Focus On

Tamoxifen: using pharmacogenetics to rediscover an old drug

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Abstract Tamoxifen can be considered a classic 'pro-drug', requiring metabolic activation to elicit pharmacological activity. Our findings suggest that both genetic and drug-induced factors that alter CYP2D6 enzyme activity influence the clinical response to tamoxifen. Given the small differences in disease-free survival comparing tamoxifen to third-generation aromatase inhibitors, knowledge of the genetic and environmental factors that influence CYP2D6 enzyme activity may provide a robust tool to individualize the hormonal therapy to breast cancer.

Introduction

Breast cancer is the most common female malignancy in the Western world. Two-thirds of all breast cancers are estrogen receptor (ER) positive, a phenotypic characteristic that is prognostic of disease-free survival (DFS) and predictive of response to endocrine therapy.

While tamoxifen is still the primary endocrine therapy for the prevention and treatment of pre-menopausal breast cancer, aromatase inhibitors (AI) are now commonly administered for post-menopausal breast cancer. However, the small absolute gain in DFS (2–3%) and the lack of an overall survival advantage observed in the head-to-head trials continue to fuel the debate as to the role of tamoxifen for post-menopausal breast cancer [1,2]. In addition, the repeated demonstration that sequencing of hormonal therapy prolongs survival compared to 5 years of tamoxifen [3,4] has provided additional impetus for the continued use of tamoxifen in this setting.

While a plethora of tumor or somatic factors, including gene expression profiles [5–8], have been associated with tamoxifen resistance, the best-studied biomarkers (e.g. progesterone receptor, HER1, and HER2) appear to predict resistance to endocrine therapy in general (e.g. both tamoxifen and AI), rather than resistance to a specific drug [9,10]. However, recent discoveries regarding the pharmacology and pharmacogenetics of tamoxifen indicate that genetically inherited variation in the enzyme responsible for the metabolic activation of tamoxifen may account for a portion of 'de novo' or early tamoxifen resistance. In this review, we will focus on the tamoxifen metabolic pathway, the clinical implications for treatment of endocrine-positive breast cancer, and the use of pharmacogenetics as a tool to individualize endocrine therapy.

Tamoxifen metabolism

For over 30 years, multiple pre-clinical and clinical studies have demonstrated that tamoxifen and its metabolites competitively bind to the ER in both

breast and non-breast tissues to produce a complex phenotype of both agonist and antagonist effects. While researchers have consistently demonstrated that tamoxifen has minimal anti-proliferative activity in vitro [11–14] and in breast tumor xenograft models [11,15], paradoxically, clinically significant tumor responses are observed when tamoxifen is administered to women with estrogen-positive breast cancer. For example, the clinical response rate observed in tamoxifen neoadjuvant trials is 35–40% [16,17]. In spite of the wide interindividual variability in the concentrations of tamoxifen and its metabolites, previous studies have not demonstrated a link between the plasma concentrations of tamoxifen or its metabolites with clinical response [18,19].

For many years, tamoxifen clinical activity was felt to be substantially related to 4-OH tamoxifen [20,21], a primary tamoxifen metabolite with a 100-fold greater affinity for ERs and 30–100-fold more potency than tamoxifen in suppressing estrogen-dependent cell proliferation [22,23]. However, human steady-state concentrations of 4-OH tamoxifen are negligible (5–10 nanomolar (nM)) compared to tamoxifen (370 nM) or its most abundant metabolite, N-desmethyl-tamoxifen [(NDM; 650 nM)] [24].

Recently, the characterization of a secondary metabolite, 4-OH N-desmethyl-tamoxifen (endoxifen), originally described nearly 20 years ago as 'metabolite Bx' by Lien and colleagues [25,26], has led to the premise that endoxifen plays a central role in the activity of tamoxifen. Compared to 4-OH tamoxifen, endoxifen has equivalent potency in vitro in ER-α and ER-β binding [13], in suppressing ER-dependent breast cancer proliferation [13,27] and in global ER-responsive gene expression [28], but importantly is present at concentrations up to 10-fold higher than 4-OH tamoxifen [29]. Further studies evaluating the effect of tamoxifen and its metabolites on the anti-proliferative TGFβ signal transduction pathway in human breast cancer cells have demonstrated that only 4-OH tamoxifen and endoxifen have significant anti-proliferative activity and were able to induce TGFβ2 and TβRII in MCF-7 cells [14]. Thus, in summary, 4-OH tamoxifen and endoxifen have the greatest affinity towards the ER and are tamoxifen metabolites exhibiting the most significant anti-proliferative activity.

Cytochrome P450 metabolism of tamoxifen

Tamoxifen is extensively hepatically metabolized by the cytochrome P450 system to several primary and secondary metabolites [30–32]. The major metabolites of primary tamoxifen metabolism are NDM, 4-OH tamoxifen, and α-hydroxy-tamoxifen. NDM,
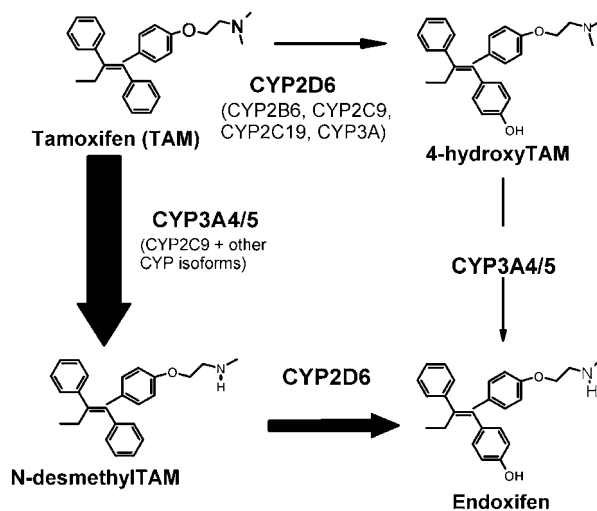

Figure 1.
Selected transformation pathways of tamoxifen and the main CYP enzymes involved. The relative contribution of each pathway to the overall oxidation of tamoxifen is shown by the thickness of the arrow, and the principal P450 isoforms responsible are highlighted in larger fonts (modified in part from Jin et al. [24]).

resulting from the CYP3A4/5-mediated catalysis of tamoxifen, is quantitatively the major primary metabolite of tamoxifen, accounting for more than 90% of primary tamoxifen oxidation [32], whereas the steady-state concentrations of 4-OH tamoxifen (accounting for less than 10% of tamoxifen oxidation) are extremely low (5–10 nM) (Fig. 1)

Recent work by Desta et al. [32] has led to a more complete characterization of the secondary pathway of tamoxifen metabolism. NDM is predominantly biotransformed to α-hydroxy N-desmethyl-, N-didesmethyl-, and endoxifen. CYP2D6 is the rate-limiting enzyme responsible for the formation of endoxifen [32] (Fig. 1). In contrast to 4-OH tamoxifen, wherein multiple CYP450 isoforms are responsible for the oxidation of tamoxifen to 4-OH tamoxifen, CYP2D6 is the primary enzyme responsible for the catalysis of NDM to endoxifen [32,33]. Recent clinical studies have demonstrated that common *CYP2D6* genetic variation (leading to reduced or absent CYP2D6 activity) or the inhibition of CYP2D6 activity significantly lowers the concentrations of endoxifen [24,33]. Specifically, plasma levels of endoxifen are up to 10-fold higher than 4-OH tamoxifen, and range from 20 nM in patients with no CYP2D6 activity (CYP2D6 *poor metabolizers*) up to 180 nM in patients with normal or increased CYP2D6 activity (CYP2D6 *extensive* or *ultra-rapid metabolizers*) [29]. This makes endoxifen the most abundant active tamoxifen metabolite.

CYP2D6 and genetic variation

The *CYP2D6* gene is localized on chromosome 22q13.1. The genetic polymorphism of *CYP2D6* is documented by the fact that 63 different major polymorphic *CYP2D6* alleles are known, many of which are associated with increased, decreased, or abolished function of the final gene product. Some of the most common and important variant alleles distributed in different ethnic groups are listed in Table 1, and all variant alleles are presented at the home page of the human CYP allele nomenclature committee (http://www.imm.ki.se/cypalleles/cyp2d6. htm). Carriers of any two of about 20 known null alleles are phenotypic *poor metabolizers*, representing about 5–10% of the individuals of European and North American Caucasian populations. Individuals at the higher end of the activity spectrum (*ultra-rapid metabolizers*) carry alleles with gene duplications and multiduplications, which lead to higher CYP2D6 expression and enzyme activity.

Tamoxifen pharmacogenomics and breast cancer relapse

Knowing that CYP2D6 is the rate-limiting enzyme responsible for the production of endoxifen and, additionally, contributes to the production of 4-OH tamoxifen, we hypothesized that genetic variation and/or drug-induced variation in CYP2D6 leading to the *poor metabolizer* state could alter breast cancer outcomes in women administered tamoxifen. We performed a retrospective analysis of a prospective adjuvant tamoxifen trial (NCCTG 89-30-52) in post-menopausal women with surgically resected ER-positive breast cancer (stages I–III). Because of the difficulty in amplifying DNA from formalin-fixed paraffin-embedded tissue, only the *CYP2D6* *4 (the most common null allele contributing to the *poor-metabolizer* state) and the *6 (a rare allele with a frequency less than 1%) were studied. The *4 and *6 alleles account for 75–80% of CYP2D6 *poor*

metabolizers in a predominantly Caucasian population [35]. As expected, no *6 variants were detected. Women with the CYP2D6 *4/*4 genotype had shorter relapse-free time and worse DFS compared with either the *4/wt or the wt/wt genotype (log rank $P = 0.030$ and $P = 0.020$, respectively). In a multivariate analysis, *4/*4 patients still tended to have worse DFS (HR 1.86, $P = 0.089$) [36]. Additionally, we observed differences in the incidence of moderate or severe hot flashes in patients homozygous for the *4 allele (0%) vs. those with 1 or 2 functional alleles (20%) ($P = 0.06$). These data supported the hypothesis that the CYP2D6-mediated formation of the potent anti-estrogens may lead not only to differences in response but also to side-effects.

CYP2D6 inhibitors and their effect on tamoxifen activation

The measurement of enzyme activity for phase I and II drug metabolizing enzymes is complex, as genetic variation accounts for only one aspect of the known wide variability in the expression and activity of these enzymes. In the case of CYP2D6, many drugs, including the newer antidepressant drugs such as the selective serotonin reuptake inhibitors (SSRIs) and the serotonin and norepinephrine reuptake inhibitors (SNRIs), are commonly administered with tamoxifen to treat depression or to alleviate hot flashes [37–41]. Many SSRIs used to treat symptoms of hot flashes in tamoxifen-treated women are known inhibitors of the CYP2D6 enzyme [42]. Stearns *et al*. demonstrated that co-administration of paroxetine in tamoxifen-treated women dramatically reduced the plasma concentrations of endoxifen by nearly 65% in patients with normal CYP2D6 metabolism [33]. A follow-up prospective trial demonstrated that the co-administration of potent inhibitors of CYP2D6 (paroxetine and fluoxetine) and weak CYP2D6 inhibitors significantly lowered plasma concentrations of endoxifen [29].

Table 1. Major *CYP2D6* alleles, effect on enzyme metabolism, and allele frequencies in selected populations.

Major variant alleles	Consequence	Allele frequencies (%)		
		Caucasians	Asians	Black Africans
CYP2D6*2xn	Increased enzyme activity	1–5	0–2	2
CYP2D6*4	Inactive enzyme	12–21	1	2
CYP2D6*5	No enzyme	2–7	6	4
CYP2D6*10	Decreased activity	1–2	51	6
CYP2D6*17	Decreased activity	0	0	20–35
CYP2D6*41	Decreased activity	8–10	0–2	11–14

Modified from Ingelman-Sundberg [34].

CYP2D6 metabolism and tamoxifen treatment outcome

We recently updated our analysis of the NCCTG 89-30-52 tamoxifen trial by performing a comprehensive analysis of the combined effect of exogenous inhibition of the CYP2D6 enzyme and genetic variation on breast cancer outcomes [43]. We reviewed the medical records at each NCCTG randomizing site to determine whether the following known CYP2D6 inhibitors were co-administered with tamoxifen: fluoxetine, paroxetine, sertraline, cimetidine, amiodarone, doxepin, ticlopidine, and haloperidol. In our analysis, we used the *in vivo* data regarding the known combined effect of *CYP2D6* genotype and CYP2D6 inhibitors on endoxifen plasma concentrations [24,29]. 'Extensive' metabolizers were women without a CYP2D6 *4 allele, who did not receive a CYP2D6 inhibitor. 'Decreased' CYP2D6 metabolism was defined as patients with one or two *4 alleles or the confirmation that a CYP2D6 inhibitor was co-administered with tamoxifen (regardless of genotype).

Medication history was available in 225/256 eligible patients and we confirmed that 13 patients (6%) were co-prescribed a CYP2D6 inhibitor with a median duration of 2–3 years. Knowledge of both medication history and CYP2D6 genotype (available in 180 pts) allowed us to classify patients as having 'extensive' ($n = 115$) or 'decreased' ($n = 65$) CYP2D6 metabolism. In a multivariate analysis, patients with 'decreased' metabolism had worse relapse-free survival (RFS) ($P = 0.017$; HR = 1.74), relative to patients with 'extensive' metabolism. Additionally, based on the *in vivo* data regarding the combined effect of *CYP2D6* genotype and CYP2D6 inhibitors on endoxifen plasma concentrations [24,29], we further classified patients with 'decreased' CYP2D6 metabolism into 'intermediate' or 'poor' metabolizer categories, based on the potency of the CYP2D6 inhibitor prescribed. While women classified as having 'intermediate' metabolism tended to exhibit worse RFS (HR 1.63, $P = 0.075$), patients classified as 'poor metabolizers' had the worst outcome, with significantly worse RFS (HR 2.69, $P = 0.005$) [43], consistent with a gene dosage effect (Fig. 2).

Recently, our findings were validated by Schroth *et al.* [44], who demonstrated the significance of *CYP2D6* genetic variation in a retrospective cohort of 206 tamoxifen-treated and 280 tamoxifen-untreated breast cancer patients. Tamoxifen-treated women with one or two *CYP2D6* alleles associated with absent or reduced enzyme function (*4, *5, *10, *41) had significantly more recurrences, shorter relapse-free time (HR = 2.24; 95% CI, 1.16–4.33; $P = 0.02$), and worse event-free survival
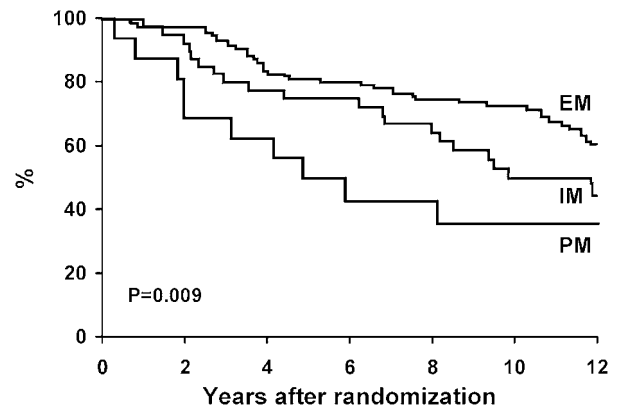

Figure 2.
Kaplan–Meier estimates of relapse-free survival based on metabolizer status (extensive, intermediate, or poor) (reprinted from Goetz et al. [43]).

(HR = 1.89; 95% CI, 1.10–3.25; $P = 0.02$) compared to carriers of functional alleles [44]. As expected, there was no association between treatment outcome and *CYP2D6* genotype in the cohort of patients that did not receive tamoxifen.

Tamoxifen and FDA relabeling

On October 18, 2006, an FDA Advisory Subcommittee was convened to review the tamoxifen research findings to date and to make a recommendation regarding a label change. The consensus of the Subcommittee was that the label should be updated to reflect the fact that postmenopausal women with ER-positive breast cancer who are CYP2D6 poor metabolizers treated with tamoxifen (by genotype or drug interaction) are at increased risk for breast cancer recurrence.

Clinical impact of tamoxifen pharmacogenomics

One of the consistent findings in tamoxifen adjuvant studies to date is the demonstration of a peak in the annual hazard rates of recurrence within the first 2–3 years of initiating tamoxifen [45]. In our study, by grouping patients according to metabolizer status, the 2-year RFS rates by metabolizer status were 98%, 92%, and 68% for *extensive*, *intermediate*, and *poor* metabolizers, respectively (Fig. 2). Furthermore, when comparing instantaneous hazard rates for RFS by CYP2D6 enzyme status (Fig. 3), our data demonstrate an immediate broad peak in the RFS hazard rate for patients with 'decreased' metabolism while the hazard rate in patients with extensive metabolism was reduced and did not peak until nearly the 4th year. These
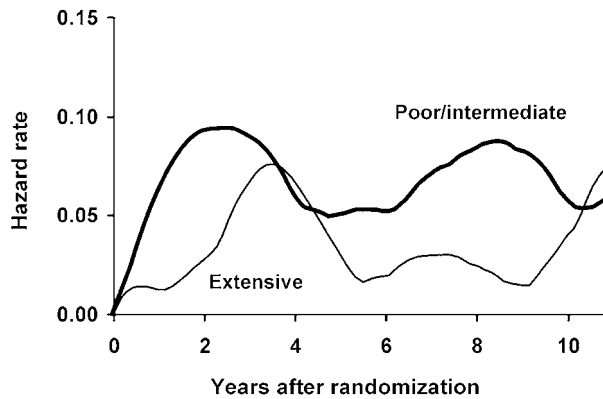
Figure 3.
Smoothed Hazard rates for relapse-free survival comparing patients with extensive vs. decreased CYP2D6 metabolism (reprinted from Goetz et al. [43]).

findings suggest that the peak in the annual hazard rates for recurrence in tamoxifen-treated women may be related to genetic or drug-induced variation in CYP2D6. Furthermore, our findings suggest that patients with decreased CYP2D6 metabolism who have not relapsed within the first 2–3 years of receiving tamoxifen therapy may be the subset that derives the greatest benefit from switching to an AI.

Our data may have profound implication for the long-term endocrine management of breast cancer, which is characterized by an unrelenting risk of relapse that carries well into the 2nd decade following diagnosis [46]. Notably, CYP2D6 is not known to be involved in the metabolism of any of the AI. In this way, *CYP2D6* genotyping may identify patients most suitable for sequencing of hormonal therapy. Given the potential implications of long-term estrogen deprivation as is currently being evaluated in studies looking at 10 years of AI, a strategy of sequencing may provide the most tolerable and efficacious strategy if CYP2D6 pharmacogenetics is validated as an early predictor or tamoxifen relapse in the head-to-head trials.

Current management of ER-positive breast cancer

It is our practice to fully inform patients of the importance of CYP2D6 as a key activator of tamoxifen. We consider CYP2D6 genotyping in settings wherein alternative therapies are known to be equivalent or superior to tamoxifen monotherapy (e.g. post-menopausal adjuvant breast cancer). However, it is important to stress that further research is needed to determine the optimal sequence of hormonal therapy (tamoxifen for some duration followed by an AI) in patients who carry one or two functional *CYP2D6* alleles. For

pre-menopausal breast cancer, there are no published data regarding *CYP2D6* genotype and treatment outcome. In the setting of chemoprevention, a small case–control study from the Italian chemoprevention group demonstrated a higher likelihood that tamoxifen-treated women homozygous for the *4 allele would develop breast cancer compared to those with the wild-type genotype [47]. These data are considered preliminary, and further research is needed in pre-menopausal women before using *CYP2D6* genotype to exclude patients from tamoxifen.

Additionally, we recommend a careful evaluation of a patient's prescription and non-prescription medications, with a recommendation for discontinuation of potent CYP2D6 inhibitors. Regarding the non-hormonal treatment of hot flashes, we remind women that hot flashes may be a biomarker of tamoxifen drug effect [48]. In this setting, administration of an SSRI or SNRI like venlafaxine with little or no effect on endoxifen plasma levels [29] appears to be a reasonable treatment of tamoxifen-induced hot flashes.

In conclusion, our findings suggest that both genetic and drug-induced factors which alter CYP2D6 enzyme activity influence the clinical response to tamoxifen. Given the small differences in DFS comparing tamoxifen to third-generation AIs, knowledge of the genetic and environmental factors that influence CYP2D6 enzyme activity may provide a robust tool to individualize the hormonal therapy of breast cancer.

Acknowledgements

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