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Potential role of UGT pharmacogenetics in cancer treatment and prevention: focus on tamoxifen and aromatase inhibitors

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Abstract

Tamoxifen (TAM) is a selective estrogen-receptor modulator that is widely used in the prevention and treatment of estrogen-receptor–positive breast cancer. Its use has significantly contributed to a decline in breast cancer mortality, since breast cancer patients treated with TAM for 5 years exhibit a 30–50% reduction in both the rate of disease recurrence after 10 years of patient follow-up and in the occurrence of contralateral breast cancer. However, in patients treated with TAM, there is substantial interindividual variability in the development of resistance to TAM therapy and in the incidence of TAM-induced adverse events, including deep-vein thrombosis, hot flashes, and the development of endometrial cancer. Aromatase inhibitors (AIs) have emerged as a viable alternative to TAM, working by inhibiting aromatase activity and blocking estrone/estrodiol biosynthesis in postmenopausal women. The current third-generation AIs, anastrozole, exemestane, and letrozole, were used initially for the treatment of metastatic breast cancer, demonstrating similar or greater benefit but less toxicity, compared with TAM, and are now being employed as adjuvant treatment for early breast cancer in postmenopausal women. This article will focus on the UDP-glucuronosyltransferases, a family of metabolizing enzymes that play an important role in the deactivation and clearance of TAM, anastrazole, and exemestane, and how interindividual differences in these enzymes may play a role in patient response to these agents.

Keywords

Breast cancer; tamoxifen; aromatase inhibitors; exemestane; anastrazole; UDP-glucuronosyltransferase; glucuronidation; metabolism; pharmacogenetics

Introduction: tamoxifen

Adjuvant endocrine therapy reduces the risk of recurrence and improves survival among women with hormone-receptor–positive breast cancer (EBCTCG, 2005). Because most breast cancers, especially those among postmenopausal women, are hormone-receptor positive, hundreds of thousands of women worldwide initiate adjuvant endocrine treatment each year.

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Tamoxifen (TAM) is a nonsteroidal antiestrogen that has been commonly used for the treatment and prevention of estrogen-dependent breast cancer (Cuzick et al., 2003; Fisher et al., 1998; Howell et al., 2003; Osborne, 1998). First approved in 1977 by the U.S. Food and Drug Administration (FDA) for the treatment of women with metastatic breast cancer, TAM is currently an established hormonal treatment for all stages of estrogen-receptor (ER)-positive breast cancer. Adjuvant TAM treatment increases recurrence-free survival and overall survival in breast cancer patients with hormone-receptor–positive tumors irrespective of their nodal status, menopausal status, or age (Howell et al., 2003; Osborne, 1998). TAM is also widely used as a chemopreventive agent in women at risk for developing breast cancer (Cuzick et al., 2003; Fisher et al., 1998).

In addition to its antiestrogenic properties, which have been related to menopausal-like symptoms including hot flashes and vaginal bleeding (Osborne, 1998), TAM also exhibits seemingly tissue-dependent partial estrogen-agonistic effects that may be linked to reduced risk for ischemic heart disease and osteoporosis (McDonald and Stewart, 1991; Rutqvist and Mattsson, 1993), but may also increase the risk for endometrial cancer (Rutqvist et al., 1995; van Leeuwen et al., 1994) and venous thromboembolism (Meier and Jick, 1998). Although TAM is generally well tolerated, significant interindividual variability has been observed in the clinical efficacy as well as toxicity, of TAM. For instance, about 30% of patients acquire TAM resistance and relapse (EBCTCG, 1998). In addition, the relative risk of endometrial cancers in patients treated with TAM is estimated to be 2- to 3-fold that of controls, and the risk increases with both the duration and cumulative dose of TAM treatment (Bergman et al., 2000; Bernstein et al., 1999; Fisher et al., 1994; Rutqvist and Mattsson, 1993).

TAM acts by binding to the ER, thereby competitively inhibiting the binding of estrogen in breast tissue (Furr and Jordan, 1984). While TAM exists in both a *trans* and a *cis* configuration, the *trans*-isomer of TAM is the pharmaceutically manufactured form of TAM used in the treatment and prevention of breast cancer. TAM is metabolized via cytochrome P450 (CYP450)-mediated pathways into several metabolites after oral administration, including the metabolites, *N*-desmethylTAM (DMT), 4-hydroxyTAM (4-OH-TAM), α-hydroxyTAM (α-OH-TAM), TAM-*N*-oxide, *N*, *N*-didesmethylTAM (DDMT), and 4-OH-*N*-desmethylTAM (endoxifen; see Figure 1). CYP3A4 has been shown to be the main CYP450 enzyme involved in the metabolism of TAM to α-OH-TAM (Boocock et al., 2002; Kim et al., 2003) and to DMT (Desta et al., 2004; Jacolot et al., 1991), although CYPs 2D6, 1A1, 1A2, 1B1, 2C9, and 2C19 may also play a role in DMT formation (Crewe et al., 2002; Jacolot et al., 1991). CYP2D6 appears to be the major CYP involved in the hydroxylation of *trans*-TAM to *trans*-4-OH-TAM (Coller, 2003; Coller et al., 2002; Crewe et al., 1997, 2002; Dehal and Kupfer, 1997; Desta et al., 2004; Hu et al., 2003) and DMT to endoxifen (Desta et al., 2004). Endoxifen formation was observed to be highest in activity assays of recombinant CYP2D6 enzyme incubated with *trans*-4-OH-TAM, although CYP3A4 activity also correlates well with endoxifen formation in human liver microsomes (HLMs) (Desta et al., 2004).

DMT has been established as the primary metabolite of TAM, as determined by *in vitro* assays performed with HLM (Desta et al., 2004) and by *in vivo* studies that have shown levels of steady-state plasma DMT to be greater than 70 times the levels of 4-OH-TAM in the serum (Lonning et al., 1992; Stearns et al., 2003). Steady-state plasma levels of endoxifen are ~6-fold the levels observed for 4-OH-TAM in TAM-treated subjects (Desta et al., 2004; Jin et al., 2005; Stearns et al., 2003). However, the major therapeutic contributors are hypothesized to be endoxifen and 4-OH-TAM. This is based on evidence that indicates that they exhibit up to 100-fold the levels of antiestrogenic activity as compared to TAM and other TAM metabolites (Furr and Jordan, 1984; Johnson et al., 2004; Jordan et al., 1977; Katzenellenbogen et al., 1984;L. Lim et al., 2004;Y. C. Lim et al., 2005; Stearns et al.,

2003), exhibit the same relative levels of antiestrogenic activity (Johnson et al., 2004; Stearns et al., 2003), inhibit expression of β-estradiol-induced ER-dependent target genes (Johnson et al., 2004;Y. C. Lim et al., 2005), inhibit global estrogen-dependent gene expression (L. Lim et al., 2004), and exhibit high affinity for the ER (α and β) in RBA assays (Johnson et al., 2004).

Non-CYP450-mediated conjugation pathways also appear to be highly important in terms of TAM's overall metabolism and activity profile. While the hydroxysteroid sulfotransferase, SULT1A2, is involved in sulfation of α-OH-TAM (Apak and Duffel, 2004), this enzyme does not exhibit activity against either the *trans*- or *cis*-isomers of 4-OH-TAM (Chen et al., 2002; Nishiyama et al., 2002). The phenol sulfotransferase, SULT1A1, appears to be the major sulfotransferase involved in the conjugation of both *trans*- and *cis*-4-OH-TAM in humans (Falany, 1997; Nishiyama et al., 2002).

Perhaps the most important route of elimination of TAM and its metabolites is via glucuronidation by the uridine diphosphate (UDP)-glucuronosyltranferases (UGTs). TAM is excreted predominantly through the bile, a process largely facilitated by TAM conjugation to glucuronic acid during the glucuronidation process (Lien et al., 1989), and TAM glucuronides have been identified in the urine and serum of TAM-treated patients (Lien et al., 1988, 1989; Poon et al., 1993). Most of the 4-OH-TAM and endoxifen found in the bile of TAM-treated patients was as a glucuronide conjugate (Lien et al., 1988, 1989) and TAM, 4-OH-TAM, and endoxifen are glucuronidated with very high activity by HLM (Sun et al., 2007). Although TAM metabolites are often found in their unconjugated form in feces, this is likely due to β-glucuronidase–catalyzed removal of glucuronic acid within the microflora of the small intestine (Lien et al., 1989).

Large interindividual variability in endoxifen plasma concentrations in women taking TAM have been observed and can be explained, in part, by CYP2D6 genotype (Jin et al., 2005; Stearns et al., 2003). Recent evidence demonstrates that the CYP2D6*4 deletion allele has been associated with decreased time until breast cancer recurrence, relapse-free survival, disease-free survival, and overall survival in patients treated with TAM (Borges et al., 2006; Goetz et al., 2005, 2007, 2008). In addition, variant alleles that result in low activity/expression in CYPs 2D6, 2B6, and 2C9 were correlated with levels of *trans*-4-OH-TAM formation in HLM from individual subjects (Coller et al., 2002). These data suggest that the levels of circulating active TAM metabolites may differ between individuals, based upon metabolizing enzyme genotype.

TAM glucuronidation

Microsomes from human liver specimens exhibit high glucuronidating activities toward TAM to form TAM-N^+-glucuronide, and 4-OH-TAM to form 4-OH-TAM-N^+-glucuronide and 4-OH-TAM-*O*-glucuronide (Kaku et al., 2004; Nishiyama et al., 2002; Sun et al., 2006). Both isomers of endoxifen are *O*-glucuronidated; however, unlike 4-OH-TAM, no *N*-glucuronidation of endoxifen isomers was detected in assays for either HLM or individually overexpressed UGTs (Sun et al., 2007), suggesting that the demethylation of the electrophilic amine on the 4-OH-TAM side chain to form endoxifen results in a lack of *N*-glucuronidation by UGTs.

One of the major UGTs involved in the glucuronidation of TAM and its metabolites is the hepatic enzyme, UGT1A4 (Kaku et al., 2004; Nishiyama et al., 2002; Sun et al., 2006), which catalyzes the formation of a quarternary ammonium-linked glucuronide with TAM's and 4-OH-TAM's *N*, *N*-dimethylaminoalkyl side chain (Kaku et al., 2004; Sun et al., 2006). This pattern of ammonium-linked glucuronidation is consistent with UGT1A4's glucuronidation activity against primary, secondary, and tertiary amines present in a variety

of carcinogenic compounds, androgens, progestins, and plant steroids (Breyer-Pfaff et al., 2000; Green and Tephly, 1996, 1998; Wiener et al., 2004a). In addition to UGT1A4, UGTs 1A1, 1A3, 1A8, 1A9, 2B7, and 2B15 overexpressing baculosomes exhibited detectable activity against 4-OH-TAM (Kaku et al., 2004). In a comprehensive characterization and kinetic analysis of the glucuronidating enzymes responsible for *O*-glucuronidation of TAM metabolites (Sun et al., 2007), UGTs 2B7≃1A8>1A10 exhibited the highest overall activity against *trans*-4-OH-TAM as determined by V_{max}/K_m, with the hepatic enzyme, UGT2B7, exhibiting the highest binding affinity and lowest K_m (3.7 μM; Table 1). UGTs 1A10≃1A8>UGT2B7 exhibited the highest overall glucuronidating activities as determined by V_{max}/K_m for *trans*-endoxifen, with the extrahepatic enzyme, UGT1A10, exhibiting the highest binding affinity and lowest K_m (39.9 μM), but with UGT2B7 again demonstrating the highest activity of the hepatic UGTs. These data suggest that several UGTs, including UGTs 1A10, 2B7, and 1A8, could play an important role in the metabolism of 4-OH-TAM and endoxifen. Interestingly, while UGT1A4 is active against TAM and 4-OH-TAM, no activity was observed for UGT1A4-overexpressing cell homogenates against endoxifen isomers, a pattern that is consistent with the lack of *N*-glucuronidation observed for endoxifen in HLM (Sun et al., 2007). Also, this pattern of UGT1A4 substrate selectivity is consistent with UGT1A4 activity toward the tertiary amine, imipramine, but not its *N*-desmethyl metabolite, desipramine (Green and Tephly, 1998).

While high antiestrogenic activity has been reported for both 4-OH-TAM and endoxifen, studies examining the effects of glucuronide conjugation of these metabolites were only recently described (Y. Zheng et al., 2007). E_2-mediated induction of the gene encoding the progesterone receptor (PGR) was determined in MCF-7 cells by real-time reverse-transcriptase polymerase chain reaction (RT-PCR) for individual TAM metabolites and isomers. While E_2 (1×10^{-10} M) induction of PGR mRNA was 6-fold after a 12-hour incubation, unconjugated TAM metabolites (i.e., *cis*- and *trans*-isomers of 4-OH-TAM and endoxifen) inhibited this effect. A similar dose-dependent inhibition of E_2-induced PGR gene expression was found for both the *trans*- and *cis*-isomers of 4-OH-TAM and endoxifen, with maximal inhibition attained at 1×10^{-6} M of TAM metabolite. In contrast, the glucuronide conjugates of all 4-OH-TAM and endoxifen isomers exhibited no effect on E_2-mediated induction of PGR expression at all concentrations of TAM metabolite conjugates examined in this study. These data indicate that isomers of both 4-OH-TAM and endoxifen exhibit roughly equipotent antiestrogenic effects on E_2-induced gene expression, and that glucuronide conjugates of the same metabolites effectively negate this activity.

More recent studies have focused on ER-binding activities of TAM metabolites (Lazarus et al., 2009). Similar to that observed for PGR induction, the *trans*-isomers of both 4-OH-TAM and endoxifen exhibit similar relative binding activities (RBA), as compared to E_2, while their glucuronide counterparts exhibited 57–130-fold decreases in RBA as compared to their unconjugated counterparts. While a similar pattern was also observed for *cis* TAM metabolite isomers and their glucuronides, the RBA of these unconjugated *cis*-isomers were 35–67-fold lower than their *trans*-unconjugated counterparts. These data were similar to that observed previously for 4-OH-TAM and endoxifen (Johnson et al., 2004), but was the first assessment of individual isomers and glucuronide conjugates. The *trans*-4-OH-TAM and *trans*-endoxifen isomers exhibited 30–37-fold higher RBA than *trans*-TAM in this study. Overall, these differences suggest that the *trans*-isomers of 4-OH-TAM and endoxifen are the major active antiestrogenic metabolites of TAM, that glucuronides of TAM metabolites are relatively inactive, and that *cis*-isomers may be inhibiting E_2-induced activities by mechanisms other than competitive binding to the ER. This could have important implications in how metabolic pathways are targeted in terms of augmenting the therapeutic efficacy of TAM.

Effect of UGT polymorphisms on TAM glucuronidation activities in UGT-overexpressing cell lines

Missense polymorphisms have been identified in the UGTs active against TAM metabolites, including nonsynonymous SNPs at codons 24 and 48 of the UGT1A4 gene (Wiener et al., 2004b), at codon 268 of the UGT2B7 gene (Coffman et al., 1998), at codon 139 SNP in the UGT1A10 gene that is prevalent in African Americans (Blevins-Primeau et al., 2009; Elahi et al., 2003), and at codons 173 and 277 of the UGT1A8 gene (Huang et al., 2002). To determine whether any of these SNPs result in differential activities against the *trans*-isomers of 4-OH-TAM or endoxifen, *in vitro* kinetic analysis of HEK293 cells overexpressing the wild-type or variant isoforms of each of these four UGT enzymes was performed (Blevins-Primeau et al., 2009; Sun et al., 2006). The UGT1A8$^{173Gly/277Cys}$ variant exhibited no difference in overall glucuronidation activity (V_{max}/K_m) against *trans*-4-OH-TAM and exhibited a small (1.25-fold), but significant ($P < 0.05$), decrease in overall activity (manifested primarily by a higher K_m) against *trans*-endoxifen, as compared to wild-type UGT1A8$^{173Ala/277Cys}$ (Table 2). In contrast, the UGT1A8$^{173Ala/277Tyr}$ variant exhibited no detectable glucuronidation against the *trans*-isomers of either 4-OH-TAM or endoxifen.

For UGT1A4, kinetic analysis demonstrated that higher *N*-glucuronidation activities were observed for UGT1A4$^{24Pro/48Val}$-overexpressing cell microsomes as compared to microsomes from wild-type UGT1A4$^{24Pro/48Leu}$-overexpressing cells against *trans*-4-OH-TAM, with a significantly ($P \leq 0.02$) lower K_m observed for *trans*-4-OH-TAM for the UGT1A4$^{24Pro/48Val}$ variant (Table 2). No significant effect on enzyme kinetics was observed for the UGT1A4$^{24Thr/48Leu}$ variant against *trans*-4-OH-TAM. In addition, no difference in overall glucuronidation activity was observed for the UGT1A10^{139Lys} variant versus wild-type UGT1A10 against the *trans*-isomers of both 4-OH-TAM and endoxifen (Table 2).

For UGT2B7, kinetic analysis demonstrated that significantly higher glucuronidation activities were observed for the wild-type UGT2B7^{268His}, as compared to the UGT2B7^{268Tyr} variant, against the *trans*-isomers of both 4-OH-TAM ($P < 0.05$) and endoxifen ($P < 0.01$; Table 2). This was manifested by a higher K_m (2.4-fold) and a lower V_{max}/K_m (2.4-fold) for 4-OH-TAM, as well as a lower V_{max} (5.5-fold) and lower V_{max}/K_m (5.0-fold) for endoxifen by the UGT2B7^{268Tyr} variant.

UGT genotypes and TAM glucuronidation phenotype in HLM

To determine the rate of *O*- and *N*-glucuronidation of *trans*-4-OH-TAM and *trans*-endoxifen, glucuronidation assays were performed for a series of HLM and analyzed by ultrapressure liquid chromatography (Blevins-Primeau et al., 2009). The mean rate of formation of TAM-4-*O*-glucuronide, 4-OH-TAM-N^+-glucuronide, and endoxifen-*O*-glucuronide in 111 HLM specimens was 141 ± 45, 175 ± 52, and 168 ± 66 pmol·min^{-1}·mg^{-1}, respectively. A 4.5-, 10- and 17-fold range in glucuronide formation was observed for TAM-4-*O*-glucuronide, 4-OH-TAM-N^+-glucuronide, and endoxifen-*O*-glucuronide, respectively. The range of the ratio of TAM-4-*O*-glucuronide:4-OH-TAM-N^+-glucuronide in the HLM samples was 8.0-fold. These data suggest that significant differences in glucuronidation capacity exist between individual HLM against TAM metabolites. After stratifying by UGT2B7 codon 268 genotype, there was a near-significant ($P = 0.059$) 13% decrease in TAM-4-*O*-glucuronide formation in HLM with the UGT2B7 (His268Tyr) genotype and a significant ($P < 0.001$) 28% decrease in TAM-4-*O*-glucuronide formation in HLM with the UGT2B7 (Tyr268Tyr) genotype, as compared to HLM with the UGT2B7 (His269His) genotype (Figure 2, panel A). A significant ($P = 0.01$) 17% decrease in TAM-4-*O*-glucuronide formation was observed in HLM with the UGT2B7 His268Tyr

genotype versus HLM with the UGT2B7 (Tyr268Tyr) genotype. A significant trend of decreasing *O*-glucuronidation of *trans*-4-OH-TAM was observed in HLM with increasing numbers of the UGT2B7^{268Tyr} allele ($P < 0.001$).

Similar to that observed for *trans*-4-OH-TAM, a significant ($P = 0.002$) 27% decrease in *O*-glucuronidation of *trans*-endoxifen was observed in HLM with the UGT2B7 (Tyr268Tyr) genotype, as compared to HLM with the UGT2B7 (His268His) genotype (Figure 2, panel B). A significant trend of decreasing *O*-glucuronidation of *trans*-endoxifen was observed in HLM with increasing numbers of the UGT2B7^{268Tyr} allele ($P = 0.009$). No *N*-glucuronidation of endoxifen was observed for any of the HLM specimens analyzed in these studies.

Aromatase inhibitors (AIs)

Aromatase, a product of the CYP19 gene, is a CYP450 enzyme complex that catalyzes the last step in several reactions for estrogen biosynthesis. Aromatase is found in many human tissues, including the ovaries (Means et al., 1991), testes (Tsai-Morris et al., 1985), adipose tissue (Bulun and Simpson, 1994), placenta (Means et al., 1991), brain (Roselli et al., 1985), muscle (Matsumine et al., 1986), skin fibroblasts (Berkovitz et al., 1987), and osteoblasts of bone (Shozu and Simpson, 1998) and facilitates the conversion of androstenedione and testosterone via three hydroxylation steps to estrone and estradiol. This conversion increases as a function of age, obesity, and aromatase activity in adipose tissue and is the major source of estrogen in postmenopausal women (Nelson and Bulun, 2001). In the past decade, a number of AIs have been developed as an alternate approach to TAM for the treatment of estrogen-receptor–positive breast cancer. The current third-generation of AIs, anastrozole, exemestane, and letrozole, are highly specific for the aromatase enzyme, with fewer side effects than previous generations of AIs and are sufficiently long acting to be administered on a daily basis. Evidence from several clinical trials indicates that AIs may be superior to TAM as first-line therapy for postmenopausal women with metastatic breast cancer (Ferretti et al., 2006). Results from at least eight major clinical trials indicate that AIs are associated with longer disease-free survival than therapy with TAM alone (Eisen et al., 2008), and this supports the use of AIs as first-line therapy or as second-line therapy after treatment with TAM. The fact that the incidence of contralateral breast cancer was significantly reduced in most of these trials is also supportive of a potential role for AIs in cancer prevention.

Serious adverse events were shown in the ATAC trial to occur more frequently with TAM than with anastrozole (Buzdar et al., 2006; Forbes et al., 2008). However, TAM was associated with reduced cholesterol, hypercholesterolemia, and other lipid metabolism disorders, as compared to AIs (Boccardo et al., 2005; Thurlimann et al., 2005). In addition, patients receiving exemestane had increased LDL levels, but lower or unchanged triglycerides, while those receiving TAM had decreased LDL levels, but higher triglycerides (Hozumi et al., 2006; Markopoulos et al., 2005). Perhaps the major toxicities associated with AIs are joint pain and bone loss, the propensity for bone fractures, and increased risk for osteoporosis (Coates et al., 2007; Coleman et al., 2007; Coombes et al., 2007; Howell et al., 2005; Howell and Group, 2006), a pattern consistent with the significantly lower hip and lumbar spine bone mineral density observed in patients on AIs (Perez et al., 2006). While not as severe as TAM, other toxicities reported for AIs include a variety of gynecological events, including vaginal bleeding or discharge and hot flashes. Variability in changes to lipid profiles, manifestation of osteoporosis, and time of recurrence were observed in patients in many of the different clinical trials (Eisen et al., 2008), and the mechanism underlying this variability in response to AIs and to their toxicities remains unclear.

AI metabolism

Limited studies have been performed examining AI metabolism. Anastrozole is eliminated mainly through N-dealkylation, hydroxylation via the CYP3A4 enzyme (Antoniou and Tseng, 2005; Dowsett et al., 2001), and glucuronidation via the UGT family of enzymes (Mareck et al., 2006). The major metabolites of anastrozole identified in urine and plasma are triazole, OH-anastrozole, the glucuronide of OH-anastrozole, and the *N*-glucuronide of anastrozole (Figure 3, panel A) (AstraZeneca, 2006). While triazole is the major circulating metabolite of anastrozole in serum of anastrozole-treated patients (AstraZeneca, 2006), 40% of excreted anastrozole is as a glucuronide. Triazole lacks pharmacologic activity (AstraZeneca, 2006); while little is known about the pharmacologic activity of OH-anastrozole, it is likely that the removal of the triazole moiety results in an inactivation of the hydroxyanastrozole derivative. Anastrozole can be directly glucuronidated to form anastrozole-N^+-glucuronide in HLM (Lazarus and Sun, unpublished results). In a recent screening of cell lines individually overexpressing each of the known human UGT1A and 2B enzymes (except UGTs 1A5 and 2B28), only UGT1A4 exhibited glucuronidating activity against anastrozole, with an apparent K_m of 637 ± 40 μM and a V_{max}/K_m of 5.6 ± 0.6 nl·min^{-1}·μg^{-1} (Lazarus and Sun, unpublished results).

For exemestane, studies of human liver preparations suggest that cytochrome CYP3A4 is the principal enzyme involved in exemestane oxidation (Pfizer, 2007; Anonymous, 2000), but reduction of the 17-keto group to form 17-dihydroexemestane is the major pathway for exemestane metabolism (Figure 3, panel B) (Pfizer, 2007). Unchanged exemestane is less than 1% in urine and less than 10% in plasma of the total exemestane dose (Pfizer, 2007). Urinary and fecal excretion of exemestane was similar, both around 42%, with the glucuronide of 17-dihydroexemestane (at the 17-*O*-position) a major metabolite found in urine (Pfizer, 2007). The glucuronidation pathway of excretion of exemestane is important, because 17-di-hydroexemestane was reported to exhibit significant antiaromatase activity *in vitro* (Buzzetti et al., 1993). Recent studies have shown that several UGTs exhibit activity against 17-dihydroexemestane (Table 3), with UGT2B17>UGT1A10>UGT1A8≃UGT1A4 (Lazarus and Sun, submitted). UGT2B17 exhibited the highest binding affinity against 17-dihydroexemestane, as reflected by the lowest K_m (14.5 ± 2.7 μM), which is 8.5-fold lower than that of UGT1A10, and the highest overall activity, as reflected by the highest V_{max}/K_m (137 nl·min^{-1}·mg^{-1}), which is 14-fold higher than that of UGT1A8.

CYP3A4 was shown to metabolize letrozole to the carbinol metabolite (2° alcohol metabolite), while CYP2A6 formed this metabolite and its ketone analog (FDA, 2004). However, while glucuronidation of the alcohol metabolite was found to be the predominant species in urine (Sioufi et al., 1997), this conjugation is of inactive metabolites.

Effect of UGT polymorphisms on anastrozole and exemestane glucuronidation activities

Recent kinetic studies demonstrate that while UGT1A4$^{24Pro/48Val}$-overexpressing homogenates exhibited a significantly ($P < 0.01$) higher K_m (~1.6-fold), against anastrozole as compared with the wild-type UGT1A4$^{24Pro/48Leu}$ isoform, no differences in overall glucuronidation activity (V_{max}/K_m) was observed (Table 4; Lazarus and Sun, unpublished results). Similarly, no difference in anastrozole glucuronidation kinetics was observed for the UGT1A4$^{24Thr/48Leu}$ variant, as compared to wild-type UGT1A4. This suggests that genetic variations in the hepatic UGT1A4 may not be playing a large role in variability in response to anastrozole.

Previous studies identified a prevalent polymorphic whole-gene deletion for the UGT2B17 gene (Wilson et al., 2004). Similar to that described above for TAM glucuronidation and UGT2B7 genotype, a potential association between the UGT2B17 deletion and liver 17-dihydroexemestane glucuronidation activity was assessed in a panel of 110 HLMs. As shown in Figure 4 (panel A), there was a significant ($P < 0.001$) 14-fold decrease in glucuronidation activity against 17-dihy-droexemestane in HLM from subjects exhibiting the homozygous UGT2B17 deletion (*2/*2) genotype, as compared with HLM from subjects wild type (*1/*1) for UGT2B17 (Lazarus and Sun, submitted). A significant ($R^2 = 0.72$) correlation was observed between hydroexemestane-17-*O*-glucuronide formation in HLM and UGT2B17 expression in the same HLM (Figure 4, panel B). In addition, the average K_m for two HLMs against 17-dihydroexemestane from subjects wild type for UGT2B17 was 9 μM (Table 5), which is similar to the apparent K_m for UGT2B17 against 17--dihydroexemestane *in vitro* (14.5 μM; see Table 3). HLM from subjects exhibiting the UGT2B17 (*2/*2) genotype exhibited an average 2.3-fold higher K_m and an average 12-fold lower V_{max}/K_M than HLM from subjects with the UGT2B17 */*1) genotype. Together, these data suggest that the UGT2B17 deletion may significantly alter *in vivo* glucuronidation of a major active exemestane metabolite.

UGTs and TAM/AI pharmacogenetics: focus on the hepatic UGTs

Glucuronidation plays a major role in the metabolism of TAM, anastrozole, and exemestane, with specific UGT enzymes performing either *N*- or *O*-glucuronidation of the active TAM metabolite, anastrozole, and the active exemestane metabolite, 17-dihydroexemestane. UGT2B7 appears to be the most active hepatic UGT against TAM. UGT2B7 expression has been detected in a variety of tissues, including liver, the gastrointestinal tract, and breast (Blevins-Primeau et al., 2009; Nakamura et al., 2008; Ren et al., 2000; Strassburg et al., 1999; Turgeon et al., 2001;Z. Zheng et al., 2002). Therefore, variations in UGT2B7 function or expression could potentially significantly impact individual response to drugs or chemotherapeutic agents. The *O*-glucuronidation of both *trans*-4-OH-TAM and *trans*-endoxifen in HLM was significantly associated with UGT2B7 genotype, with lower activities correlated with increasing numbers of the $UGT2B7^{268Tyr}$ allele. These data are consistent with the observation that HEK293 cells that overexpressed the $UGT2B7^{268Tyr}$ variant exhibited lower activity *in vitro* against both TAM metabolites, as compared to cells overexpressing wild-type $UGT2B7^{268His}$. These results are also consistent with a functional role for this polymorphism against other substrates, including tobacco carcinogen metabolites, such as 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) (Wiener et al., 2004b).

Similarly, UGT2B17 is hepatically expressed (Turgeon et al., 2001) and is the primary UGT involved in the glucuronidation of the major active exemestane metabolite, 17-dihydroexemestane. Homozygous deletion of the UGT2B17 gene is associated with significantly reduced levels of hydroexemestane-17-*O*-glucuronide formation in HLM. Since both the UGT2B7 codon 268 SNP and the UGT2B17 deletion are highly prevalent in the population (~0.50 and 0.30 prevalence, respectively) in Caucasians (Gallagher et al., 2007; McCarroll et al., 2006; Murata et al., 2003; Wiener et al., 2004b; Wilson et al., 2004), both polymorphisms could potentially affect overall response to these agents in a large segment of the population.

Conclusions

Additional studies examining the effect of UGT genotypes on breast microsomal glucuronidation activity against TAM and exemestane metabolites, plasma levels of TAM or

exemestane metabolites, and overall patient response will be required to further examine the role of UGT polymorphisms on the therapeutic efficacy of these agents.

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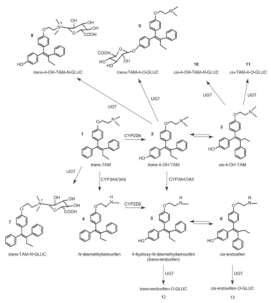

Figure 1.
Schematic of TAM metabolism.

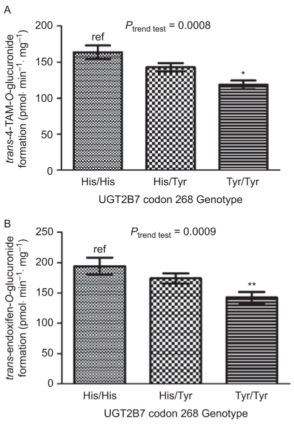

Figure 2.

Analysis of glucuronidation activities against *trans*-4-OH-TAM and *trans*-endoxifen in HLM stratified by UGT2B7 genotypes. Glucuronidation assays were performed and 4-OH-TAM and endoxifen-glucuronides separated by ultrapressure liquid chromatography. (A) *trans*-4-OH-TAM and UGT2B7 codon 268 genotypes. (B) *trans*-endoxifen and UGT2B7 codon 268 genotypes. Comparative analysis was performed by using HLM from subjects with the homozygous wild-type UGT2B7^{268His} genotype as the referent. *$P < 0.001$; **$P < 0.002$; error bars represent standard error.

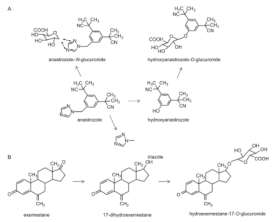

Figure 3.
Schematic of (A) anastrozole and (B) exemestane metabolism. Dashed lines between anastrozole-*N*-glucuronide and anastrozole reflect the potential binding of the glucuronide moiety to either amine group on the triazole ring.

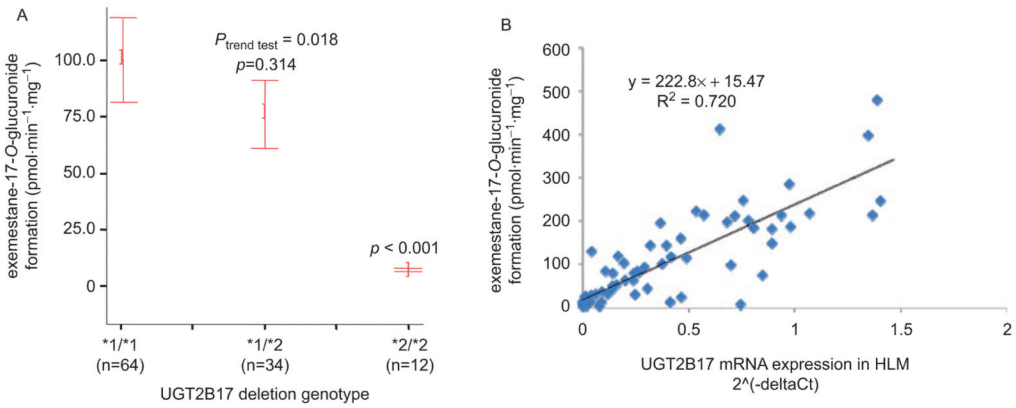

Figure 4.

HLM 17-dihydroexemestane glucuronidation activity and UGT2B17 expression stratified by UGT2B17 genotypes. (A) Exemestane-17-*O*-glucuronide formation versus UGT2B17 genotype in HLM. Glucuronidation assays were performed by using 9.4 μM 17-dihydroexemestane, and hydroexemestane-17-*O*-glucuronide was separated from parent 17-dihydroexemestane by ultraperformance liquid chromatography. The *1 and *2 alleles refer to the UGT2B17 wild-type and deleted alleles, respectively. Actual hydroexemestane-17-O-glucuronide rates are 100.2, 76.1, and 7.0 pmol·min^{-1}·mg^{-1}, respectively, for HLM from subjects exhibiting the UGT2B17 (*1/*1), UGT2B17 (*1/*2), and UGT2B17 (*2/*2) genotypes, respectively. Comparative analysis was performed by using HLM from subjects with the wild-type UGT2B17 (*1/*1) genotype as the referent; error bars represent standard error. (B) UGT2B17 expression versus UGT2B17 genotype in human liver. UGT2B17 expression was determined relative to PPIA as the “housekeeping” gene by real-time polymerase chain reaction by using total RNA from the same livers for whom HLMs were prepared. Comparative analysis was performed by using the UGT2B17 (*1/*1) genotype group as the referent, with the *P*-value shown for the UGT2B17 (*2/*2) genotype group; error bars represent standard error.

Table 1

Kinetic analyses of *O*-glucuronidation of *trans*-4-OH-TAM or *trans*-endoxifen by UGTs.[a]

	***trans*-4-OH-TAM**			***trans*-endoxifen**		
UGT variant	V_{max}**(pmol·min^{-1}·μg^{-1})**[b]	K_m**(μM)**	V_{max}/K_m**(μL·min^{-1}·μg^{-1})**[b]	V_{max}**(pmol·min^{-1}·μg^{-1})**[b]	K_m**(μM)**	V_{max}/Km**(μL·min^{-1}·μg^{-1})**[b]
1A1	3.4 ± 0.2	124 ± 16	0.028 ± 0.004	2.3 ± 0.3	333 ± 60	0.007 ± 0.0005
1A3	1.9 ± 0.3	94 ± 18	0.02 ± 0.001	2.9 ± 0.4	158 ± 29	0.02 ± 0.001
1A7	1.2 ± 0.2	166 ± 27	0.0074 ± 0.0002		Low activity[c]	
1A8	3.2 ± 0.2	23 ± 2.4	0.14 ± 0.02	12 ± 1.4	101 ± 13	0.12 ± 0.01
1A9	3.0 ± 0.1	319 ± 38	0.009 ± 0.001		Low activity[c]	
1A10	4.7 ± 0.3	96 ± 8.0	0.049 ± 0.006	5.7 ± 0.7	40 ± 3.0	0.14 ± 0.005
2B7	0.55 ± 0.18	3.7 ± 0.6	0.15 ± 0.03	3.0 ± 0.4	101 ± 17	0.03 ± 0.004
2B17	0.02 ± 0.001	41 ± 6	0.001 ± 0.0001		No detectable activity[d]	

[a] All data are the mean ± standard deviation, based on three independent experiments.

[b] Data are expressed per μg UGT protein, as determined by Western blot analysis.

[c] Low activity describes the fact that although some glucuronidation activity was observed for a UGT enzyme against a particular TAM metabolite, the level of detection was below sensitivity for kinetic studies.

[d] In addition to no detectable glucuronidation activity observed for homogenates of UGT2B17-overexpressing cells observed against *trans*-endoxifen, homogenates from cells overexpressing UGTs 1A6, 2B4, 2B10, 2B11, or 2B15 exhibited no detectable glucuronidating activity against *trans*-4-OH-TAM or *trans*-endoxifen.

Table 2

Kinetic analyses of *O*-glucuronidation of the *trans*-isomers of 4-OH-TAM and endoxifen by UGT variants.[a]

	***trans*-4-OH-TAM**			***trans*-endoxifen**		
UGT variant	V_{max}**(pmol·min^{-1}·μg^{-1})**[b]	K_m**(μM)**	V_{max}/K_m**(μL·min^{-1}·μg^{-1})**[b]	V_{max}**(pmol·min^{-1}·μg^{-1})**[b]	K_m**(μM)**	V_{max}/K_m**(μL·min^{-1}·μg^{-1})**[b]
UGT1A8$^{173Ala/277Cys}$	2.3 ± 0.1	23 ± 2	0.10 ± 0.02	5.4 ± 0.2	98 ± 9	0.06 ± 0.004
UGT1A8$^{173Gly/277Cys}$	5.4 ± 0.2**	43 ± 7**	0.13 ± 0.03	5.9 ± 0.4	135 ± 26	0.04 ± 0.005*
UGT1A8$^{173Ala/277Tyr}$	No detectable activity			No detectable activity		
UGT1A4$^{24Pro/48Leu}$	62 ± 5.8	2.2 ± 0.4	29 ± 2.7	No detectable activity		
UGT1A4^{24} r/48Leu	55 ± 11	1.6 ± 0.1	33 ± 4.9	No detectable activity		
UGT1A4$^{24Pro/48Val}$	49 ± 2.8	1.2 ± 0.1[b]	41 ± 1.4[c]	No detectable activity		
UGT1A10^{139Glu}	4.7 ± 0.3	96 ± 8	0.05 ± 0.006	5.7 ± 0.7	40 ± 3	0.14 ± 0.005
UGT1A10^{139Lys}	2.1 ± 0.2**	52 ± 6**	0.04 ± 0.006	1.9 ± 0.2**	13 ± 2**	0.14 ± 0.004
UGT2B7^{268His}	0.55 ± 0.18	3.7 ± 0.6	0.15 ± 0.03	3.0 ± 0.44	101 ± 17	0.03 ± 0.004
UGT2B7^{268Tyr}	0.54 ± 0.09*	8.7 ± 0.8**	0.062 ± 0.01**	0.55 ± 0.01**	101 ± 15	0.006 ± 0.001**

[a] All data are the mean ± standard deviation, based on three independent experiments. Homogenates from cells overexpressing UGT1A8$^{173Ala/277Tyr}$ exhibited no detectable activity against *trans*-4-OH-TAM and *trans*-endoxifen. Homogenates from cells overexpressing any of the UGT1A4 variants exhibited no detectable activity against *trans*-endoxifen.

[b] Data are expressed per μg UGT protein, as determined by Western blot analysis.

* $P \leq 0.05$;

** $P < 0.01$.

Table 3

Kinetic analyses of *O*-glucuronidation of 17-dihydroexemestane by human UGTs.[a]

UGT	V_{max}(pmol·min^{-1}·μg^{-1})[b]	K_m(μM)	V_{max}/K_m(nl·min^{-1}·μg^{-1})[b]
UGT1A1		No detectable activity	
UGT1A3		No detectable activity	
UGT1A4	0.27 ± 0.01	34 ± 3.9	8.1 ± 0.9
UGT1A5		No detectable activity	
UGT1A6		No detectable activity	
UGT1A7		No detectable activity	
UGT1A8	0.30 ± 0.06	14 ± 3.9	22 ± 2.1
UGT1A9		No detectable activity	
UGT1A10	12 ± 1.8	124 ± 15	100 ± 7.9
UGT2B4		No detectable activity	
UGT2B7		No detectable activity	
UGT2B10		No detectable activity	
UGT2B11		No detectable activity	
UGT2B15		No detectable activity	
UGT2B17	2.0 ± 0.25	14 ± 2.7	137 ± 17

[a] All data are the mean ± standard deviation, based on three independent experiments.

[b] Data are expressed per μg UGT protein, as determined by Western blot analysis.

Table 4

Kinetic analyses of *O*-glucuronidation of anastrozole by UGT1A4 variants.[a]

UGT	V_{max}(pmol·min^{-1}·μg^{-1})[b]	K_m (μM)	V_{max}/K_m(nL·min^{-1}·μg^{-1})[b]
UGT1A4$^{24Pro/48Leu}$	3.6 ± 0.58	637 ± 40	5.6 ± 0.58
UGT1A4$^{24Thr/48Leu}$	3.2 ± 0.46	802 ± 137	4.1 ± 1.0
UGT1A4$^{24Pro/48Val}$	5.0 ± 1.3	1045 ± 131*	4.8 ± 0.88

[a] All data are the mean ± standard deviation, based on three independent experiments. Homogenates from cells overexpressing UGT1A8$^{173Ala/277Tyr}$ exhibited no detectable activity against *trans*-4-OH-TAM and *trans*-endoxifen. Homogenates from cells overexpressing any of the UGT1A4 variants exhibited no detectable activity against *trans*-endoxifen.

[b] Data are expressed per μg UGT protein, as determined by Western blot analysis.

* $P < 0.01$.

Table 5

Kinetic analysis of 17-dihydroexemestane glucuronidation by individual HLM specimens from subjects stratified by UGT2B17 genotype.

HLM #	UGT2B17 genotype	V_{max}(pmol·min^{-1}·mg^{-1})	K_m(μM)	V_{max}/K_m(μL·min^{-1}·mg^{-1})
972	*1/*1	51.6	10.6	4.9
1603	*1/*1	20.2	7.4	2.7
4118	*1/*2	63.6	12.1	5.3
1270	*1/*2	7.6	10.2	0.75
416	*2/*2	5.6	17.2	0.33
145	*2/*2	7.5	23.3	0.32