Letter to the Editor

Which CYP2B6 Variants Have Functional Consequences for Cyclophosphamide Bioactivation?

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Introduction

We read with interest the recent publications by Ariyoshi et al. (2011) and Raccor et al. (2011). The role of a number of cytochrome P450 (P450) isozymes in the 4-hydroxylation of cyclophosphamide has been debated for several years. There is evidence that both CYP2B6 and CYP2C19 are important in the biotransformation and activation of this prodrug in vitro (Chang et al., 1993, 1997; Huang et al., 2000; Griskevicius et al., 2003; Xie et al., 2003; Chen et al., 2004) and in vivo (Timm et al., 2005; Xie et al., 2006; Nakajima et al., 2007; Helsby et al., 2010; Afsar et al., 2011). However, whereas there is some evidence to support altered therapeutic response and/or toxicity in patients who have single nucleotide polymorphisms (SNP) variants of either of these P450 enzymes (Takada et al., 2004; Elmaagacli et al., 2007; Singh et al., 2007; Tran et al., 2008; Bray et al., 2010; Melanson et al., 2010; Afsar et al., 2011; Black et al., 2012), not all studies confirm this effect (Petros et al., 2005; Ekhart et al., 2008; Low et al., 2009; Yao et al., 2010; Winoto et al., 2011). This lack of consistency in the published literature may in part relate to invalid assumptions regarding the functional changes associated with each CYP2B6 genotype and hence misclassification of metabolizer categories. The data provided by both Ariyoshi et al. (2011) and Raccor et al. (2011) may clarify the situation.

This clarification is important because a recent report (Melanson et al., 2010) categorized the predicted cyclophosphamide metabolizer status of the patients based on CYP2B6 variants as follows: ultrarapid (*4), extensive (*5), and poor (*6 and/or *7). More recently, these same CYP2B6 genotypes were grouped together as “variant,” irrespective of whether individual genotypes led to increased or decreased function (Gor et al., 2010; Afsar et al., 2011). Such errors can lead to invalid analysis of the data, which emphasizes the importance of the correct assignment of CYP2B6 genetic variants with respect to cyclophosphamide metabolizer status (Helsby and Tingle, 2011).

The classic approach of determining intrinsic clearance in vitro and/or determining a metabolic ratio (drug/metabolite) in patients by area under the curve ratio or a single time point concentration is often under-used in the field of pharmacogenetic research. The recent data reported by both Ariyoshi et al. (2011) and by Raccor et al. (2011) reinforce the requirement for directly validating the functional role of SNP variants of P450 isoforms in the biotransformation of the drug of interest before undertaking genotype-therapeutic response association studies. Moreover, the substrate-specific catalytic consequences of individual SNP variants of CYP2B6 highlight the importance of obtaining this evidence for this particular P450 isozyme (Table 1). In addition to clarifying the role of CYP2B6 variants in cyclophosphamide bioactivation, this information may also be important for pharmacogenetic studies focused on other drug substrates of CYP2B6 such as efavirenz, selegiline, methadone, and propofol (Zanger et al., 2007).

The publications by Ariyoshi et al. (2011) and Raccor et al. (2011) also highlight the importance of full haplotype analysis to determine CYP2B6 genotype rather than reliance on one single SNP variant. The SNPs associated with common CYP2B6 genotypes are as follows: CYP2B6*4 (A785G), CYP2B6*5 (C1459T), and CYP2B6*6 (G516T and A785G). These amino acid changes are also found in the CYP2B6*7 (G516T, A785G, and C1459T) allele. Some studies investigating association of CYP2B6-predicted metabolizer status with therapeutic response to cyclophosphamide have used single SNP rather than haplotypes. For example, analyzing response relative to G516T without determining the presence or absence of A785G in the allele (Bray et al., 2010; Yao et al., 2010). This approach should be undertaken with caution. The amino acid change associated with the A785G SNP (K262R) appears to result in substantial differences in cyclophosphamide biotransformation depending on the presence or absence of the Q172H amino acid change (G516T SNP), (Ariyoshi et al., 2011; Raccor et al., 2011).

Recently, we have demonstrated that there is a significantly decreased intrinsic clearance of cyclophosphamide in human livers that are carriers of CYP2B6*5 compared with homozygous wild-type livers (Helsby et al., 2010). The R487C amino acid change associated with this SNP (C1459T) results in an approximately 50% decrease in the intrinsic clearance of cyclophosphamide (Raccor et al., 2011) (Table 1). CYP2B6.5 variant protein also has decreased artemether and bupropion intrinsic clearance (Honda et al., 2011; Zhang et al., 2011). However, in marked contrast, there is an increase in the biotransformation of efavirenz (Zhang et al., 2011) with this variant (Table 1). Consistent with decreased in vitro bioactivation of cyclophosphamide by CYP2B2.5, there is a lack of therapeutic response to cyclophosphamide in individuals homozygous for CYP2B6*5 (Takada et al., 2004; Black et al., 2012). The cytotoxic agent cyclophosphamide is a prodrug, and CYP2B6*5 has also been significantly associated with a decrease in adverse events (Takada et al., 2004; Singh et al., 2007; Black et al., 2012). However, a higher incidence of dose delay (implying increased bioactivation to the cytotoxin) is associated with CYP2B6*5 in patients treated with a combination of cyclophosphamide and doxorubicin (Bray et al., 2010). Unfortunately, this SNP was overlooked and hence not investigated in other studies of adverse events (Elmaagacli et al., 2007; Low et al., 2009; Yao et al., 2010). Further in vitro investigation of the effect of this R487C amino acid change is required to confirm whether it is associated with a significant decrease in function with respect to cyclophosphamide biotransformation.

Moreover, it is imperative that the substrate-specific effects of other nonsynonymous amino acid changes associated with other CYP2B6 genotypes are also investigated. In particular, C64T, rs8192709 (R22C) and A415G, rs12721655 (K139E) have been
reported to influence outcome in breast cancer patients receiving cyclophosphamide and doxorubicin (Bray et al., 2010). The effect of the Q172H amino acid change (rs3745274) on cyclophosphamide bioactivation alone (CYP2B6*9) and in combination with K262R (rs2279343) and R487C (rs3211371) also requires investigation. The CYP2B6*18 (rs2839949, I328T) and CYP2B6*16 (I328T, Q172H, K262R) variants lead to slow efavirenz metabolism; however, whether these genotypes are associated with increased cyclophosphamide bioactivation similar to CYP2B6*6 is currently not known.

Data from studies such as those of Ariyoshi et al. (2011) and Raccor et al. (2011) provide further and important clarification as to which CYP2B6 variants lead to increased or decreased function with respect to cyclophosphamide bioactivation. This data will facilitate the appropriate study of these SNPs in gene-association studies. However, it is also important to note that both CYP2B6 and CYP2C19 have roles in cyclophosphamide bioactivation. Roles for either variant CYP2B6 (Chang et al., 1993; Huang et al., 2000; Xie et al., 2003, 2006; Chen et al., 2004; Nakajima et al., 2007), variant CYP2C19 (Chang et al., 1997; Griskevicius et al., 2003; Timm et al., 2005; Afsar et al., 2011), or a combination of both CYP2C19 and CYP2B6 loss-of-function variants (Helsby et al., 2010) have been reported. Hence, in addition to clarifying the functional variants of CYP2B6, analysis of the haplotype with respect to both these genes should be considered. As a minimum, clinical studies investigating cyclophosphamide pharmacogenetics should identify and report each individual patient’s genotype for CYP2B6*4, CYP2B6*5, CYP2B6*6, CYP2C19*2, CYP2C19*3, and CYP2C19*17.

Wide variation in the prevalence of CYP2B6 variants and CYP2C19 in different ethnicities adds to the complexity of the associations between genotype and cyclophosphamide effect in different clinical study populations. For example, the frequency of the CYP2B6*5 allele is low in Asian populations (0–0.8%) and ~15-fold higher in white populations (12.2%). In contrast, the CYP2B6*6 allele is observed at a frequency of 28.2% in whites and 15.9 to 18.4% in Asians (Davaalkham et al., 2009). Knowledge of the pharmacogenetics of cyclophosphamide is a constantly evolving field, and variants such as CYP2B6*16 and CYP2B6*18, which are essentially absent in white and Asian populations, may be important in clinical studies in populations of African ancestry.

As a further difficulty, genotype-phenotype discordance of CYP2C19 in cancer patients has been reported (Williams et al., 2000; Helsby et al., 2008). This finding suggests that an additional role for changes in hepatic enzyme regulation may further complicate cyclophosphamide pharmacogenetic studies. More extensive study of the factors that influence the cyclophosphamide biotransformation phenotype in patients could lead to improvement in the prediction of therapeutic response or toxicity with this agent.

### Table 1

<table>
<thead>
<tr>
<th>Drug Substrate and Reaction</th>
<th>%Activity Relative to Wild-Type</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclophosphamide (hydroxylation)</td>
<td>CYP2B6.4 K262R (rs2279343)</td>
<td>72.6 n.d.</td>
</tr>
<tr>
<td></td>
<td>CYP2B6.5 R487C (rs3211371)</td>
<td>69.6 52</td>
</tr>
<tr>
<td></td>
<td>CYP2B6.6 Q172H, K262R (rs3745274, rs2279343)</td>
<td>164 n.d.</td>
</tr>
<tr>
<td></td>
<td>CYP2B6.7 Q172H, K262R, R487C (rs3745274, rs2279343, rs3211371)</td>
<td>96 138 20</td>
</tr>
</tbody>
</table>

Wild-type activity = 100%; n.d., not determined; rs, reference SNP number.

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**References**


Honda M, Muroi Y, Tamaki Y, Saigusa D, Suzuki N, Tomioka Y, Matsubara Y, Oda A, Hirasawa N, and Hiratsuka M (2011) Functional characterization of CYP2B6 allelic variants with respect to cyclophosphamide metabolism; however, whether these genotypes are associated with increased cyclophosphamide bioactivation similar to CYP2B6*6 is currently not known.

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