Tamoxifen and Metabolite Concentrations in Serum and Breast Cancer Tissue during Three Dose Regimens in a Randomized Preoperative Trial

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ABSTRACT

Purpose: Both therapeutic and adverse effects of tamoxifen may be related to its tissue concentrations. We investigated concentrations of tamoxifen, 4-hydroxytamoxifen, N-desmethyltamoxifen, and N-didesmethyltamoxifen in serum, normal breast, and breast cancer tissues during conventional dosage and two low-dose regimens. Furthermore we studied tamoxifen effects on the cancer proliferation marker Ki-67, and on sex hormone-binding globulin (SHBG).

Experimental Design: From September 1999 to August 2001, 120 breast cancer patients were randomized to 20-, 5-, or 1-mg tamoxifen daily. We measured serum and tissue concentrations of tamoxifen and three metabolites after 28 days of treatment, and the changes between baseline and post-treatment levels of SHBG and Ki-67.

Results: The median (range) tamoxifen concentrations (ng/ml) at doses of 1, 5, and 20 mg daily (n = 38, 37, and 36) were 7.5 (2.9–120.9), 25.2 (1.9–180.9), and 83.6 (8.7–134.4) in serum, and 78.2 (35.9–184), 272.3 (122–641), and 744.4 (208.6–2556) in breast cancer tissue, respectively. Tamoxifen levels followed a dose-concentration relationship. The concentrations of tamoxifen and metabolites were related to each other. Serum and tissue concentrations of tamoxifen were associated with corresponding changes of SHBG levels, whereas changes of Ki-67 levels were not related.

Conclusions: Estrogen agonistic effects of tamoxifen on SHBG decreased with lower dosage, whereas tamoxifen effects on Ki-67 expression did not change. This together with a >10-fold variation in serum tamoxifen concentrations and a serum to tissue concentration relationship suggest that tamoxifen treatment may be improved by administration of lower doses and therapeutic drug monitoring.

INTRODUCTION

The nonsteroidal selective estrogen receptor modulator tamoxifen is a first-line drug in the treatment of breast cancer (1). The drug is also used for breast cancer prevention in high-risk subjects (2). Although two trials have shown that the nonsteroidal aromatase inhibitors anastrozole and letrozole may be superior to tamoxifen in the treatment of breast cancer of postmenopausal women (3, 4), the long-term safety of these agents is unknown with possible detrimental effects at bone metabolism and cognitive disorders due to chronic estrogen deprivation in the brain tissue (5, 6). Likewise, the long-term safety of tamoxifen is also a subject of controversy, because its estrogen agonistic properties may cause side effects like endometrial cancer and thromboembolic diseases (5, 7), which may limit its use in healthy women. Whereas large trials have shown that tamoxifen significantly reduces breast cancer incidence in high-risk women, data on the overall safety of tamoxifen have shown mixed results (5, 7–9).

Tamoxifen metabolism shows considerable interindividual variation (10, 11). Its side effects may be dose and concentration dependent (12), and an increased risk of endometrial cancer has been associated with duration of treatment and accumulated dose (13–15). Furthermore, the activity and side effects of tamoxifen may be attributed not only to concentrations of the parent drug but also to its biologically active metabolites and their accumulation in target tissues. Moreover, doses lower than the conventional 20 mg/day may have a more favorable safety profile on serum biomarkers of cardiovascular disease (16), suggesting that a dose reduction may decrease side effects of tamoxifen.

The metabolism of tamoxifen is complex (17), and a wide interindividual variation in tamoxifen metabolizing enzyme activity has been reported (18). The tamoxifen demethylating enzyme, cytochrome P-450 (CYP) 3A4, is inducible (19), whereas the hydroxylation CYP 2D6 has a polymorphic distribution that divide populations into “slow” or “rapid” metabolizers (20). In vitro studies using recombinant human CYPs have also indicated a contribution of CYP 2C9 in the hydroxylation process of tamoxifen (20), although the findings seem inconclusive (18, 21). The metabolite 4-hydroxytamoxifen (4OHtam) is highly potent because its affinity for the estrogen receptor (ER) is up to 140 times higher than tamoxifen in in vitro systems (22, 23). However, serum concentrations of 4OHtam are only
and bone fractures were also determined at baseline and at blood risk biomarkers of breast cancer, cardiovascular disease, expression of the cancer proliferation antigen Ki-67. Levels of examine the effects of low-dose tamoxifen regimens on the protocol and main results have been described in detail else-

PATIENTS AND METHODS

Patient Selection and Treatment Protocol. The study protocol and main results have been described in detail elsewhere (24). Briefly, the primary goal of the study was to examine the effects of low-dose tamoxifen regimens on the expression of the cancer proliferation antigen Ki-67. Levels of blood risk biomarkers of breast cancer, cardiovascular disease, and bone fractures were also determined at baseline and at surgery. A total of 120 subjects, aged ≥45 years, with ER-positive and/or progesterone receptor-positive cancers were randomly assigned to a conventional regimen of 20 mg of tamoxifen daily or a bolus dose of 20 mg of tamoxifen on day 1, followed by 1 or 5 mg daily for a total of 28 days before surgery. In addition, we recruited two groups of control subjects who did not take tamoxifen, one group formed by 29 subjects with ER-positive tumors and the other by 34 subjects with ER-negative tumors. Ten of these subjects were used as negative controls in the “blinding” of tamoxifen and metabolite assays.

Given that a period of at least 4–6 weeks is necessary for tamoxifen to reach steady-state levels (27, 28) the bolus dose of 20 mg of tamoxifen was given to patients on the 1 and 5 mg regimens to shorten the time required to achieve drug effects. Due to results from single dose studies (29, 30) and given a tamoxifen half-life of 7 days, the predicted contribution of the bolus dose after 4 weeks of treatment corresponded to a serum tamoxifen concentration of 2.2 ng/ml.

Patient history of diseases and current intake of other drugs were recorded at baseline. Compliance was assessed by pill count, and adverse events were assessed at 2 and 4 weeks using the National Cancer Institute Common Toxicity Criteria (31).

Sample Collection. Serum and plasma aliquots were collected at baseline and after an overnight fasting at the day of surgery between 7 a.m. and 9 a.m., and stored at −80°C until analysis. At surgery, representative samples of breast cancer tissue and normal breast tissue were obtained from the operative specimen and stored at −80°C. The mean interval from the last drug intake to blood sampling was ~24 h. This study was approved by the local ethics committee and performed according to good clinical practice.

Drugs and Chemicals. The 1, 5, and 20 mg tamoxifen citrate tablets were a generous gift from Laboratori MAG (Milan, Italy; Food and Drug Administration drug master file number 6735). The tamoxifen, 4OHtam, NDtam and N-desmethyltamoxifen (NDtam) standards with purity >98% were gifts from Imperial Chemical Industries, PLC Pharmaceuticals Division (Macclesfield, United Kingdom). The “high-pressure liquid chromatography grade” acetonitrile (LichroSolv) was purchased from Merck KgaA (Darmstadt, Germany). Acetic acid (HiperSolv) was supplied from BDH Laboratory Supplies (Poole, England).

High-Pressure Liquid Chromatography Method and Sample Processing. Serum and tissue concentrations of tamoxifen and its metabolites were measured “blinded” as to treatment allocation, using a method described previously (29). The within-day precision for tamoxifen and the metabolites measured were 0.7–5.6% for concentrations between 10 and 800 ng/ml. The detection limits were 1 ng/ml and the recovery from human serum ranged between 100% and 108%, whereas recovery from human tissues ranged between 79% and 99% (32). The assay was modified to improve the separation and sensitivity of the potent metabolite, 4OHtam (33). A representative high-pressure liquid chromatography trace is depicted in Fig. 1.

Tissue samples were prepared as described previously (32). Due to the small size of most tissues, the dilution factor for tissue samples ranged between 5 and 30 times (w:v), whereas all of the serum samples were diluted twice. We tested for the influence of breast tissue dilution on concentration at a dilution range between 5 and 35 times, and found no influence of dilution on the drug concentrations detected (data not shown).

Statistics. The results are described as ratios, percent-
ages, mean, and SDs, or medians and ranges. Two-tailed Spearman correlation rank tests were used to examine: (a) the correlation between change in Ki-67 and tamoxifen or metabolite levels; (b) the intercorrelations between tamoxifen and metabolite concentrations in serum, normal tissue, and breast cancer tissue; (c) the correlation between tamoxifen and metabolites with SHBG changes during treatment; and (d) the correlation between serum and tissue levels of tamoxifen and metabolites over the three tamoxifen dosing groups. The Kruskall-Wallis test was used to compare the median metabolite levels and ratios over the three dose groups. A repeated measures ANOVA of the NDtam/tam and NDDtam/tam ratios were used to compare the ratios in serum, normal tissue, and tumor tissue over the three dose groups. Contrasts were used to compare the ratios in serum to the ratios in breast cancer tissue, and the ratios in normal tissue to the ratios in cancer tissue. These were within-subject comparisons. Between subject contrasts were used to compare the daily doses of 5 mg and 20 mg of tamoxifen with 1 mg. A
Low-Dose Tamoxifen in Breast Cancer Patients

were available for the normal breast tissue samples, and 10 untreated control subjects.

71.8% were nonsmokers and 17.3% were current smokers.

RESULTS

Patient Characteristics. Age, menopausal status, BMI, smoking habits, and concomitant medications are summarized in Table 1. The three treatment groups were well balanced with respect to demographic characteristics. Of the analyzed patients, 71.8% were nonsmokers and 17.3% were current smokers. Except for 1 patient assigned to the 1 mg/day, and another assigned to the 5 mg/day groups who did not return any drug blisters, all of the subjects exhibited >90% compliance by pill count. No tamoxifen or metabolite concentrations were detected in 1, 3, and 2 subjects in the 1, 5, and 20 mg/day tamoxifen groups, respectively. These subjects and 3 subjects who did not take tamoxifen pills for the last 5 days were excluded from the pharmacokinetic part of the study.

Tamoxifen and Metabolite Concentration Profile. A total of 108 serum samples, 62 breast cancer tissue and 52 normal breast tissue samples, and 10 untreated control subjects were available for the “blinded” analysis of tamoxifen and metabolite concentrations. The number of patients with undetectable 4OHtam levels in the 1, 5, and 20 mg/day dose groups were 14, 9, and 6 in breast cancer tissues, respectively.

Tamoxifen levels showed a wide variation, with the median (mean) concentrations in the 1, 5, and 20 mg/day dose groups being 7.5 (16.5), 25.2 (30.8), and 83.6 (77.1) ng/ml, respectively, assuming 100% bioavailability of tamoxifen and using results from earlier studies (34) and the formula for estimating steady-state concentrations of drugs in serum (35). The median concentrations of tamoxifen and its demethylated metabolites in the tumor tissues were 5–11 times higher than those observed in serum (Table 2). The median concentrations of tamoxifen in breast cancer tissues were 26 times higher than those of 4OHtam (data not shown).

A statistically significant dose-concentration relationship was observed for tamoxifen and the demethylated metabolites in the serum and tissues examined (P < 0.001, Table 2).

There were high intercorrelations among tamoxifen, 4OHtam, NDtam, and NDDtam in serum, where the smallest Spearman correlation was 0.733 (Table 3). Unlike 4OHtam levels, tamoxifen and the demethylated metabolites in serum were related to their levels in normal breast tissue and breast cancer tissues (P < 0.001; Table 4). In normal tissue there were lower correlations between 4OHtam and tamoxifen, NDtam, and ND-Dtam (0.26–0.40), although these latter three were inter-related with correlations ranging from 0.79 to 0.80 (data not shown). In tumor tissue there were high intercorrelations, with lower values for those involving 4OHtam (data not shown).

In the three studied sites, the concentrations of tamoxifen, NDtam, and NDDtam were correlated to each other, although correlations involving 4OHtam were smaller and there was little evidence that normal tissue 4OHtam concentration in particular was related to other metabolites at other sites.

Concentration Ratios in Serum, Normal Tissue, and Breast Cancer Tissue. The repeated measures ANOVA of the 4OHtam/tam ratios in serum, normal breast, and cancer tissues over the three dose groups was not carried out, because 4OHtam levels were below detection in several of the normal and cancer tissues. Normal plots confirmed that the ratios were reasonably normally distributed, and no transformation im
three dose groups of tamoxifen (\(P\) 0.001 for both). There was no evidence for a trend among the 

\[
P_{\text{H11005}}
\]

in serum (Table 5; evidence that the NDDtam/tam and NDtam/tam ratios were highest 

\[
P_{\text{H11021}}
\]

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dence that the NDDtam/tam and NDtam/tam ratios were highest 

\[
P_{\text{H11021}}
\]

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Tamoxifen and metabolite con-

4OHtam or Its Metabolite Levels. Tamoxifen and metabolite con-

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with baseline-to-post-treatment changes in serum SHBG lev-

els, with correlations ranging from 0.28 to 0.42 (\(P < 0.005\) for serum; Table 3), 0.35 to 0.44 in the normal tissue, and 

0.35 to 0.55 in the tumor tissue (\(P < 0.013\) for both; data not shown).

There was no association between the percentage change in 

Ki-67 or the absolute change in Ki-67 (baseline to post-treat-

ment) and concentrations of tamoxifen or any of its metabolites 

in serum, normal tissue, or breast cancer tissue (\(P = 0.550\), 

0.883, and 0.886 for the percentage change in Ki-67 versus 

serum tamoxifen in the 20, 5, and 1 mg/day dose groups respec-

tively). This was true over the three dose groups and also for the 

dthree dose groups, separately.

Relationships between SHBG or Ki-67 and Tamoxifen 

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serum tamoxifen in the 20, 5, and 1 mg/day dose groups respec-

tively). This was true over the three dose groups and also for the 

dthree dose groups, separately.

Table 2  Concentrations and tissue:serum ratios of tamoxifen and metabolites (median and range) after 28 days of tamoxifen treatment

<table>
<thead>
<tr>
<th>Dose (mg/day)</th>
<th>Serum</th>
<th>Normal tissue</th>
<th>Cancer tissue</th>
<th>Breast tissue/serum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(ng/ml)</td>
<td>(ng/g)</td>
<td>(ng/g)</td>
<td>Normal</td>
</tr>
<tr>
<td>Tam (r^2)</td>
<td>1 37</td>
<td>7.5 (2.9–120.9)</td>
<td>100.5 (33.1–464)</td>
<td>78.2 (35.9–184)</td>
</tr>
<tr>
<td>5 36</td>
<td>25.2 (1.9–180.9)</td>
<td>218.7 (34.5–601.6)</td>
<td>272.3 (122–641)</td>
<td>8.7 (1.3–81.8)</td>
</tr>
<tr>
<td>20 35</td>
<td>83.6 (6.7–134.4)</td>
<td>866.5 (413.4–1466)</td>
<td>744.4 (208.6–2556)</td>
<td>8.0 (4.1–64.1)</td>
</tr>
<tr>
<td>(P)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

4OHtam or Its Metabolite Levels. Tamoxifen and metabolite con-

centrations in serum and tissues were associated significantly 

with baseline-to-post-treatment changes in serum SHBG lev-

els, with correlations ranging from 0.28 to 0.42 (\(P < 0.005\) for serum; Table 3), 0.35 to 0.44 in the normal tissue, and 

0.35 to 0.55 in the tumor tissue (\(P < 0.013\) for both; data not shown).

There was no association between the percentage change in 

Ki-67 or the absolute change in Ki-67 (baseline to post-treat-

ment) and concentrations of tamoxifen or any of its metabolites 

in serum, normal tissue, or breast cancer tissue (\(P = 0.550\), 

0.883, and 0.886 for the percentage change in Ki-67 versus 

serum tamoxifen in the 20, 5, and 1 mg/day dose groups respec-

tively). This was true over the three dose groups and also for the 

dthree dose groups, separately.

Table 3  Relationship between levels of Tam\(a\), 4OHtam, NDtam, NDDtam, and SHBG in serum

<table>
<thead>
<tr>
<th>Tam</th>
<th>4OHtam</th>
<th>NDtam</th>
<th>SHBG</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>r</td>
<td>P</td>
<td>n</td>
</tr>
<tr>
<td>Tam</td>
<td>108</td>
<td>0.865</td>
<td>0.000</td>
</tr>
<tr>
<td>4OHtam</td>
<td>108</td>
<td>0.807</td>
<td>0.000</td>
</tr>
<tr>
<td>NDtam</td>
<td>108</td>
<td>0.970</td>
<td>0.000</td>
</tr>
<tr>
<td>SHBG</td>
<td>108</td>
<td>0.393</td>
<td>0.000</td>
</tr>
</tbody>
</table>

\(a\) Tam, tamoxifen; 4OHtam, 4-hydroxytamoxifen; NDtam, N-desmethyltamoxifen; and NDDtam, N-didesmethyltamoxifen.

\(b\) Spearman rank correlation coefficient. Values below the measurable level were set as 0.4.

Table 4  Relationship between Tam\(a\) and metabolite levels in serum and the identical compounds in normal breast tissue and breast cancer tissue

<table>
<thead>
<tr>
<th>Serum</th>
<th>Tam</th>
<th>4OHtam</th>
<th>NDtam</th>
<th>NDDtam</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>r</td>
<td>P</td>
<td>n</td>
<td>r</td>
</tr>
<tr>
<td>Normal tissue</td>
<td>52</td>
<td>0.499</td>
<td>0.000</td>
<td>52</td>
</tr>
<tr>
<td>Breast cancer tissue</td>
<td>62</td>
<td>0.654</td>
<td>0.000</td>
<td>62</td>
</tr>
</tbody>
</table>

\(a\) Tam, tamoxifen; 4OHtam, 4-hydroxytamoxifen; NDtam, N-desmethyltamoxifen; and NDDtam, N-didesmethyltamoxifen.

\(b\) Spearman rank correlation coefficient. Values below the measurable level were set as 0.4.
**Table 5** Concentration ratios \(^a\) (mean ± SD) in serum, normal tissue, and breast cancer tissue

<table>
<thead>
<tr>
<th>Dose (mg/day)</th>
<th>n</th>
<th>4OHtam/tam (^b)</th>
<th>NDtam/tam</th>
<th>NDDtam/tam</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>37</td>
<td>0.05 ± 0.07</td>
<td>1.5 ± 0.40</td>
<td>0.17 ± 0.09</td>
</tr>
<tr>
<td>5</td>
<td>36</td>
<td>0.08 ± 0.12</td>
<td>1.5 ± 0.30</td>
<td>0.17 ± 0.11</td>
</tr>
<tr>
<td>20</td>
<td>35</td>
<td>0.05 ± 0.03</td>
<td>1.6 ± 0.40</td>
<td>0.16 ± 0.08</td>
</tr>
<tr>
<td>Normal tissue</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>37</td>
<td>0.01 ± 0.01</td>
<td>0.4 ± 0.20</td>
<td>0.03 ± 0.03</td>
</tr>
<tr>
<td>5</td>
<td>36</td>
<td>0.00 ± 0.01</td>
<td>0.5 ± 0.30</td>
<td>0.05 ± 0.05</td>
</tr>
<tr>
<td>20</td>
<td>35</td>
<td>0.01 ± 0.01</td>
<td>0.4 ± 0.30</td>
<td>0.05 ± 0.02</td>
</tr>
<tr>
<td>Tumor tissue</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>37</td>
<td>0.05 ± 0.10</td>
<td>0.9 ± 0.06</td>
<td>0.09 ± 0.06</td>
</tr>
<tr>
<td>5</td>
<td>36</td>
<td>0.03 ± 0.03</td>
<td>1.2 ± 1.40</td>
<td>0.10 ± 0.04</td>
</tr>
<tr>
<td>20</td>
<td>35</td>
<td>0.04 ± 0.07</td>
<td>1.1 ± 0.60</td>
<td>0.12 ± 0.06</td>
</tr>
</tbody>
</table>

\(^a\) Ratio: metabolite/tam ratios. Values below detection were set as 0.4.

\(^b\) tam, tamoxifen; 4OHtam, 4-hydroxytamoxifen; NDtam, N-desmethyltamoxifen; and NDDtam, N-didesmethyltamoxifen.

**Other Diseases and Concomitant Medications.** Only 34% of the patients used tamoxifen alone, whereas 41% were treated with two or more additional drugs for different medical conditions (Table 1). A total of 64% of the patients used drugs for other disorders, mostly cardiovascular (conditions (Table 1). A total of 64% of the patients used drugs for other disorders, mostly cardiovascular (n = 25), and endocrine (n = 14; Tables 1 and 6). Drugs used that may interfere with tamoxifen metabolizing enzymes (36, 37) are shown in Table 6. In most cases no significant difference was observed between tamoxifen concentrations in the patients who used concomitant medications as compared with those who used tamoxifen alone.

However, 3 patients who used calcium-channel blockers, known to be strong inhibitors of both CYP 2D6 and CYP 3A4 (38), had extremely low serum tamoxifen and metabolite levels compared with patients in the same treatment group. One patient using both an inducer (spirolonactone) and an inhibitor (amiodipine) of CYP 3A4 (38) also had extremely low levels. Four patients on the CYP 3A4-inducing drugs simvastatin, carbamazepine, and spirolonactone (39) had tamoxifen and metabolite profile that did not deviate from the profile observed in their treatment groups.

**Adverse Effects, BMI, and Smoking.** The number of subjects complaining of adverse events, all of grade 1, were 9, 12, and 11 in the 1 mg, 5 mg, and 20 mg treatment groups, respectively. No serious side effects were observed. Of the anticipated effects of tamoxifen, hot flashes (central nervous system effects) were recorded in 32%, 36%, and 50% of the subjects and vaginal discharge in 26%, 22%, and 47% of the subjects in the 1 mg, 5 mg, and 20 mg/day groups, respectively. The concentrations of tamoxifen and metabolites in patients with side effects were within the range of their treatment groups in serum as well as in tissues.

The average BMI values for the three dosing groups are shown in Table 1. Adjusting for the tamoxifen group and multiple comparisons, there were no significant correlations between BMI values and serum or tissue levels of tamoxifen and its metabolites, except for a weak correlation with NDDtam (r = −0.201; P = 0.037).

The number of current smokers in the 1, 5, and 20 mg dosing groups were 12, 4, and 3, respectively (Table 1). Tamoxifen and metabolite concentrations in smokers did not differ from those of nonsmokers. The low number of smokers did not allow the statistical evaluation of differences in tamoxifen kinetics between smokers and nonsmokers to be performed. Mean changes in SHBG levels were not higher in the smokers than in nonsmokers. The median (mean) SHBG difference with 1 mg, 5 mg, and 20 mg/day were: −2.9 (−2.49), 16.0 (17.18), and 25.4 (24.23) ng/ml in smokers and 1.7 (−0.04), 7.1 (1.49), and 18.6 (15.47) ng/ml in nonsmokers, respectively.

**DISCUSSION**

The present pharmacokinetic study is part of a clinical trial that investigated the effects of normal dose or low-dose tamoxifen regimens on the expression of the proliferation antigen Ki-67 and several circulating surrogate end point biomarkers (24). Patients were randomly allocated to either the conventional dose of 20 mg/day, or 5 mg/day or 1 mg/day for 28 days before surgery. The post-treatment levels of Ki-67 were significantly lower in the tamoxifen-treated groups compared with the control groups, but there was no evidence of a dose-response relationship (24).

A main finding of the present study is that the tamoxifen and metabolite concentrations in breast cancer tissue, normal breast tissue, and serum were inter-related. The average concentrations of tamoxifen in serum approximated to the estimated concentrations in serum was observed among subjects in each of the dosing groups. The observed intersubject variability compares well with those observed in earlier studies with 20 mg/day (10, 40, 41). This variability together with the observed serum to tissue concentration relationships suggest that therapeutic drug monitoring may possibly be used to optimize tamoxifen treatment.

Our results show a lack of correlation between Ki-67 changes and tamoxifen or metabolite levels both among all of the dose groups and also for each dose group separately. This lack of association strengthens the notion that lower drug concentrations exert a clinically significant antiproliferative effect on breast cancer cells. Similarly, there were no correlations between Ki-67 and the ratios 4OHtam/tam, NDtam/tam, or NDDtam/tam.

The metabolite profile differed among serum, normal breast tissue, and breast cancer tissue as demonstrated by the differences between metabolite:parent drug ratios at the different sites. The NDtam/tam and NDDtam/tam ratios were highest in serum followed by breast cancer tissue and normal tissue, which may be explained by the preferential accumulation of the most lipophilic compound, tamoxifen, in lipid-rich tissues. However, differences among serum, normal tissue, and breast cancer tissue ratios were the same at all levels of the tamoxifen doses, indicating a linear relationship between tamoxifen doses and the levels of NDtam and NDDtam.

The half-life of tamoxifen has been estimated to be ~7 days and that of NDtam 13 days, with near steady-state concentrations being achieved after 4–6 weeks of continuous treatment (34, 42, 43). The tamoxifen and metabolite concentrations meas-
ured in the present study with doses of 20 mg/day were ~40% lower compared with our previous study where tamoxifen had been administered for 2 months (44). This suggests that steady state conditions may not have been attained after 28 days of treatment. Moreover, tamoxifen itself has inducing activities on its demethylating enzyme CYP 3A4 (45). The induction process may additionally delay time to reach steady state.

A major proportion of patients used additional drugs that may influence tamoxifen metabolism. However, in patients using drugs known to inhibit CYP3A4 and/or CYP2D6, or inducing CYP3A4, we did not observe abnormal trends in drug levels or metabolite profiles in serum. Thus, no effect of CYP 3A4 induction was observed. For instance 10 patients on benzodiazepines, drugs known to be CYP 3A4 substrates (46), had concentrations of tamoxifen and metabolites that were not different from that of the other patients. Conversely, a trend to lower than expected drug concentrations were noted in subjects using CYP 3A4-inducing drugs. However, the present study was not designed to investigate drug interactions, and our results must be interpreted cautiously. The observation that tamoxifen, 4OHtam, and NDtam also are inhibitors of CYP 2D6 (47, 48), as well as inducers of CYP 3A4 (45, 49) additionally complicate the kinetics of tamoxifen.

Whereas tamoxifen itself and many of its metabolites have anticancer effects in in vitro systems (50, 51), 4OHtam binds the ER with an affinity 50 to >140 times higher than that of tamoxifen (23, 52–54). The median concentrations of tamoxifen in breast cancer tissues were 26 times higher than those of 4OHtam. Thus, the overall effects of the metabolites and tamoxifen itself.

Our findings are in agreement with those of de Lima et al. (55) who studied the effects of low doses (5 or 10 mg daily) of tamoxifen for 50 days in women with nonmalignant breast disorders and demonstrated comparable antiproliferative activity between conventional and low doses of tamoxifen in normal epithelial breast tissue.

Because of lack of standards, we were not able to measure metabolite 4-hydroxy-N-desmethyltamoxifen in the present study. We and others have observed this metabolite in humans (56, 57). Stearns et al. (58) demonstrated recently the influence of CYP2D6 genotype and activity on the conversion of tamoxifen to this metabolite, which is, like 4OHtam, highly potent in in vitro studies (23, 58).

Because side effects may be related to tissue concentrations, and tamoxifen at low doses seems to be as active as the conventional dose on tumor cell proliferation (24), the positive correlation between serum and tissue concentrations of tamoxifen found in this study supports the argument that therapeutic drug monitoring may be introduced to optimize tamoxifen treatment. Because serum concentrations remain relatively unchanged for years during chronic dosing (10), only a few serum measurements seem necessary in each patient.

The changes in SHBG levels were related to tamoxifen and metabolite levels in serum as well as in breast tissues. Because SHBG levels are known to reflect tamoxifen estrogenicity in the liver (59), high tamoxifen concentrations might possibly predict estrogen agonistic adverse events. In the IBIS-I prevention study, tamoxifen showed a significant decrease in breast cancer risk by 32%, although a significant increase in all-cause mortality was reported in the tamoxifen-treated arm (60). However, the combined evidence from all of the primary prevention trials

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**Table 6** Drugs used by the patients that may interfere with tamoxifen metabolizing enzymes

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Inducers</th>
<th>Inhibitors</th>
<th>Substrates</th>
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<tr>
<td>Cytochrome P-450 3A4</td>
<td>Simvastatin (n = 2)</td>
<td>Enalapril (n = 5)</td>
<td>Simvastatin (n = 2)</td>
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<td></td>
<td>Carbamazepine (n = 1)</td>
<td>Nifedipine (n = 4)</td>
<td>Enalapril (n = 5)</td>
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<td>Amiodipine (n = 3)</td>
<td>Fosinopril (n = 5)</td>
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<td>Ramipril (n = 2)</td>
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<td>Lisinopril (n = 1)</td>
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* For references see text.

* Number of patients.
of tamoxifen indicated that all-cause mortality decreased by 9% on tamoxifen treatment (9). Our results suggest that adverse events caused by the estrogen agonistic effects of tamoxifen may be reduced by decreasing the doses.

There are limitations to the present study. First, steady state levels may not have been attained during the short study period of 28 days. However, for ethical reasons, it was not possible to prolong the treatment time between tamoxifen initiation and surgery. Second, we were not able to detect or measure 4OHtam in a number of samples, especially in the low-dose regimens. Lastly, the protocol was not primarily designed for a pharmacokinetic study. However, the study gives insight into the real life situation of a breast cancer patient population with concomitant diseases and a parallel intake of drugs that may interact with the pharmacokinetics of tamoxifen.

Conclusively, a >10-fold variation in serum concentrations of tamoxifen was observed in a clinical situation. The tissue concentrations were related to those observed in serum. Changes in SHBG levels during treatment were positively associated with serum levels of tamoxifen, whereas no association with Ki-67 change was observed. The present results suggest that therapeutic drug monitoring may be used to optimize tamoxifen treatment, and that low dose tamoxifen regimens deserve to be explored further in clinical trials.

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REFERENCES


30. Adam, B. A review of the pharmacokinetics and metabolism of “Nolvadex” (tamoxifen). In: RL Sutherland, Jordan VC, editors. Non-
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