped with TurboIonSpray.

**CYPD6 genotype determination**

Genomic leukocyte DNA was extracted from EDTA anti-coagulated whole blood using the QIAamp DNA Blood Mini
Kit (Qiagen, Hilden, Germany). The CYP2D6 genotypes were tested previously and subdivided into four groups and ranked according to their predicted increasing enzymatic activity: poor metaboliser [subjects with any combination of two non-functional variant type (Vt) alleles *3, *4, *5, and *6], intermediate metabolizer [heterozygous; wild type/variant type], wild type/wild-type (Wt/Wt) extensive metabolizer (EM) CYP2D6, and ultra-rapid metabolizer [heterozygous; Vt allele *2 \times 2/wild type] [10].

Statistical analysis

Two-tailed Spearman correlation rank tests were used to examine the correlations between tamoxifen and metabolite concentrations in serum and breast cancer tissues. \( p < 0.05 \) was considered statistically significant. The Kruskal–Wallis test was used to compare samples from different age groups. All \( p \) values were two-sided. Statistical analyses were performed with IBM SPSS Statistics software, version 19 (SPSS Inc, Chicago, Illinois).

Results

One hundred fifty-one breast cancer patients were included in the study. The median (range) age of the patients was 57 (32–85) years. Fifteen of the patients were premenopausal, 17 perimenopausal, and 119 postmenopausal. We observed a more than tenfold inter-patient variation in serum concentrations of tamoxifen and some of its metabolites (Table 1). The concentrations of tamoxifen, 4OHNDtam, and NDDtam were positively correlated to age (Table 1). The number of poor, intermediate, extensive, and ultra-rapid metabolizers were 11, 49, 86, and 5, respectively. The overall CYP2D6 genotype distribution was in accordance with an expected Hardy–Weinberg equilibrium.

To circumvent effects of CYP2D6 polymorphisms we also studied these correlations exclusively in EMs. Although the number of patients included in this calculation was reduced from 151 to 86, the positive correlation between 4OHNDtam and age increased and the \( p \) value improved (Table 1). In the total subgroup of postmenopausal women (\( n = 119 \)) the relationship between 4OHNDtam and age was not significant (\( p = 0.107 \), correlation coefficient 0.148). However, in the subgroup of CYP2D6 extensive metabolizers (\( n = 69 \)), the correlation was highly significant (\( p = 0.007 \), correlation coefficient 0.322) (Fig. 1).

Table 1: Serum concentrations of tamoxifen and its metabolites correlate with age

| Serum concentrations (ng/ml) | Tamoxifen | 4OHtam | 4OHNDtam | NDTam | NDDtam | TamNox |
|-----------------------------|-----------|--------|----------|--------|--------|--------|
| All (\( n = 151 \))         | 89.9 (27.1–302.1) | 5.7 (1.7–17.2) | 49.6 (24.3–184.8) | 225.7 (90.1–690.9) | 37.0 (11.4–93.4) | 9.4 (2.7–37.9) |
| Wt/Wt EM (\( n = 86 \))     | 90.5 (33.9–291.1) | 5.8 (2.2–17.2) | 52.3 (24.3–184.8) | 216.7 (90.1–596.3) | 37.7 (11.4–93.4) | 9.0 (3.2–29.0) |

Correlations with age (\( R(p) \))

| All (\( n = 151 \))         | 0.163 (0.045) | 0.120 (0.141) | 0.195 (0.017) | 0.207 (0.011) | 0.260 (0.001) | 0.112 (0.172) |
| Wt/Wt EM (\( n = 86 \))     | 0.203 (0.060) | 0.190 (0.080) | 0.285 (0.008) | 0.250 (0.020) | 0.263 (0.014) | 0.191 (0.079) |

\( a \) 4OHtam 4-hydroxytamoxifen, 4OHNDtam 4-hydroxy-N-demethyltamoxifen, NDTam N-demethyltamoxifen, NDDtam N-dedimethyltamoxifen, TamNox tamoxifen-N-oxide, Wt/Wt EM wild type/wild type extensive metabolizer CYP2D6
\( b \) Median (range)
\( c \) \( R \) and \( p \) values are derived from two-tailed Spearman correlation rank test

Discussion

We observed in the present study that the serum concentrations of the active tamoxifen metabolite, 4OHNDtam (endoxifen), increase with age during steady-state tamoxifen treatment. Our findings are in line with a study published earlier this year by Teft et al. [21]. Peyrade et al. [27] and Sheth et al. [28] have previously observed that the serum concentrations of tamoxifen and its demethylated metabolites increased significantly with age.

Another important finding is that the high inter-patient variation in serum concentrations of 4OHNDtam was most pronounced in the oldest patient group, probably due to...
more concomitant diseases and increased use of additional drugs that may interact with tamoxifen metabolism. As creatinine clearance declines gradually with age and urinary elimination represents a major excretory route for 4OHNDtam [29], this may also cause higher 4OHNDtam levels in older patients [30]. The observation of higher 4OHNDtam levels and a high inter-patient variation of 4OHNDtam in the oldest patient group may in part explain the inconsistencies between studies linking CYP2D6 polymorphisms to outcome. This may further explain the finding of Margolin et al. [31] that the effect of CYP2D6 seems to derive mainly from premenopausal patients.

Tamoxifen dosage is based on the one-dose-fits-all approach. The up to tenfold variation in serum and tissue concentrations of tamoxifen and its metabolites [10, 32], which have been observed in multiple clinical studies, may influence the outcome and occurrence of adverse effects. It is still not sufficiently defined which concentrations are needed for optimal effects and at which concentrations side effects develop. Decensi et al. [33] observed in a preoperative study, using 1, 5, or 20 mg tamoxifen daily for 4 weeks, that the expression of the tumor proliferation marker Ki-67 decreased to the same extent irrespective of dose. In line with this, Moi et al. [34] observed in tumor tissues that an increase of coactivator mRNA levels seems to be an early response to tamoxifen without dose–response relationship in the 1- to 20-mg range.

Madlensky et al. [35] identified a threshold for the serum concentrations of 4OHNDtam suggesting that the women in the upper four quintiles of 4OHNDtam concentrations had a lower recurrence rate than women in the bottom quintile. Furthermore, Love et al. [36] suggested a J-shaped relationship between 4OHNDtam concentrations in individual patients and likelihood of recurrence. Quite recently, Gong et al. [37] using human MCF7 xenografts in athymic nu/nu mice observed a relationship between endoxifen concentrations and tumor growth inhibition.

Side effects of tamoxifen seem to be related to the serum levels of tamoxifen and its metabolites [38, 39]. Two studies report that the proportion of tamoxifen and its demethylated metabolites in serum was higher in patients with toxicity versus those not experiencing toxicity [9, 27]. In order to increase the serum level of 4OHNDtam in patients with decreased CYP2D6 activity, Kiyotani et al. [40] increased the daily doses of tamoxifen from 20 to 30 or 40 mg for at least 8 weeks, with no increase of side effects except from hyperhidrosis. Furthermore, some side effects like endometrial carcinoma and retina disease usually appear after long-term treatment and may be related to the cumulative dose [40]. Thus, we need more studies to better define the therapeutic range of 4OHNDtam serum levels. Are the high levels of tamoxifen and its metabolites in postmenopausal women of any clinical relevance after the advent of aromatase inhibitors?

Tamoxifen may still be a first line drug in the adjuvant treatment to some groups of postmenopausal women and could certainly be used in the last 3 years of the 5-year endocrine treatment period. In a recent study where 90% of the patients included were postmenopausal, it was observed that prolonged tamoxifen treatment from 5 to 10 years halved breast cancer mortality during the second decade after diagnosis [41]. In the BIG 1-98 randomized trial, about half of the patients were node negative, i.e., at a

![Fig. 1 Serum levels of 4OHNDtam and age in postmenopausal patients with wt/wt CYP2D6 alleles (EM)](image)

### Table 2

| Age (years) | Tamoxifen | 4OHtam | 4OHNDtam | NDtam | NDDtam | TamNox |
|------------|-----------|--------|----------|-------|--------|--------|
| 30–49 (n = 33) | Median (ng/ml) | 75 | 5.5 | 44 | 187 | 26 | 7.8 |
| | Range (min–max) | 176 (27–203) | 9.5 (1.7–11.3) | 65 (24–89) | 473 (90–563) | 77 (11–89) | 19 (2.7–21.2) |
| 50–69 (n = 83) | Median (ng/ml) | 95 | 5.8 | 51 | 229 | 38 | 9.5 |
| | Range (min–max) | 271 (31–302) | 10 (2.8–13.0) | 116 (25–141) | 575 (116–691) | 78 (16–93) | 34 (3.5–38.0) |
| >69 (n = 35) | Median (ng/ml) | 96 | 5.5 | 54 | 255 | 42 | 10 |
| | Range (min–max) | 188 (36–224) | 15 (2.2–17) | 159 (26–185) | 415 (97–512) | 72 (18–90) | 21 (3.9–25) |
low risk [42]. In this group the overall survival favored tamoxifen, although insignificantly, compared with treatment with the aromatase inhibitor letrozole. Moreover, osteoporosis and bone fractures were increased with letrozole compared to tamoxifen mono-therapy.

Tamoxifen treatment may be further improved by dose adjustment based on direct measurement of the active tamoxifen metabolites. Moreover, direct blood measurements may help to detect low compliance, which is a problem at least outside clinical studies [43]. Common criteria for therapeutic drug monitoring are fulfilled for tamoxifen, such as major inter-individual variation in metabolism, difficulties in observing toxicity clinically, and possibility of interactions and problems related to compliance.

In summary, we observed that the serum concentrations of 4OHNDtam (endoxifen) in breast cancer patients increase with age during steady-state tamoxifen treatment. The observed high inter-patient range in serum concentrations of tamoxifen and its metabolites, especially in the highest age group, suggest that use of therapeutic monitoring of tamoxifen and its metabolites is warranted inside as well as outside clinical studies.

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Conflict of interest The authors declare they have no conflict of interest.

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