Adjusting the dose of tamoxifen in patients with early breast cancer and CYP2D6 poor metabolizer phenotype

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ABSTRACT

Background: CYP2D6 is a key enzyme in tamoxifen metabolism, transforming it into its main active metabolite, endoxifen. Poor CYP2D6 metabolizers (PM) have lower endoxifen plasma concentrations and possibly benefit less from treatment with tamoxifen. We evaluated tamoxifen dose adjustment in CYP2D6 PM patients in order to obtain plasma concentrations of endoxifen comparable to patients with extensive CYP2D6 metabolism (EM).

Patients and methods: Comprehensive CYP2D6 genotyping and plasma tamoxifen metabolite concentrations were performed among 249 breast cancer patients in adjuvant treatment with tamoxifen. Tamoxifen dose was increased in PM patients to 40 mg and to 60 mg daily for a 4-month period each, repeating tamoxifen metabolite measurements on completion of each dose increase. We compared the endoxifen levels between EM and PM patients, and among the PM patients at each dose level of tamoxifen (20, 40 and 60 mg).

Results: Eleven PM patients (4.7%) were identified. The mean baseline endoxifen concentration in EM patients (11.30 ng/ml) was higher compared to the PM patients (2.33 ng/ml; p < 0.001). In relation to the 20 mg dose, increasing the tamoxifen dose to 40 and 60 mg in PM patients significantly raised the endoxifen concentration to 8.38 ng/ml (OR 3.59; p = 0.013) and to 9.30 ng/ml (OR 3.99; p = 0.007), respectively. These concentrations were comparable to those observed in EM patients receiving 20 mg of tamoxifen (p = 0.13 and p = 0.64, respectively).

Conclusion: In CYP2D6 PM patients, increasing the standard tamoxifen dose two-fold or three-fold raises endoxifen concentrations to levels similar to those of patients with EM phenotype.

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Introduction

Tamoxifen is the drug of choice for the prevention and treatment of estrogen receptor (ER)-positive breast cancer in premenopausal women, and although aromatase inhibitors (AIs) are generally preferable for post-menopausal patients, some women do not tolerate these drugs. However, about 25% of patients with early breast cancer who receive adjuvant tamoxifen relapse and eventually die from the disease. Tamoxifen is a pro-drug which undergoes significant metabolism beginning with the biotransformation into two main metabolites, 4-hydroxytamoxifen and N-desmethyltamoxifen. Cytochrome P450 2D6 (CYP2D6) further metabolizes N-desmethyltamoxifen to 4-hydroxy-N-desmethyltamoxifen or endoxifen [1,2]. Plasma levels of endoxifen are 5–10 times higher than 4-hydroxytamoxifen, so endoxifen is considered the main active metabolite of tamoxifen [3–5]. The isomers (Z)-4-hydroxytamoxifen and (Z)-endoxifen are more active than tamoxifen itself, due to their 50-fold higher affinity to ER and greater ability to inhibit estrogen-dependent cellular proliferation [3,4]. In contrast, the (Z’) isomers of endoxifen and 4-hydroxytamoxifen only show ~10% of the respective (Z) isomers activity. The prior methods of quantifying tamoxifen and its metabolites did not differentiate between the isomers (Z) and (Z’), and the published concentrations of endoxifen and 4-hydroxy-tamoxifen reflected their total concentrations. The current methods make possible the quantification of these isomers.

The CYP2D gene is highly polymorphic, with more than 100 variants described. Based on the metabolic capacity of CYP2D6, the population can be divided into four metabolizer phenotypes: ultrafast (UM), extensive metabolizers (EM), intermediate metabolizers (IM) and poor metabolizers (PM). Approximately 7% of Caucasian women are PM [6]. The variants CYP2D6*3, *4, *5 and *6 are the main null alleles identified in the Caucasian race.

Several research groups showed that the loss of CYP2D6 function led to lesser benefit of tamoxifen therapy [7–11], probably due to lower endoxifen plasma concentrations [2,12]. However, negative results have also been published [13–15] and recent retrospective analyses of three large randomized studies have yielded conflicting results with regard to the association between the CYP2D6 genotype and clinical outcomes [16–19]. On-going trials will help to resolve this controversy.

PM patients receiving tamoxifen may therefore need an alternative endocrine strategy. In this prospective study we examined the hypothesis that increasing the tamoxifen dose in early breast cancer patients with the CYP2D6 PM phenotype, raises endoxifen plasma concentrations to “effective” concentrations similar to those of EM patients.

Patients and methods

Study design and population

Women ≥18 years with ER-positive early breast cancer receiving adjuvant tamoxifen in two Spanish hospitals were prospectively included and were tested for CYP2D6 genotype. Patients with contraindications to tamoxifen or receiving CYP2D6 inhibiting drugs were excluded. Written informed consent was obtained from all the participants prior to inclusion. For all the patients of Hospital Provincial de Castellón receiving tamoxifen for at least 4 months (ensuring that they had reached the steady state of the drug), baseline plasma concentrations of tamoxifen, 4-hydroxytamoxifen and endoxifen were measured. In the Hospital Universitario San Cecilio these concentrations were only measured in the 5 PM patients. Once genotyped, patients with UM, EM or IM phenotypes continued tamoxifen at standard doses (20 mg/day). For patients with PM phenotype, participation in the tamoxifen dose escalation phase was offered, provided that they presented an adequate performance status (ECOG 0–1), normal liver, kidney, cardiac and bone marrow function. Eligible patients signed a new informed consent specific to this phase. The tamoxifen dose was subsequently increased to 40 mg for the following 4 months and then to 60 mg for 4 additional months, measuring plasma concentrations of tamoxifen and its metabolites at completion of each dose level. Patients who escalated tamoxifen dose were periodically monitored at the study visits, systemically evaluating adherence to treatment (through self-reports from patients), concomitant medication and toxicity (based on the Common Toxicity Criteria of the National Cancer Institute, NCI-CTCAE v.3.0). Once the third dose level (60 mg) was completed and blood tests were taken, the patient concluded the study and continued endocrine therapy at the discretion of their attending physician.

The primary objective of the study was to compare the mean endoxifen concentrations of PM patients at the 40 mg and 60 mg tamoxifen dose levels to the concentration at the basal dose (20 mg), as well as to compare the concentration of each dose level of tamoxifen (20, 40 and 60 mg/day) in PM patients to EM patients receiving the standard dose (20 mg). The study was approved by each hospital’s institutional review board and by the Agencia Española del Medicamento y Productos Sanitarios (Spanish drug regulatory agency), and is registered in the EudraCT database (https://www.clinicaltrialsregister.eu/ctr-search/trial/2007-002942-40/ES).

CYP2D6 genotype and phenotype definition

Genomic DNA was extracted from a peripheral blood sample using Qiagen DNA extraction kit (Qiagen, Valencia, CA, USA). Determination of the CYP2D6 genotype was undertaken in the molecular biology laboratories of both institutions using the FDA-cleared, CE-IVD–approved AmpliChip CYP450 test (Roche Diagnostics, Indianapolis, IN, USA), according to the manufacturer’s instructions. This is a highly reliable method for CYP2D6 genotyping which identifies 33 CYP2D6 alleles (including variants associated to impaired enzyme activity and seven gene duplications) using the Affymetrix microarray platform [20]. Once the genotype was known, according to the conventional classification system, the AmpliChip CYP450 test predicted the metabolizer phenotype as PM if they carry two non-functional alleles; IM if they carry one non-functional allele and one associated with reduced activity or two reduced activity alleles; EM if they carry at least one functional allele and UM if they carry at least three copies of a functional allele [21,22].

Determination of the plasma concentration of tamoxifen and its metabolites

Analysis of plasma concentrations of tamoxifen and its metabolites was undertaken at Fundación Tejeirina (Madrid, Spain) using liquid chromatography associated to tandem mass spectrometry (LC-MS/MS). The Supplementary Data details a full description of this method. The intra- and inter-day variability in precision for all compounds (expressed as the coefficient of variation) ranged from 0.8 to 11% and from 2 to 8%, respectively. The average accuracies for them were between 95.5% and 106.7%.

Statistical analysis

Frequency distribution of CYP2D6 polymorphisms were expressed as absolute and relative frequency, and were assessed for Hardy–Weinberg equilibrium using the χ² test. For the evaluation

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of the differences in the patients’ baseline characteristics and adverse effects among the different genotypes groups, Fisher’s exact test was used. The Student’s t-test for paired data and the Mann–Whitney test were used for comparisons of endoxifen concentrations. Compliance with the normality assumption of data (through Kolmogorov–Smirnov and Shapiro–Wilk tests) and equality of variances across groups (through the Levene test) were checked. The two-sided significance level was considered to be 0.05. All statistical analyses were performed independently by the clinical research organization (CRO) Experior S.L. (Valencia, Spain), using R statistical software, version 2.11.1.

Results

Between 2007 and 2010, 249 patients were enrolled: 156 from Hospital Provincial de Castellón and 93 from Hospital Universitario San Cecilio. The majority of patients had an EM phenotype (n = 189; 80%), 30 patients (13%) had an IM phenotype, 11 (4.7%) a PM phenotype and 6 (2.5%) an UM phenotype. Of the 11 PM patients, 2 were withdrawn from the study, one due to toxicity (after having completed 4 months at the 40 mg tamoxifen dose level) and another one because of disease progression (at the 60 mg dose level) (Fig. 1). All the patients included were Caucasian, except for one of Hispanic origin. Table 1 shows the main baseline characteristics of the women included in the study. The CYP2D6 allele and genotype frequencies from our population meet the Hardy–Weinberg equilibrium (p = 0.255) and are presented in Table 2 and Supplementary Table 1, respectively. The genotypes of 10 women were unable to be determined, and 3 patients had a genotype whose corresponding metabolizer phenotype had not yet been characterized.

Endoxifen concentrations

Among the 156 patients of the Hospital Provincial de Castellón, 50 patients (40 EM, 5 IM and 5 unknown phenotype patients) were excluded of the endoxifen analysis for different reasons: administration of tamoxifen for less than 4 months (n = 25), no blood sample drawn (n = 12), technical failure (n = 5), unknown phenotype (n = 5) and concomitant use of CYP2D6 inhibitors.

Table 1

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>UM (N = 6)</th>
<th>EM (N = 189)</th>
<th>IM (N = 30)</th>
<th>PM (N = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average age (years)</td>
<td>42 (37–47)</td>
<td>45 (25–78)</td>
<td>42 (29–50)</td>
<td>45 (33–56)</td>
</tr>
<tr>
<td>Hormonal state</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-/perimenopausal (%)</td>
<td>5 (83.3)</td>
<td>159 (87.8)</td>
<td>27 (93.1)</td>
<td>10 (90.9)</td>
</tr>
<tr>
<td>Postmenopausal (%)</td>
<td>1 (16.7)</td>
<td>22 (12.1)</td>
<td>2 (6.9)</td>
<td>1 (9.1)</td>
</tr>
<tr>
<td>Tumour size</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>5</td>
<td>90</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>T2</td>
<td>1</td>
<td>62</td>
<td>14</td>
<td>2</td>
</tr>
<tr>
<td>T3</td>
<td>0</td>
<td>20</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>T4</td>
<td>0</td>
<td>5</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Node status</td>
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<td></td>
</tr>
<tr>
<td>N0</td>
<td>4</td>
<td>101</td>
<td>15</td>
<td>5</td>
</tr>
<tr>
<td>N1</td>
<td>2</td>
<td>56</td>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td>N2</td>
<td>0</td>
<td>15</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>N3</td>
<td>0</td>
<td>8</td>
<td>1</td>
<td>0</td>
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<tr>
<td>Prior chemotherapy</td>
<td></td>
<td></td>
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<tr>
<td>Yes</td>
<td>4</td>
<td>146</td>
<td>24</td>
<td>9</td>
</tr>
<tr>
<td>No</td>
<td>2</td>
<td>43</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>Average duration of prior Tamoxifen (months)</td>
<td>12.8</td>
<td>14.2</td>
<td>23.8</td>
<td>7.6</td>
</tr>
</tbody>
</table>

Fig. 1. CONSORT diagram of patients included in the study.

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Table 2

CYP2D6 allele frequencies (N = 249).

<table>
<thead>
<tr>
<th>Allele</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>*1</td>
<td>165</td>
<td>33.13</td>
</tr>
<tr>
<td>*2</td>
<td>98</td>
<td>19.68</td>
</tr>
<tr>
<td>*3</td>
<td>44</td>
<td>8.92</td>
</tr>
<tr>
<td>*4</td>
<td>42</td>
<td>8.51</td>
</tr>
<tr>
<td>*5</td>
<td>24</td>
<td>4.84</td>
</tr>
<tr>
<td>*9</td>
<td>12</td>
<td>2.44</td>
</tr>
<tr>
<td>*10</td>
<td>9</td>
<td>1.80</td>
</tr>
<tr>
<td>*5 × N</td>
<td>5</td>
<td>1.00</td>
</tr>
<tr>
<td>No Call</td>
<td>4</td>
<td>0.80</td>
</tr>
<tr>
<td>Others</td>
<td>35</td>
<td>7.02</td>
</tr>
</tbody>
</table>

(n = 3) (Fig. 1). At the Hospital Universitario San Cecilio only endoxifen concentrations for the 5 PM patients were measured. The results of baseline endoxifen concentrations (receiving 20 mg/day of tamoxifen) for the 111 evaluable patients are summarized in Table 3.

As expected, the mean baseline concentration of endoxifen was significantly higher in EM compared to PM patients (11.3 vs. 2.3 ng/mL; p < 0.0001). When tamoxifen dose was increased to 40 mg/day in the 11 PM patients, endoxifen concentrations statistically significantly raised compared to the baseline values for those same patients (8.4 vs. 2.3 ng/mL; p = 0.003). Similarly, the increase in endoxifen concentration to 60 mg for an additional 4-month period also significantly raised the endoxifen concentration with respect to baseline levels (9.3 vs. 2.3 ng/mL; p = 0.001). However, no statistically significant differences were observed between levels obtained at the 60 mg dose and the 40 mg tamoxifen dose (9.3 vs. 8.4 ng/mL; p = 0.38) (Table 4; Fig. 2).

Endoxifen levels reached after the dose escalation to 40 and to 60 mg were close to the mean endoxifen concentration in the group of PM patients receiving standard dose (20 mg), with no statistically significant differences (8.4 vs. 11.3 ng/mL; p = 0.13; and 9.3 vs. 11.3 ng/mL, p = 0.67, respectively) (Fig. 3).

Tamoxifen and 4-hydroxytamoxifen concentrations

As expected, no differences were observed in baseline tamoxifen concentrations between EM and PM patients (97.2 vs. 104.4 ng/mL, p = 0.8) (Table 3). The tamoxifen dose escalation to 40 and 60 mg in the PM patients increased their tamoxifen concentrations compared to the baseline values in the same PM patients (269.2 vs. 104.4 ng/mL, p < 0.0001; and 418.9 vs. 104.4 ng/mL, p < 0.0001, respectively) (Fig. 2), and compared to the patients of the control group with an EM phenotype (269.2 vs. 97.2 ng/mL, p < 0.0001; and 418.9 vs. 97.2 ng/mL, p < 0.0001, respectively).

Similarly to the analysis with endoxifen, there were differences of a small but statistically significant magnitude between mean baseline concentrations of 4-hydroxytamoxifen in EM patients compared to PM patients (1.8 vs. 1.0; p = 0.03). The increase in tamoxifen doses to 40 and 60 mg in PM patients led to a statistically significant rise in 4-hydroxytamoxifen levels compared to the baseline values in the same PM patients (4.6 vs. 1.0 ng/mL, p < 0.0001; and 5.4 vs. 1.0 ng/mL, p < 0.0001, respectively), but in contrast to the results for endoxifen, the levels also increased when compared to the baseline values for the EM patients (4.6 vs. 1.8 ng/mL, p < 0.0001; and 5.4 vs. 1.8 ng/mL, p < 0.0001, respectively).

Toxicity

During the study one PM patient was hospitalized due to disease progression presenting with brain, bone and ovarian metastases with a fatal outcome. There was no thromboembolic or any other serious adverse event. The most frequent adverse event was mild fatigue which was present in 31% of women at some point during the study. Tamoxifen doses of 40 mg and 60 mg were generally well tolerated, and no differences in the incidence of adverse events were observed after increasing the tamoxifen dose. One PM patient who had received a tamoxifen dose of 40 mg was withdrawn from the study due to uterine bleeding caused by a hyperplasic endometrial polyp. No other woman was withdrawn from the study due to toxicity. No differences were observed in the average number of daily hot flashes between PM phenotype patients and EM patients when standard tamoxifen doses were used (3.67 vs. 0.13; p = NS), not even when the dose was increased to 40 mg (3.25; p = NS) or to 60 mg (2.00; p = NS). Other adverse events were, in general, mild and infrequent.

Discussion

This study shows the feasibility of individualizing tamoxifen dose according to the CYP2D6 genotype. Our data demonstrates that a two-fold or three-fold increase in the standard tamoxifen dose (20 mg/day) in patients with a PM CYP2D6 phenotype significantly raises the endoxifen plasma concentration, reaching similar levels to those of patients with an EM phenotype treated with standard doses. However, while doubling the dose of tamoxifen (40 mg) significantly increases endoxifen concentrations, our results suggest that tripling the dose (60 mg) does not increase the concentration of endoxifen much more, and, in fact, there are no statistically significant differences in the mean endoxifen concentrations between the 40 and 60 mg doses. These dose increases were well tolerated and not associated with a higher incidence of adverse events, although the very small sample size prevents rule out a potential increased toxicity of this strategy. Therefore our study suggests that in patients with the PM CYP2D6 phenotype, doubling tamoxifen doses to 40 mg/day (authorized in the USA and Europe) could be a reasonable and safe option. Notwithstanding, the true clinical utility of this approach remains to be shown.

Several retrospective studies have shown that the loss of CYP2D6 activity is associated with poorer clinical outcome in patients receiving adjuvant tamoxifen [7–11] or in the setting of metastatic disease [23]. However, other studies have not confirmed these results [13–15]. There are multiple factors that can explain these discrepancies, reviewed elsewhere [17,24]. Recently, retrospective sub-analyses from three large randomized studies on adjuvant hormone therapy (ATAC, BIG 1–98 and ABCSG 8) have
been published [16–18]. Two of these sub-studies have not demonstrated the association between the CYP2D6 genotype and the benefit of tamoxifen therapy. However, these analyses were clearly underpowered to detect significant differences in outcome [10,19]. Furthermore, a major concern has been raised after the observation of massive departures from Hardy–Weinberg equilibrium (HWE, or static allele frequencies in a population) for CYP2D6 alleles in both ATAC and BIG 1–98 analyses [25,26], possibly due to genotyping errors. Conversely, a matched case–control study from the ABCSG 8 trial recently published did show the association of PM phenotype with a poor prognosis [18]. All of these contradictory results have raised controversy as to the value of CYP2D6 as an efficacy biomarker for tamoxifen [19,27]. Future prospective clinical studies like TEXT (Tamoxifen and Exemestane Trial) and SOFT
also in such as CYP2C9 or the UDP-glucuronosyl-transferases (UGTs) can between lower plasma concentrations of endoxifen (lowest quintile treated with tamoxifen in the study WHEL showed an association cacy than CYP2D6 itself. In fact, one analysis of 1370 patients doses in PM patients. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(Suppression of Ovarian Function Trial) which are also addressing the pharmacogenetics of tamoxifen in premenopausal patients, could provide further data to resolve this controversy.

But the CYP2D6 genotype only explains up to 40% of the vari-ability in plasma concentrations of endoxifen [4]. Other enzymes such as CYP2C9 or the UDP-glucuronosyl-transferases (UGTs) can also influence its concentration. For this reason, the endoxifen concentration might be a more decisive marker of tamoxifen effi-cacy than CYP2D6 itself. In fact, one analysis of 1370 patients treated with tamoxifen in the study WHEL showed an association between lower plasma concentrations of endoxifen (lowest quintile of <5.97 ng/ml) and a higher risk of relapse. This same association was not found for tamoxifen, 4-hydroxy-tamoxifen nor N-des-methyl-tamoxifen. Although the majority of patients with the PM phenotype had low levels of endoxifen, 24% reached ‘therapeutic’ levels of endoxifen despite their phenotype, which further strengthens the idea that CYP2D6 alone is not sufficient to deter-mine the efficacy of tamoxifen [28].

In our study we focused on patients with PM phenotype because we doubted that they could actually achieve an appropriate concentra-tion of endoxifen due to the absence of enzymatic activity. Furthermore, previous studies had revealed a negative clinical impact of tamoxifen primarily in these PM phenotype patients. We have shown that by increasing the dose of tamoxifen in PM patients, the endoxifen concentrations do reach similar levels to those with the EM phenotype, perhaps reflecting metabolism by other enzymes in the pathway. The mean endoxifen concentration for the IM and the PM phenotype, perhaps re-

![Box and whisker diagram of evolution over time of the mean endoxifen concentration in patients with PM phenotype (blue), showing an increase in endoxifen concentration with the dose escalation of tamoxifen that approaches the basal EM phenotype (red). The two ends of the boxes indicate the first and third quartiles, the median is the middle band inside the box, the mean is the small black circle protruding boxes and the whiskers represent the upper and lower fence of the data (15% inter-quartile range [IQR] of the upper quartile, and the 1.5*IQR of the lower quartile). Observations outside the fences are identified as outliers with a white circle. The blue line connects the mean endoxifen concentrations of the three tamoxifen doses in PM patients. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)]

Fig. 3. Box and whisker diagram of evolution over time of the mean endoxifen concentration in patients with PM phenotype (blue), showing an increase in endoxifen concentration with the dose escalation of tamoxifen that approaches the basal EM phenotype (red). The two ends of the boxes indicate the first and third quartiles, the median is the middle band inside the box, the mean is the small black circle protruding boxes and the whiskers represent the upper and lower fence of the data (15% inter-quartile range [IQR] of the upper quartile, and the 1.5*IQR of the lower quartile). Observations outside the fences are identified as outliers with a white circle. The blue line connects the mean endoxifen concentrations of the three tamoxifen doses in PM patients. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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Conflict of interest statement

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.breast.2014.02.008.

References


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