Pharmacogenomics of Tamoxifen in a Nutshell—And Who Broke the Nutcracker?

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Imagine biting into one of south Texas's most inviting sweets, a pecan pie, but with all the pecans still in their shells—not only insipid, but potentially dangerous. At the 2003 San Antonio Breast Cancer Symposium (San Antonio, TX, December 3-6, 2003), my heart sank when I learned that the activity of tamoxifen was almost completely inhibited when paroxetine was coadministered—a pairing I so frequently used for control of vasomotor symptoms. Subsequently, Stearns et al¹ published their evolved understanding of tamoxifen pharmacokinetics; it attracted the attention of the Cable News Network (CNN), and it was viewed widely by patients and their families. However, conversations with colleagues indicated that this more sophisticated understanding of the peculiar pharmacokinetics of tamoxifen may not have been incorporated into clinical patient care, especially when patients receive medications from other nononcology physicians.

The purpose of this article is to review one small segment of the rapidly expanding understanding of adverse drug interactions, namely the interaction of tamoxifen with selective serotonin-reuptake inhibitors (SSRIs) that may block any therapeutic benefit. For the full story, go to Wilkinson et al,² Jin et al,³ and the informative Web site of David A. Flockhart, MD, PhD, <http://medicine.iupui.edu/flockhart/table.htm>.⁴ For those who never read the book but only see the movie, go to the Center for Drug Evaluation and Research's Web site, <http://www.fda.gov/cder/drug/drugReactions/default.htm>,⁵ and see Jin et al.³

Background

Do you remember mixing watercolors together? Although the first two colors often produced a wonderful chromogenic effect—red and blue made purple!—the addition of any other colors always seemed to turn to black in my hands. Likewise, coadministration of more than two drugs can often result in an adverse drug interaction instead of a therapeutic benefit. As noted by Jacubeit et al,⁶ the rate of adverse drug interactions increases exponentially when four or more medications are coadministered. A cartoon from one of my patients showed a pharmacist dispensing “the medicine your doctor gave you for your problem” and four other medicines “to prevent side effects from the medicine your doctor gave for your original problem.” Verily, for many of us treating young women with tamoxifen, no prescription was ever written without a corresponding script for paroxetine, sertraline, venlafaxine, or gabapentin, because Adelson et al⁷

demonstrated a dramatic percentage of reduction in otherwise often incapacitating vasomotor symptoms. At that time, tamoxifen metabolism was poorly understood.

New Understanding of Tamoxifen Metabolism: In a Nutshell

The work of V. Craig Jordan, OBE, PhD, DSc, provided much of our early understanding of tamoxifen metabolism.⁸ Tamoxifen is metabolized in the liver to N-desmethyl-tamoxifen and 4-hydroxytamoxifen (4HT). Tamoxifen and the N-desmethyl metabolite have equal antiestrogenic properties. However, 4HT, the “nut” in the nutshell, is 100-fold more active than tamoxifen, though present only in minute concentrations. Jin et al³ discovered another metabolite, 4-hydroxy-N-desmethyltamoxifen (endoxifen), that is more abundant than 4HT but is equally active, which is 100 times more potent than the parent compound, tamoxifen (another “nut” within the tamoxifen shell).³ This newer understanding clearly suggests tamoxifen can be thought of as a prodrug that must be activated to achieve the therapeutic effect. The revised metabolic pathway for generation of endoxifen, the real “business end” or the effector molecule of tamoxifen is shown in Figure 1. Using Jin's data,³ Table 1 shows the relative biologic activity of tamoxifen and its metabolic products. Clearly, endoxifen is responsible for the antiestrogenic activity, and cytochrome P450 2D6 is responsible for generation of endoxifen.

Cytochrome P450: The Superfamily, the Heavy Lifters, and 2D6

Welcome to the Superfamily

The cytochrome P450 enzymes (CYPs) are a superfamily of metabolic enzymes present in the endoplasmic reticulum of liver cells and the brush border of the intestines. The CYPs are enzymes responsible for biosynthesis and degradation of endogenous steroids, lipids, and vitamins as well as many exogenous substances. They are chemically distinct and, reminiscent of high school biology's “kingdom, phylum, family, genus, species,” are similarly named in a specific format based on their genetic origins but totally unrelated to their function. The particular CYP of interest to this article, 2D6, exemplifies the standard nomenclature: first, a number, 2, identifies the family; then, a letter, D, identifies the genetic subfamily; and, finally, another number, 6, identifies the specific gene. There are six forms of CYP450, called isoforms, that are important for human drug metabolism: CYP1A2, CYP3A, CYP2C9, CYP2C19, CYP2D6, 2E1.⁹ Each of these CYP enzymes, like most proteins, is produced by a specific gene. Each gene for a specific CYP is composed of two alleles,

Figure 1. Metabolic pathways of tamoxifen. The size of the arrow indicates the magnitude of the reaction. The most utilized pathway involves conversion of tamoxifen to N-desmethyltamoxifen by CYP3A. N-desmethylTAM is then converted to the most active antiestrogenic molecule, endoxifen, by CYP2D6. Adapted from Jin et al.³

one maternal and one paternal. Some of the CYP genes have mutations that result in more than one form of the gene—polymorphisms—which, in turn, produce enzymes that, like siblings, are very similar but have entirely different personalities. These sibling enzymes are called isozymes. Three of the CYPs have polymorphisms: 2D6, 2C19, and 2C9.

CYP Heavy Lifters

As seen in Figure 2, CYP3A is the isoenzyme family that metabolizes more than half of all drugs, whereas the CYP2D6 isoenzyme family metabolizes approximately a quarter of all drugs. CYP3A is present in about 31% of liver microzymes, whereas the CYP2D6 family barely registers as present at 2%. Thus, the stage is set for significant effects on drug metabolism because of small changes in the function of this relatively scarce, but functionally very prominent, isoenzyme, CYP2D6.

CYP2D6: The Tamoxifen “Nutcracker”

CYP2D6 has 78 variants (many different styles of nutcracker), but most of these variant forms make the enzyme inactive. Indeed, alternative genetic variants of the 2D6 family result in four distinct patient groups: (1) the normal group, termed “extensive” metabolizers; (2) a “poor” metabolizer group; (3) an intermediate group halfway between normals and poor metabolizers; and, (4) an ultra-rapid metabolic group (URM). The poor-metabolizer group has lower than average metabolic power. This means that drug clearance may be slower, resulting in more toxicity or, conversely, if metabolism is necessary for activation, then less therapeutic benefit, if any. Approximately 5% to 10% of whites and 1% to 2% of

Southeast Asians are poor metabolizers. On the other side, ultra-rapid metabolism is caused by gene duplication of three to 13 copies resulting in “superpower” metabolic efficacy. Whites of Northern European descent rarely have gene duplication mutations, whereas nearly 30% of Ethiopians have multiple copies of 2D6.¹

When the Nutcracker Is Defective or Doesn’t Work Normally

Phenocopying is when a drug (or food) affects 2D6 to make one phenotype (eg, normal) look like another (eg, a poor metabolizer). After defining the central role of endoxifen as the effector molecule of tamoxifen, it was observed that the concentrations of endoxifen varied depending on the basis of a patient’s CYP2D6 genotype and use of paroxetine.³ Recognizing the immense importance of any process or drugs that would, in effect, turn up or turn down the effect of tamoxifen, Jin et al undertook a formal study in 80 women with breast

cancer who had completed surgery, chemotherapy, and/or radiation, and who were ready to begin undergoing treatment with tamoxifen.³ At initiation of tamoxifen treatment, 17% of the 80 patients were receiving SSRIs, but by 4 months later, 29% were receiving SSRIs. Blood for analysis was drawn at baseline and at 4, 8, and 12 months.

Distribution of Genotypes

Of these 80 patients, approximately 90% were white and 60% had two normal alleles (homozygous wild type, *wt/wt*), one from each parent; they would be called extensive (normal) metabolizers. Approximately 35% had one normal allele and one variant (*vt*) allele (heterozygous *wt/vt*). Only three patients (approximately 4%) had no normal alleles (homozygous *vt/vt*) and this corresponds to expected population frequencies reported greater than 5% to 10%.

Table 1. Most of the biologic effect of tamoxifen is via endoxifen

Molecular Concentration	Plasma Effect	Biologic Activity	Effective Species
Tamoxifen	370	1	370
N-desmethyltamoxifen	650	1	650
Endoxifen	60	100	6,000
4-hydroxy-tamoxifen	9	100	900

NOTE. Plasma effect × biologic activity = effective species. Adapted from Jin et al.³

Figure 2. Major subclasses of cytochrome P450 (CYP) isoenzyme families. (A) Distribution of CYP isoenzymes based on number of known drugs they metabolize. (B) Distribution of CYPs in the liver. Adapted from Flockhart,⁴ in Shimada et al.⁹

Endoxifen Concentrations

Four findings were noted: (1) It took at least 4 months, not 1 month, to achieve steady state concentrations. Endoxifen, and the levels of the other two metabolites, were statistically

Figure 3. Selective serotonin reuptake inhibitors decrease plasma endoxifen concentrations. Paroxetine is the most potent inhibitor and causes reduction of endoxifen concentrations to levels expected from a homozygous variant (null) without any significant 2D6 activity. Adapted from Jin et al.³

significantly higher at 4 months. (2) CYP3A functionality did not affect endoxifen levels. (3) Endoxifen levels were statistically significantly different on the basis of genotype: Homozygous *wt/wt*, heterozygous *wt/vt*, and homozygous *vt/vt* (no functional 2D6) levels were, respectively, 78.0, 43.1, and 20.0 nM ($P < .001$) with nonoverlapping 95% CIs. Finally, (4) even within each genotype, there was a huge variation in levels.

2D6 Inhibitors (“damaged nutcrackers”)

The next obvious step was to determine whether patients were using any other medications that would inhibit 2D6, and to obtain the patients’ concomitant medication history. Of 78 patients who had a complete list of concomitant medications, 30% were receiving various SSRIs including paroxetine, fluoxetine, sertraline, citalopram, and venlafaxine. Conspicuously absent is escitalopram, although the package insert suggests caution regarding use of escitalopram and drugs metabolized by CYP2D6.¹⁰ Figure 3 shows the relative inhibition of CYP2D6 by each of the aforementioned drugs. Venlafaxine is the weakest inhibitor, with essentially no inhibition of 2D6. In contrast, in patients who are receiving both tamoxifen and paroxetine, paroxetine is such an effective inhibitor of 2D6 that endoxifen levels are not significantly different from those that would occur in patients who have two variant alleles (homozygous *vt/vt*)—effectively, no functional 2D6 isozyme.

Significance

Although no clinical outcomes data were provided for this study, the implications are obvious. Concomitant administration of SSRIs, especially paroxetine, effectively inhibits conversion of tamoxifen to the active metabolite. The patient may as well be taking a placebo.

Discussion

Tamoxifen: A Prodrug Inactive Until Converted to Endoxifen

Tamoxifen can, in some ways, be thought of as a prodrug that must be activated by the CYP2D6 isoenzyme to produce endoxifen, the therapeutic molecular effector. Poorly functioning or

Table 2. Effect of the “dud” genotype compared with at least one normal gene (n = 191)

Genotype	5-Year Relapse-Free Survival		5-Year Disease-Free Survival	
	%	95% CI	%	95% CI
wt/wt or wt/vt	84	78 to 89	83	78 to 89
vt/vt	56	33 to 95	46	26 to 83

Abbreviations: wt, wild type; vt, variant.

nonfunctional isoenzymes as a result of genetic mutations result in lower endoxifen levels and decreased or absent antiestrogenic activity. Moreover, concomitant medications that inhibit CYP2D6, such as the SSRI antidepressants can have the same effect as mutant genotypes, and the use of these inhibitors in a patient who is heterozygous for 2D6 may effectively result in a null phenotype—complete lack of conversion of tamoxifen into the active agent endoxifen.

The frightening prospect of basically administering a placebo was shown in a clever analysis by Goetz et al,¹¹ who looked at patients in the tamoxifen-only arm of the North Central Cancer Treatment Group adjuvant breast cancer trial 89-30-52, which compared tamoxifen alone with tamoxifen plus 1 year of fluoxymesterone. Paraffin tumor samples were available for DNA extraction in 224 of 257 patients who had a median follow up of 10.4 years (range, 5.2 to 13.1 years) and a 5-year disease-free survival of 79% (95% CI, 74% to 84%). CYP2D6 was amplified in 191 of these 224 patients. The normal, or wild-type (wt) enzyme is 2D6 (wt/wt); the nonfunctional or variant enzyme is the 2D6*4/*4 mutant (vt/vt), and the 2D6*4/wt is the heterozygote. Seven percent of patients had the “dud” genotype 2D6*4/*4 (vt/vt). Patients who had at least one normal gene (wt) had significantly better disease-free and relapse-free survival than the “dud” (vt/vt) genotype, as shown in Table 2.

Because only 7% of patients had the “dud” phenotype, so what? The problem is that if a patient with a normal or heterozygous pattern receives tamoxifen with a drug that inhibits 2D6, such as paroxetine, that patient effectively turns her normal metabolic machinery into a “dud” incapable of releasing endoxifen from its tamoxifen “shell.”

The onus is on oncologists not only to take a complete medication history at every visit, but also to educate our patients and consulting and referring physicians about potential drug interactions that may potentially nullify treatment with tamoxifen.

Drug Interactions

There are two other aspects of this issue that will become a part of our daily practice of not only oncology, but also medicine in general. The first is the broader issue of drug interactions that may be fatal. The dramatic presentation of

sudden death caused by torsade de pointe arrhythmia due to the interaction of terfenadine (Seldane, Hoechst Marion Roussel and Baker Norton Pharmaceuticals) with ketoconazole caused the U.S. Food and Drug Administration (FDA) to remove terfenadine from the U.S. market in 1998.^{12,13} Multiple withdrawals followed, most recently cisapride (Propulsid, Janssen-Ortho, Inc.; withdrawn from the U.S. market in 2000).

However, more frequently used drugs represent a bigger threat (e.g., erythromycin, which prolongs QT intervals, with CYP3A inhibitory drugs such as diltiazem or verapamil). And, sadly, the recognition that drinking only one 8-oz. glass of grapefruit juice will inhibit CYP3A for 24 to 48 hours.¹

Gene Expression Profiles to Predict Response or Resistance to Therapy

The second issue is specific to oncology and relates to development of genetic prediction tests—somewhat like a urine culture and sensitivity. One aspect of this is simply to know whether a patient has the metabolic machinery to activate specific drugs. Petros et al¹⁴ looked at this issue of drug-metabolism genotype and chemotherapy pharmacokinetics and correlated these with overall survival in breast cancer patients undergoing high-dose chemotherapy with stem-cell rescue using high-dose cyclophosphamide, cisplatin, and carmustine. Plasma levels of drugs and metabolites were measured. Cyclophosphamide is a prodrug and must be activated by CYP3A. Thus, patients who had higher levels of the parent, inactive cyclophosphamide were those patients who had a less active form of CYP3A (i.e., a polymorphic variant). Survival of patients with this variant CYP3A was shorter (1.3 years) than patients who had a normal gene (2.7 years). Similar findings were seen with the genes that metabolized cisplatin and carmustine. Recognizing the immense importance of knowing exactly how a patient will metabolize specific drugs, in December 2005, the FDA approved the AmpliChip CYP450 (Roche Diagnostics, Basel, Switzerland), which will define a patient's 2D6 and 2C19 isozymes. The AmpliChip CYP450 will allow customized drug dosing for drugs that are cleared by these two enzymes.¹⁵

The other aspect of this prediction model, which relates even more directly to outcome, is determination of the specific genes within a tumor that correlate with tumor response or resistance to specific therapies. Sorlie et al¹⁶ got the ball rolling in breast cancer when they grouped breast cancers into five major groups on the basis of similarity of the gene expression profiles. This is akin to taking the spoken word “to” and showing that the same sound can signify “toward” (to), “also” (too), or “a pair” (two)—same sound, but different meaning because the letters are different. With breast cancer, it's the same diagnosis but the genes are

Table 3. Drug interaction tools

Name	Location	Cost
Epocrates	www.epocrates.com	\$59.95/year
P450 Table ⁴	http://medicine.iupui.edu/flockhart/table.htm	Free
The Medical Letter	www.themedicalletter.com	Free

different in each of the major groups. Ayers et al¹⁷ pushed the ball even further when they looked at the gene expression profiles of patients being treated with preoperative chemotherapy for early-stage breast cancer and identified a pattern of 70 genes that correlated with and/or predicted a pathologic complete response to a specific chemotherapy regimen, paclitaxel plus fluorouracil, doxorubicin, and cyclophosphamide.

Chang et al¹⁸ performed gene expression profiling in 24 patients treated with neoadjuvant single-agent docetaxel and identified 92 genes that correctly identified 10 of 11 sensitive tumors and 11 of 13 resistant tumors. To further refine predictors of resistance to docetaxel, they subsequently evaluated the molecular patterns of the residual tumors after 3 months of docetaxel and found these patterns amazingly similar despite initial sensitivity or resistance. In fact, these genes identified a specific “escape route” (the mammalian target of rapamycin [mTOR]) for which inhibitors are currently under development.

My own group has nearly completed a community-based trial in collaboration with Ayers et al, evaluating gene expression profiles of patients before treatment with neoadjuvant chemotherapy with fluorouracil, epirubicin, cyclophosphamide followed by weekly docetaxel with capecitabine to identify the gene expression profile(s) associated with response and to correlate these with in vitro chemotherapy sensitivity testing.

The Bottom Line: Do Not Administer Any SSRI With Tamoxifen

The future is exciting with the possibility of developing truly customized, 100%-effective therapies for our patients. For the present, however, besides entering our patients onto well-designed clinical trials asking important questions, we must not encumber our currently available therapies with combinations of drugs that effectively make them a placebo. The bottom line is this: Do not administer any SSRIs (fluoxetine [Eli Lilly and Company, Indianapolis, IN], paroxetine [Paxil, GlaxoSmithKline, Pittsburgh, PA], sertraline [Zoloft, Pfizer, New York, NY], citalopam [Celexa, Forest Laboratories, Inc., New York, NY], or escitalopam [Lexapro, Forest Laboratories, Inc.]) concurrently with tamoxifen. When in doubt, check it out. Sources are listed in Table 3. However, because this interaction is so recently

documented, it is not yet included in many of the standard references. Be the first to tell your colleagues.

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