Tamoxifen, CYP2D6 and endoxifen in the treatment of hormone sensitive breast cancer: demystifying the connections

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Abstract

The role of the selective oestrogen receptor modulator, tamoxifen, is well established in the treatment of hormone sensitive breast cancer. The metabolism of tamoxifen to its active metabolites is however complex. Despite much research, a conclusive stance on the clinical implications of CYP2D6, active metabolites including endoxifen in efficacy and toxicity, is yet to be reached. Herein we examine the literature to clarify the connections between tamoxifen, CYP2D6 and endoxifen with resultant clinical recommendations.

As the first targeted systemic therapy in the history of solid tumour oncology, tamoxifen has a prominent place as an effective anti-cancer treatment (endocrine therapy) for hormone-sensitive breast cancer.¹ Although somewhat superseded by aromatase inhibitors in the treatment of disease in postmenopausal women, tamoxifen still has an important role. This is particularly the case in the treatment of pre and peri menopausal women, as well as in cases where toxicities and pre-existent comorbidities do not permit use of an aromatase inhibitor.

Tamoxifen set the early benchmark for endocrine therapies in breast cancer. A multitude of studies and subsequently the Oxford Overview clearly established that when used in the adjuvant setting, this selective oestrogen receptor modulator will reduce breast cancer recurrence by a half and breast cancer specific mortality by a third.² However, despite being in use for over three decades, there is no conclusive, unified stance regarding the therapeutic implications of its metabolism and its impact on efficacy.

In brief, tamoxifen itself is only weakly anti-oestrogenic and is biologically not effective until it is metabolised to produce active metabolites. Therefore, tamoxifen is a prodrug which is extensively metabolised by hydroxylation, demethylation and conjugation, giving rise to several metabolites. Endoxifen (4-OH-N-desmethyltamoxifen) and 4OH tamoxifen are the most active, and endoxifen is the most abundant.³,⁴ It has been known for some time that genetic variability of the CYP2D6 gene has an impact on the levels of active metabolites. However, CYP3A4, CYP2C9/19, UDP-glucuronosyltransferases and sulfotransferases as well as compliance, absorption and concomitant medications that inhibit the CYP2D6 enzyme also have an impact on tamoxifen metabolism.⁵,⁶,⁷ Thus simply looking at CYP2D6 genotype is unlikely to describe tamoxifen metabolism accurately. A simplified representation of tamoxifen metabolism is depicted in figure 1.

Endoxifen

Endoxifen, as the most abundant and active anti-oestrogenic metabolite of tamoxifen, has been recognised as mediating the vast majority of both tamoxifen effects and anti-cancer benefits in the treatment of hormone-sensitive breast cancer.⁸⁻¹⁰ It is therefore possible that it is the level of endoxifen that is important for clinical benefit rather than the dose of tamoxifen, yet this is not a parameter we routinely measure.
In the pre-clinical setting, endoxifen dose certainly matters. Cell culture studies indicate that the tamoxifen effect on breast cancer cells is not only endoxifen-dependent but also concentration-dependent, with data suggesting that endoxifen levels above 40nM are required for optimal oestrogen receptor blockade. Mouse MCF7 breast cancer cell xenograft models have demonstrated that 97% of growth inhibition is attainable at 40nM, as compared to 83% at 15nM.

In women treated with tamoxifen, endoxifen levels vary widely, and are loosely correlated with genotype. There are clear trends in level according to whether patients are “poor”, “intermediate” or “extensive” metabolisers. However, there is significant heterogeneity of endoxifen level despite genotype.

Despite the wide acknowledgement of the importance of endoxifen, due to a lack of studies examining this question, there are no prospectively collected data correlating absolute endoxifen levels with survival or recurrence free survival. However, there are two retrospective studies of adjuvant tamoxifen examining the effect of endoxifen level: the first, the WHEL (Women’s Healthy Eating and Living) group, examined 1370 women and showed a 26% worse disease-free survival in women in the lowest quintile of endoxifen (<15nM) compared to those in the upper four quintiles. Similarly, Saladores et al demonstrated in 306 women that those in the lowest quintile (endoxifen <15nM) had worse disease free survival than those who had levels >35nM.

**CYP2D6**

As discussed above, CYP2D6 is an important factor in the conversion of tamoxifen to endoxifen, and polymorphisms result in variable levels. However, the literature pertaining to CYP2D6 is somewhat discordant and requires careful examination of the details.

Selected publications report CYP2D6 genotype derived from analyses of tumour DNA rather than germline DNA. This has been a cause of debate as to whether it is valid to assess patients’ inherent metabolism of tamoxifen via tumour DNA, as metabolism of tamoxifen by CYP2D6 occurs in the liver and therefore germline rather than tumour DNA is more relevant. This practice is also problematic in view of the Hardy Weinberg Equilibrium, which predicts the stability of allelic and genotypic frequencies through the generations in the absence of outside factors.

The method by which CYP2D6 genotype is determined also requires careful consideration. Commercial availability of CYP2D6 genotyping does not guarantee that a comprehensive panel of assessment is offered. We have found varying degrees of comprehensiveness for CYP2D6 single nucleotide polymorphisms tested amongst commercially available panels for genotyping CYP2D6, which renders standard evaluation of these problematic. As such, the panel below lists alleles and haplotypes that are required to be measured to classify phenotype according to the current categorisation recommended by the International Clinical Pharmacogenetics Implementation Consortium (figure 2).

The current phenotype categorisation of CYP2D6 was devised according to the metabolism of the drug codeine, so it is uncertain whether it can equally be applied to tamoxifen metabolism. Historically, CYP2D6 had been phenotyped by categorisation systems derived from debrisoquine, sparteine and other drugs. The recent categorisation system for CYP2D6 using codeine shifted several haplotypes from one phenotype to the other without evidence for this in regards to tamoxifen. This raises the question whether it is valid to use a system derived from codeine metabolism and apply it to tamoxifen metabolism.

Direct measurement of CYP2D6 enzyme activity, however, can be inferred by the N-desmethyl-tamoxifen/endoxifen ratio, as N-desmethyl-tamoxifen is converted to endoxifen by CYP2D6. We devised a categorisation system utilising this measure of protein activity by calculating the ratio and dichotomising patients to wild-type or variant metabolisers, which we found was superior to the current codeine devised classification system in predicting CYP2D6 protein activity. Though our categorisation was demonstrated in our cohort of 106 patients to be superior to the pre-existent codeine classification system, we still propose that the most accurate measure of tamoxifen effect by endoxifen is to directly assess the endoxifen level rather than infer tamoxifen activity from genotype or phenotype.
As we have previously acknowledged, there is a trend for CYP2D6 poor metabolisers to have lower endoxifen, and extensive metabolisers to have higher endoxifen.\textsuperscript{24-26} However a high degree of overlap in endoxifen levels with CYP2D6 genotype and phenotype has been replicated in other groups. Therefore CYP2D6 genotype alone does not accurately estimate endoxifen level and whether potential therapeutic levels have been achieved.

There are also conflicting data linking CYP2D6 genotype and disease free and overall survival in tamoxifen-treated women,\textsuperscript{16,27-29} presumably in part because of the overlap mentioned above. Therefore, it is not possible to predict survival or response to tamoxifen based only on CYP2D6 genotype.

We therefore support the use of direct endoxifen measurement rather than measuring genotype to guide therapeutic efficacy of tamoxifen effect.

**Dosing of tamoxifen**

The current standard dose of tamoxifen is 20mg per day as established from clinical trials. This remains despite the known variability in CYP2D6 and more importantly, endoxifen levels. We and others have examined the role of dose escalation in the face of low levels and found that with dose escalation of tamoxifen, endoxifen levels will always increase, though the rate of increment varies according to phenotype.\textsuperscript{15}

We also conducted a study in a small cohort of tamoxifen-treated women who were experiencing intolerable toxicity that threatened compliance, to determine whether dose reduction improved hot flushes.\textsuperscript{29} Firstly, we found that the distribution of CYP2D6 genotype was similar to that in our original 122 patient cohort, suggesting that CYP2D6 is not the cause of intolerable hot flushes, supporting our findings in the original cohort. Furthermore, we found that upon dose reduction from 20mg to 10mg, endoxifen levels halved and that a larger proportion of women were below the purported therapeutic level of 15nM. Although some patients reported subjective improvements in hot flushes upon dose reduction, when tested by the validated Loprinzi instrument,\textsuperscript{30} there were no statistically significant differences in hot flushes between dose levels. Therefore, we recommend that dose should not be changed according to CYP2D6 genotype or phenotype nor to hot flush toxicity of tamoxifen.

Endoxifen levels however will change according to dose alteration, albeit to differing degrees and impacted to varying degrees to patients’ CYP2D6 phenotype as well as environmental and other factors.\textsuperscript{31,32}

Therefore, to summarise:

1) **Anti-oestrogenic activity**
   - Tamoxifen itself is a weakly active prodrug that is converted by cytochrome p450 enzymes to produce active metabolites, of which endoxifen is the most potent and abundant.

2) **CYP2D6**
   - CYP2D6 is the cytochrome p450 enzyme that has wide pharmacogenetic variability and impacts on conversion of tamoxifen to endoxifen.
   - CYP2D6 genotyping assessment should be performed on germline DNA and not tumour DNA.
     - The current systems for phenotyping CYP2D6 are based on codeine metabolism.
     - There are trends for patients with poor CYP2D6 metaboliser phenotype to have lower levels of endoxifen and for patients with extensive CYP2D6 metaboliser phenotype to have higher levels, however there is a great deal of overlap.
     - CYP2D6 genotype and recurrence-free survival have not been consistently correlated.
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3) **Endoxifen level**
   - In two retrospective cohorts of tamoxifen-treated women with early breast cancer, worse recurrence-free survival was seen with levels lower than 15nM.
   - Endoxifen therapeutic monitoring may be of value though currently this is not available in Australia in commercial laboratories outside of the research setting.
4) **Dose of Tamoxifen**
- Changing tamoxifen dose impacts endoxifen level, with dose escalation causing increase and dose reduction a decrease in level. The degree of endoxifen level change is influenced by CYP2D6 genotype.

5) **Hot flushes**
- Neither endoxifen level nor CYP2D6 genotype is associated with the severity of hot flush and this toxicity should not be used as a surrogate for tamoxifen efficacy or to estimate genotype or endoxifen level.

Ultimately, further research is required to ascertain the robustness of the therapeutic endoxifen level and the utility of this in a clinical setting. We recommend overall however, that CYP2D6 testing should not be used to determine whether a woman should be treated with tamoxifen or at what dose. If a threshold endoxifen level is demonstrated more clearly, then endoxifen level testing may become important.
**Figure 1:** Simplified representation of tamoxifen metabolism to endoxifen depicting key cytochrome P450 enzymes.\(^{28,29}\)

**Figure 2:** The current CYP2D6 phenotypic categorisation according to Crews et al for codeine, as recommended by the International Clinical Pharmacogenetics Implementation Consortium.\(^{18}\)

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EM: extensive metabolizer; IM: intermediate metabolizer; PM: poor metabolizer; UM: ultrarapid metabolizer
References

21. Inherited amplification of an active gene in the cytochrome P450 CYP2D locus as a cause of ultrarapid metabolism of debrisoquine (geneamplification/ sparteine/ bufuralol/ pharmacogenetics/ drugtherapy)
23. Lee CI, Low ASK, Fox P. Simplified CYP2D6 metabolizer phenotype categorization of patients treated with tamoxifen: Role for endoxifen level monitoring. J Clin Oncol. 2016;34 (suppl; abstr 536)