Cytochrome P450 Polymorphism as a Predictor of Ovarian Toxicity to Pulse Cyclophosphamide in Systemic Lupus Erythematosus

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ABSTRACT. ***Objective.*** Ovarian toxicity is a major concern with cyclophosphamide (CYC) therapy. CYC is a prodrug that is activated by cytochrome P450 (CYP) enzymes to its active metabolites that are responsible for ovarian toxicity. The amount of active metabolites produced depends on polymorphism in CYP 450 genes. We studied the association of CYP2C19 and CYP2B6 genetic polymorphism with ovarian toxicity in patients with systemic lupus erythematosus (SLE) treated with CYC.

Methods. Thirty-five patients with SLE who had exposure to CYC were genotyped for variant alleles of CYP2B6 and CYP2C19. Ovarian toxicity included ovarian insufficiency, defined as lack of menses for 4 months, and ovarian failure (premature menopause) as amenorrhea lasting > 12 months before the age of 45 years.

Results. The mean age at start of CYC was 24.5 + 8.5 years and the cumulative dose of CYC received was 9.3 ± 2.8 g. At the time of study the median followup after CYC treatment was 3 (1–6) years. A total of 17 patients developed ovarian toxicity, of whom 11 patients had ovarian insufficiency and 6 had premature menopause. The frequencies of variant alleles CYP2B6*5 and CYP2C19*2 were 8.5% and 21%, respectively. Patients who were homozygous or heterozygous for variant allele CYP2C19*2 had a significantly lower risk of developing ovarian toxicity when compared to patients with wild-type allele CYP2C19*1 (3/13 vs 14/22; OR 0.136, 95% CI 0.028–0.653; $p < 0.01$). No association was seen with CYP2B6 polymorphism.

Conclusion. Presence of the variant allele CYP2C19*2 is associated with lower risk of ovarian toxicity in Indian patients treated with CYC. (First Release Feb 15 2007; J Rheumatol 2007;34:731–3)

Key Indexing Terms:
PHARMACOGENOMICS DRUG TOXICITY IMMUNOSUPPRESSIVES

Systemic lupus erythematosus (SLE) predominantly affects young women. Therapy with cyclophosphamide (CYC) is required in 15%–20% of patients with SLE with major organ disease[1,2]. Pulse intravenous CYC is now considered a standard protocol for administering CYC[2,3].

The major side effects of CYC include increased risk of infection, hemorrhagic cystitis, and ovarian toxicity. Among these, ovarian toxicity is of major concern in premenopausal women, and 12%–59% develop premature ovarian failure. Younger patients and lower cumulative dose of CYC are associated with lower risk of ovarian failure[2,3]. However, these 2 factors are not sufficient in assessment of the risk to a young patient.

CYC is a prodrug and requires activation by cytochrome P450 (CYP) enzymes to its active metabolites, 4- hydroxylcyclophosphamide and aldophosphamide. Several CYP are implicated in metabolism of CYC[4]. The enzymes CYP2B6 and CYP2C19 are genetically polymorphic, with allele CYP2C19*2 causing altered splicing leading to inactive form of the enzyme, while CYP2B6*5 causes decreased activity[5,6].

In a recent study, patients with CYP2C19*2 allele had a lower risk of premature ovarian failure after adjusting for age of patient and total number of CYC pulses. Also, patients who were homozygous for CYP2B6*5 or CYP2C19*2 had higher probability of reaching endstage renal disease[7]. Since allelic frequency varies in different ethnic groups we investigated the effect of polymorphism in CYP2B6 and CYP2C19 on ovarian toxicity in Indian patients with SLE treated with pulse CYC.

MATERIALS AND METHODS

We studied 35 consecutive female patients with SLE[8] who had received at least 6 pulses of CYC and had at least 1 year followup after receiving CYC. Exclusion criteria were: postmenopausal state, oophorectomy, and presence of advanced renal failure at the time of start of CYC. All patients gave informed written consent.

The patients were interviewed with the help of a questionnaire about the age at start, mean and cumulative dose of CYC, age at completion, and total duration of CYC therapy. Ovarian toxicity included ovarian insufficiency defined as lack of menses for 4 months and ovarian failure (premature menopause) as amenorrhea lasting for more than 12 months occurring before the age of 45 years.

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Doubling of serum creatinine or development of endstage renal disease was taken as a measure of poor response.

DNA was extracted from peripheral blood by the phenol-chloroform method. Genotyping for variant alleles of CYP2B6 [CYP2B6*5 (C1459T, Ag^{487}Cys)] and CYP2C19 [CYP2C19*2 (G681A, splicing defect)] was performed by polymerase chain reaction (PCR) followed by restriction fragment length polymorphism (RFLP) using methods described[5,6]. Primers used were as follows — *CYP2B6*5*: forward: 5' TGA GAA TCA GTG GAA GCC ATA GA 3'; reverse: 5' TAA TTT TCG ATA ATC TCA CTC CTG C 3'. *CYP2C19*2*: forward: 5' CAG AGC TTG GCA TAT TGT ATC 3'; reverse: 5' GTA AAC ACA CAA CTA GTC AAT G 3'.

The statistical analysis was performed on SPSS13. Chi-square test was used to test for departure from Hardy-Weinberg equilibrium. Clinical outcomes were analyzed with chi-square test. Relative risk for ovarian toxicity was calculated for each genotype with 95% confidence interval.

RESULTS

The mean age at the onset of disease was 23.0 ± 8.2 years and the mean age at the start of CYC treatment was 24.5 ± 8.5 years. The cumulative dose of CYC used was 9.34 ± 2.87 g. The major organ involvement in these patients was nephritis (34), neuropsychiatric lupus (8), and myocarditis (1). After a median followup of 3 years (range 1–16) a total of 17 (48%) patients developed ovarian toxicity, of whom 6 patients had premature menopause and 11 had ovarian insufficiency. There was no difference in the above measures in patients with or without ovarian toxicity including dose of CYC (Table 1). Three patients had doubling of serum creatinine. None of them developed endstage renal disease.

The frequency of variant allele CYP2B6*5 was 8.5%. No patient was homozygous for variant allele CYP2B6*5, 6 patients were heterozygous, and 29 were homozygous for wild-type allele. The allele frequency for CYP2C19*2 was 21%. Two patients were homozygous for variant allele CYP2C19*2, 11 were heterozygous, and 22 were homozygous for wild-type allele. The observed allele frequencies were in Hardy-Weinberg equilibrium for the study population.

Of the 22 patients who were homozygous for wild-type allele, 14 (63%) developed ovarian toxicity as compared to 3 (23%) patients who had variant allele CYP2C19*2 (p = 0.009, OR 0.136, 95% CI 0.028–0.653). No clinically significant difference was seen in patients having either wild-type or variant allele of CYP2B6 (Table 2).

There was no difference in frequency of patients having doubling of serum creatinine among individuals with wild-type (1/22) and variant allele (2/13) of CYP2C19. All the 3 with doubling of serum creatinine had wild-type CYP2B6.

Table 1. Demographic and clinical features of patients exposed to cyclophosphamide: differences between patients with and without ovarian toxicity.

Feature (mean ± SD)	No Ovarian Toxicity (n = 18)	Ovarian Toxicity (n = 17)
Age at enrollment, yrs	28.3 ± 8.7	29.1 ± 9.7
Age at onset of SLE, yrs	20.9 ± 7.1	25.5 ± 8.8
Age at diagnosis, yrs	21.8 ± 7.6	26.1 ± 8.8
Age at onset of menstruation, yrs	14.3 ± 1.7	13.6 ± 8.6
Age at start of cyclophosphamide therapy, yrs	23.1 ± 8.5	26.12 ± 8.6
Duration of cyclophosphamide therapy, mo	20.3 ± 8.3	15.4 ± 7.2
Cumulative dose of cyclophosphamide received, yrs	9.4 ± 2.8	9.1 ± 2.9

All comparisons were nonsignificant.

DISCUSSION

Our data suggest that patients with variant allele CYP2C19*2 have a significantly lower risk of developing ovarian toxicity compared to patients who are homozygous for wild-type allele.

Our allele frequency of 8.5% for variant allele CYP2B6*5 is less than the frequency (12%–14%) reported in a Caucasian population[5,7]. No data are available for the Indian population, although data from Japan, China, and South Korea suggest low prevalence of CYP2B6*5 allele in Asian populations[9]. For CYP2C19, the allele frequency for variant allele was 21%, which is close to the figures reported in the Caucasian population[7,10], but lower than those reported from our country — 30% in north Indians[11] and 37% in a south Indian population[12], which could be related to small sample size in our study.

When we compared our patients with ovarian toxicity with patients who did not have ovarian toxicity, there were no significant differences in the age of onset of SLE, age at CYC exposure, cumulative dose of CYC, or duration of CYC therapy. Our data are at variance with other studies, which have shown that the above factors have an effect on the ovarian toxicity[2,3]. Our patients received smaller amounts of CYC and were younger at initiation of CYC compared to other studies. Further, most studies have used premature menopause as their endpoint rather than overall ovarian toxicity, which we used. The variability in the prevalence of allelic forms of the CYP2C19 might be another factor.

Our study provides confirmation of an earlier report from a Caucasian population, where patients with variant allele of CYP2C19*2 had significantly lower risk of developing ovarian failure compared to patients with wild-type alleles[7]. However, that study did not include patients with ovarian insufficiency. Does ovarian insufficiency lead to premature ovarian failure in future? In animal studies, treatment with intraperitoneal CYC has been shown to reduce the numbers of ovarian follicles; how this affects future outcome of ovarian function is not known at present[13].

Lack of any association of CYP2B6 polymorphism with ovarian toxicity could be related to small sample size and low allele frequency of CYP2B6*5. Further, we found no association of genotype with doubling of serum creatinine, in contrast to a previous study[7]. Our study has a few limitations: one is the small sample size, and another is the lower prevalence of premature ovarian failure due to shorter median followup of 3 years.

Table 2. Association between cytochrome P450 genotypes and risk of ovarian toxicity.

Genotype	Ovarian Toxicity	No Ovarian Toxicity	p	Odds Ratio (95% CI)
CYP2B6				
*1/*1 (n = 29)	15	14	0.41	0.467 (0.074–2.95)
*1/*5 (n = 6)	2	4		
*5/*5 (n = 0)	0	0		
CYP2C19				
*1/*1 (n = 22)	14	8	0.009	0.136 (0.028–0.653)
*1/*2 (n = 11)	3	8		
*2/*2 (n = 2)	0	2		

Our study indicates that it may be possible to identify patients with SLE who are at risk of ovarian toxicity on exposure to CYC by determination of CYP2C19 genotype. This polymorphism may be relevant even in patients receiving lower doses of CYC, the current trend in treatment of proliferative lupus nephritis. These patients can be offered alternative treatment options in the forms of mycophenolate mofetil or rituximab[14,15].

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