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Pharmacogenetic studies related to cyclophosphamide-based therapy

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Abstract

Cyclophosphamide is a cornerstone in the treatment of many pediatric and adult malignancies, as well as in the treatment of refractory autoimmune conditions. Genetic factors are thought to play a role in the interindividual variation in both response and toxicities associated with cyclophosphamide-based therapies. This drug focus reviews the most compelling studies conducted on the pharmacogenetics of cyclophosphamide-based therapies. Broader pharmacogenomic studies are needed and may reveal additional factors important in susceptibility to toxicity and/or response to therapy.

Cyclophosphamide (CP), an oxazophosphorine, bifunctional DNA alkylating agent, is incorporated into the treatment of most pediatric and adult malignancies and was one of the first nonhormonal agents to show anti-tumor activity in humans [1]. At lower doses, CP exerts a potent immunomodulatory effect and is used as second-line therapy in many autoimmune disorders [1]. Like all chemotherapies, variation in the efficacy and toxicity associated with CP exist. A better understanding of the pharmacogenetic factors influencing the variation in response and toxicity to CP offers the ability to individualize treatment. To date, most, if not all, studies have taken a candidate gene approach, focusing on known targets involved in CP bioactivation and/or detoxification; however, a whole-genome approach may allow for a more comprehensive, unbiased approach to identify factors important in CP clinical activity.

Cyclophosphamide is a prodrug that enters the liver and is metabolized by the hepatic P450 system to both active and inactive compounds. *N*-dechloroethylation of CP, mediated primarily by CYP3A4/3A5, gives 2-dechloroethylcyclophosphamide, which is generally believed to have no cytotoxic effects, and the neurotoxic chloroacetaldehyde [2]. Oxidation at the C-4 position of CP generates 4-hydroxycyclophosphamide; this reaction is mediated by various isoforms including CYP2A6, 2B6, 2C8, 2C9, 2C19, 3A4 and 3A5 [2–6]. 4-hydroxycyclophosphamide interconverts with aldophosphamide, which undergoes further chemical decomposition by fragmentation to phosphoramidate mustard, the active anti-tumor metabolite, and acrolein, a metabolite responsible for urotoxicity [3]. Detoxification of the metabolites of CP occurs mainly through NADPH-mediated oxidation by various aldehyde dehydrogenases (ALDH1A1 and ALDH3A1) [4]. Another detoxification pathway includes the conjugation of CP with glutathione by various glutathione *S*-transferases (GSTs; GSTA1,

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GSTM1, GSTP1 and GSTT1) [5]. GST-mediated conjugations of various CP metabolites with glutathione have been reported, but the significance of enzyme catalysis in these reactions is unclear as they readily occur spontaneously (in the absence of enzymatic intervention) [6–8]. Pharmacogenetic studies in both the *ALDH* and *GST* genes have discovered polymorphisms important to response and/or toxicity associated with CP-based therapies (see Table 1). DNA repair proteins including MGMT and ERCC [9–10], and efflux of CP and its metabolites out of the cell (MRP) [11] have been shown to be important; however, the role of genetic variants within these genes has not been extensively evaluated.

Cyclophosphamide has a relatively narrow therapeutic index, and adverse effects include cardiotoxicity, nephrotoxicity, neurotoxicity, infertility, bladder toxicity, myelosuppression and leukemogenesis. Both toxicity and response to CP is quite variable. Pharmacogenetics offers clinicians the ability to individualize therapy based on a patient's risk of untoward effects as well as their likelihood of response. This brief review aims to highlight the clinically significant pharmacogenetic discoveries pertinent to CP-based treatment regimens.

Highlights

- Cyclophosphamide is used in the treatment of most pediatric and adult malignancies as well as refractory autoimmune conditions.
- Genetic polymorphisms in the CYP, GST and ALDH families of enzymes have been found to impact response and/or toxicity associated with cyclophosphamide-based therapies.
- Polymorphisms that reduce the ability of GSTP1 to detoxify cyclophosphamide and/or its active metabolites appear to confer superior survival outcomes as well as increased risk of toxicity, including secondary malignancies.
- To date, systematic, genome-wide studies of cyclophosphamide-based therapies are lacking. Prospective, well-powered and unbiased pharmacogenomic studies are needed to verify current observations as well as to identify additional genetic markers responsible for variation in response and toxicities associated with cyclophosphamide.

Cytochrome P450 polymorphisms & cyclophosphamide effect

Bioactivation of CP requires the activity of cytochrome P450 (CYP) enzymes in the liver. CYP is a superfamily of hemoproteins that exert their enzymatic activity on a variety of endogenous and exogenous compounds. The specific CYPs involved in CP metabolism all have variant alleles, and some have been shown to be associated with varied levels of protein expression and/or metabolic activity of the expressed proteins [12–18]. To varying degrees, CYP2A6, 2B6, 2C8, 2C9, 2C19, 3A4 and 3A5 have all been implicated in the 4-hydroxylation and/or dechloroethylation of CP. To our knowledge, only selected variants in *CYP2B6*, *2C19*, *3A4* and *3A5* have been studied for their impact on CP clinical response and/or toxicity.

CYP2B6 accounts for approximately 3–5% of total microsomal CYP protein in the liver, and is also expressed in small amounts in other tissues including intestine, kidney, brain, lung and skin [19]. Isoform 2B6, which is thought to be one of the major contributors to C-4 oxidation in CP [12], is highly polymorphic and extensive interindividual variation in enzymatic activity exists [13]. Decreased enzymatic activity should theoretically lead to decreased active metabolites and, therefore, fewer toxic side effects and poorer response to therapy. Takada *et al.* demonstrated in patients receiving pulse CP for the treatment of lupus nephritis who were homozygous for *CYP2B6**5, a haplotype with decreased enzymatic function, there was a higher

probability of developing end-stage renal disease. This suggests that the haplotype results in a reduced exposure to the active drug [14]. Rocha *et al.* demonstrated in 107 allogeneic stem cell transplant recipients, most of whom received busulfan and CP conditioning, that carriers of at least one mutant allele in *CYP2B6**2A and *CYP2B6**4 had a statistically significantly higher incidence of hemorrhagic cystitis and oral mucositis, respectively [15]. The effects of these mutations on 2B6 activity have not been well described.

In vitro studies with human liver cells reveal that CYP2C19 accounts for approximately 12% of CP 4-hydroxylation [12]. The variant *2C19**2 (618G>A), creates an alternate splice site and a protein lacking the ability to activate CP. Takada *et al.* demonstrated that carriers of at least one *2C19**2 allele were protected against premature ovarian failure and that *2C19**2 homozygotes had a higher probability of developing end-stage renal disease when treated with CP alone for their lupus nephritis [14].

Involved in the metabolism of over 50% of all drugs, CYP3A4 and CYP3A5 are among the most important and most abundant *CYP* isozymes in humans [16]. In a study of 85 women with metastatic or inflammatory breast cancer treated with standard-dose chemotherapy followed by high-dose CP, cisplatin and carmustine, patients with *CYP3A4**1B or *CYP3A5**1 variants had higher area under the curve values for (parent) CP at the second and third doses (suggesting reduced activation) and a decreased median survival. To date, altered protein expression has not been demonstrated as a result of these variant alleles, and the functional significance of these variants remains controversial [17,18]. In fact, a recent study of 127 premenopausal women with breast cancer exposed to CP-based therapies demonstrated that young women who were carriers of the *CYP3A4**1B variant haplotype had an increased risk of developing premature ovarian failure [20]. These data correspond to *in vitro* cell-based assays that suggest *CYP3A4**1B, a SNP in the promoter region of the gene, is associated with increased 3A4 expression as compared with wild-type, which should lead to increased exposure to the active drug [21–22]. These findings highlight that response to therapy and risk of toxicity are likely to depend on complex interactions between multiple genetic and nongenetic factors rather than a single SNP.

Glutathione S-transferase polymorphisms & cyclophosphamide

Glutathione S-transferases are Phase II enzymes that detoxify several chemotherapeutic agents and/or their metabolites by conjugating them with glutathione. Polymorphisms in any of the *GST* genes produce significant alterations in the metabolism of chemotherapeutic agents and carcinogens [23–28]. As a result, these polymorphisms have been implicated as having a role in susceptibility to cancer as well as resistance to treatment [29–35]. The GSTs involved in CP detoxification include GSTM1, GSTT1, GSTA1 and GSTP1 [36]. Reduction in the enzymatic activity of these enzymes leads to decreased detoxification of active CP and prolonged exposure to the drug. This should, in theory, result in increased risk of adverse drug effects but, also on the other hand, lead to the possibility of improved survival.

GSTA1, the primary hepatic GST, is also expressed in the human breast and is important in detoxification of CP [37,38]. In 106 newly diagnosed patients with non-Hodgkin's lymphoma treated with CP-containing combination chemotherapy (rituximab, CP, doxorubicin, vincristine and prednisone [R-CHOP]), Rossi *et al.* demonstrated that patients with the *GSTA1* rs3957357 CT/TT genotype had significantly improved event-free survival when compared with wild-type (CC) patients. Virtually all patients with wild-type alleles and advanced disease did not respond to therapy [39]. The *GSTA1* rs3957357T minor allele, one of many linked SNPs comprising the *GSTA1**B haplotype, is located in the promoter region of the gene and has been associated with reduced levels of GSTA1 enzyme in healthy individuals, predicting for reduced detoxification of alkylating agents and increased exposure

to the active drug [40,41]. Improved outcome was also observed in breast cancer patients receiving CP-based therapy who were homozygous for the *GSTA1**B allele [42].

Polymorphic deletions in two GSTs, *GSTT1* and *GSTM1*, are quite common in the general population [43,44]. Homozygous deletions cause no detectable enzymatic function, and have been identified as important risk factors in the development of solid tumors [45]. Several studies in patients with a variety of malignancies treated with CP-containing regimens have shown that deletions in one or both of these enzymes confer improved disease-free survival, decreased risk of disease relapse and an increased incidence of chemotherapy-related side effects; all consistent with decreased detoxification of and increased exposure to the drug [29,46,47].

GSTP1, which encodes for an enzyme with significant affinity for CP metabolites, is also subject to polymorphic variations; the single-nucleotide substitution A313G results in an amino acid change at codon 105 (Ile>Val) that is associated with lower substrate specific catalytic activity and lower thermal stability for the encoded protein (GST π) [26–27,48]. Several studies have demonstrated improved clinical outcomes, including improved survival and decreased rates of relapse, for both heterozygotes and homozygotes for the ¹⁰⁵Val polymorphism in cancer patients exposed to CP in combination chemotherapy [29,49–52]. Carriers of at least one ¹⁰⁵Val polymorphism also experienced increased toxicity when exposed to CP. Patients with at least one variant allele treated with pulse CP for lupus nephritis had increased incidence of myelotoxicity and gastrointestinal toxicity in a series of studies by Zhong *et al.* [53]. Even more concerning was a report by Allan *et al.*, where carriers of at least one ¹⁰⁵Val polymorphism were overrepresented in a group of patients with therapy-related acute myeloid leukemia (AML) when compared with patients with *de novo* AML. This association was even stronger for therapy-related AML patients exposed to chemotherapies that were GSTP1 substrates, suggesting that increased exposure to the active drug played a role in leukemogenesis. In addition, patients with therapy-related AML previously exposed only to radiotherapy did not show the same overrepresentation of ¹⁰⁵Val carriers [54].

Aldehyde dehydrogenase polymorphisms & cyclophosphamide effect

Oxidations of both active (aldophosphamide) and inactive but toxic (acrolein) metabolites of CP are mediated by aldehyde dehydrogenases, primarily ALDH1A1 and ALDH3A1 [48]. Introduction of these enzymes as vectors into human and hamster ovary cells reduced their sensitivity to CP, and tumor cells resistant to CP exhibited upregulation of ALDHs [55]. These enzymes are known to have genetic polymorphisms, and although the expression of *ALDH1A1* and *3A1* is not affected by their most common polymorphisms (*1A1**2 and *3A1**2, respectively), the functional effects of these polymorphisms have not yet been elucidated [56,57].

In a series of studies by Ekhardt *et al.*, of 113 patients with various malignancies treated with high dose CP, thiotepa and carboplatin followed by autologous stem cell rescue, important toxicities were observed in patients with common variants in *ALDH1A1* and *3A1*. In patients heterozygous for the *1A1**2 polymorphism, an increased incidence of liver toxicity was observed compared with wild-type patients. In patients heterozygous for the *3A1**2 polymorphism, an increased incidence of hemorrhagic cystitis was observed compared with wild-type patients [58]. Interestingly, ALDH1A1 is expressed in the liver but not the bladder, and 3A1 is expressed in the bladder, but not the liver [57,59].

Future perspective

Cyclophosphamide is the cornerstone in the treatment of most pediatric and adult malignancies. Pharmacogenetics offers the ability to better predict response and/or toxicity based on a patient's genotype and, therefore, the potential to personalize therapy. Several important

pharmacogenetic discoveries have been made with respect to CP's effect using a candidate gene approach. These findings, with a few exceptions, have also been relatively reproducible across patient populations as well as across a diverse set of diseases. However, only certain polymorphisms in important pharmacogenes have been examined, and other genes involved in CP activation, detoxification and efflux have not yet been studied for their effect. Unfortunately, most of these studies have been conducted in patients receiving combination chemotherapies with overlapping mechanisms of metabolism and detoxification, making detection of variants specific to CP effects difficult. Genes important in the pharmacokinetic and pharmacodynamic effects of CP require discovery and validation in larger and more diverse patient populations.

One consideration is the movement beyond candidate gene-driven studies into genome-wide association studies of CP in an effort to reveal, in an unbiased manner, unknown genetic variants contributing to variation in individual response and toxicity associated with CP. These whole-genome approaches should yield more robust profiles that may better guide future genetics-based treatment. Furthermore, mechanisms beyond those that act through their effect on gene expression need to be elucidated, perhaps using proteomics technologies. Future discoveries in these areas could lead to personalized therapies that enhance therapeutic response to and minimize the adverse effects of this very important and widely used chemotherapeutic drug.

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Table 1
Pharmacogenetic associations of genetic polymorphisms with cyclophosphamide

Study	Population	Gene and gene variant	Effect	Ref.
Takada <i>et al.</i> (2004)	62 patients with proliferative lupus nephritis	<i>CYP2C19</i> *2 homozygotes	Lower risk of developing premature ovarian failure and higher probability of developing end-stage renal disease	[14]
		<i>CYP2B6</i> *5 homozygotes	Higher probability of developing end-stage renal disease	
Petros <i>et al.</i> (2005)	85 women with breast cancer (74 Caucasian, 11 African-American)	<i>CYP3A4</i> *1B	Decreased median survival (p = 0.043)	[60]
		<i>CYP3A5</i> *1	Decreased median survival (p = 0.002)	
		<i>GSTM1</i> null	Increased median survival (p = 0.041)	
Rocha <i>et al.</i> (2009)	107 patients with leukemia receiving allogeneic stem cell transplant	<i>CYP2B6</i> *4 AG or GG	Increased oral mucositis versus AA (p = 0.0067)	[15]
		<i>CYP2B6</i> *2A CT or TT	Increased hemorrhagic cystitis versus CC (p = 0.03)	
Su <i>et al.</i> (2009)	127 premenopausal women with breast cancer (113 Caucasian, 5 AA, 9 other)	<i>CYP3A4</i> *1B	Increased chemotherapy-related ovarian failure than *1A wild-type if <45 years (p = 0.03)	[20]
Rossi <i>et al.</i> (2009)	106 patients with diffuse large B-cell lymphoma treated with R-CHOP	<i>GSTA1</i> rs3957357 CT/TT	Improved event-free survival versus CC wild-type (p = 0.024)	[39]
Yang <i>et al.</i> (2004)	1034 Han Chinese females with breast carcinoma	<i>GSTP1</i> Val/Val	Improved overall survival compared with Ile/Ile (HR 0.4, p = 0.69) [‡]	[49]
Hohaus <i>et al.</i> (2003)	90 patients with Hodgkin's lymphoma [§]	<i>GSTM1</i> null and/or <i>GSTT1</i> null	Improved disease-free survival (p = 0.012)	[46]
Barahmani <i>et al.</i> (2009)	42 children with medulloblastoma (14 Hispanic, 21 Caucasian, 5 AA, 2 other)	<i>GSTT1</i> null	8.9-times more likely to have any grade III toxicity than nonnull genotype	[47]
		<i>GSTM1</i> null and/or <i>GSTT1</i> null	Significant decline in IQ scores after chemo- and radio-therapy	

Study	Population	Gene and gene variant	Effect	Ref.
Sweeney <i>et al.</i> (2003)	245 women with breast cancer (198 Caucasian, 47 AA)	<i>GSTA1</i> *B	*B/*B improved survival versus *A/*A and *A/*B (p = 0.07)	[42]
Sweeney <i>et al.</i> (2000)	240 women with breast cancer (192 Caucasian, 48 AA)	<i>GSTP1</i> Val/Val	Improved overall survival compared with Ile/Ile (HR: 0.3, p = 0.04)	[50]
Hohaus <i>et al.</i> (2005)	97 patients with Hodgkin's lymphoma	<i>GSTP1</i> Val/Val	Improved failure-free survival compared with Ile/Ile (HR: 0.4, p = 0.02) [§]	[51]
Dasgupta <i>et al.</i> (2003)	222 patients with multiple myeloma randomized to either standard or high-dose chemotherapy	<i>GSTP1</i> Ile/Val and <i>GSTP1</i> Val/Val	HR for progression-free survival compared with Ile/Ile 0.55 and 0.52, respectively, in patients on standard dose arm (p = 0.04)	[52]
Stanulla <i>et al.</i> (2000)	128 children with B-precursor ALL	<i>GSTM1</i> nul	Twofold reduction in risk of relapse (p = 0.078)	[29]
		<i>GSTT1</i> null	2.8-fold reduction in risk of relapse (p = 0.048)	
		<i>GSTP1</i> Val/Val	Threefold reduction in risk of relapse (p = 0.099)	
Allan <i>et al.</i> (2001)	89 cases t-AML, 420 cases <i>de novo</i> AML, 1022 controls	<i>GSTP1</i>	Patients with at least one ¹⁰⁵ Val allele overrepresented in t-AML group previously exposed to chemotherapies that are <i>GSTP1</i> substrates (OR: 4.34)	[54]
Zhong <i>et al.</i> (2006)	102 patients with lupus treated with pulse CP	<i>GSTP1</i> Ile/Val and <i>GSTP1</i> Val/Val	Increased risk of myelotoxicity (OR: 5, with CP > 1 gm, OR: 12.88), increased risk of gastrointestinal toxicity (OR: 3.33)	[53]
Ekhart <i>et al.</i> (2008)	113 patients with various malignancies treated with high-dose CTC	<i>ALDH3A1</i> *2	Heterozygotes had increased risk of hemorrhagic cystitis compared with wild-type (OR: 11.95, p = 0.04)	[58]
		<i>ALDH1A1</i> *2	Heterozygotes had increased risk of liver toxicity compared with wild-type (OR: 5.13, p = 0.02)	

[‡]Not statistically significant.

[§]Patients with Hodgkin's lymphoma in this study received a variety of chemotherapy regimens, all of which included an alkylating agent.

ALL: Acute lymphoblastic leukemia; AML: Acute myeloid leukemia; CP: Cyclophosphamide; CTC: Cyclophosphamide, thiotepa and carboplatin; HR: Hazard ratio; OR: Odds ratio; R-CHOP: Rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone; t-AML: Therapy-related AML.