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The Influence of GSTA1 Polymorphism to the Response to Intravenous Cyclophosphamide Therapy in the Lupus Nephritis Patients

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Abstract

Variations of clinical response of cyclophosphamide (CTX) treatment in lupus nephritis (LN) could still be recognized. LN patients with a GSTA1mutation (CT heterozygous) had a risk of none-response (P=0.005). Pharmacokinetics data indicated that patients with a GSTA1 heterozygous variant had a lower exposure to 4-OH-CTX compared to wild-type patients (P=0.023). And clinical efficacy was significantly related to higher exposure to 4-OH-CTX (P=0.038). In conclusion, LN patients with GSTA1 heterozygous genotypes had poor CTX treatment response due to less exposure to activated 4-OH-CTX.

Keywords: Lupus nephritis; Nephrology; Cyclophosphamide; GSTA1; Pharmacokinetics; Pharmacogenomics

Introduction

Pulsed low-dose CTX therapy has become the most effective approach in improving the clinical outcomes of lupus nephritis (LN) patients. However, variations in CTX therapeutic outcomes in LN patients have not been completely understood.

CTX is an inactive pro-drug that undergoes complex metabolic activation and inactivation reactions. Hydroxylation at the C4 position of CTX, an initial activation step, generates 4-hydroxycyclophosphamide (4OHCTX), and this is mediated by various cytochrome P450 (CYP) enzymes. 4OHCTX is detoxified by glutathione-S-transferase (GSTs) to form 4-glutathionylcyclophosphamide [1].

GSTA1 is involved in CTX metabolism and in vitro studies confirm that GSTA1, among human GSTs, has the highest catalytic activity for GSH conjugation of cyclophosphamide metabolites. CTX response is thought to be associated with a conjugation of aldophosphamide and phosphamide mustard with glutathione through polymorphic glutathione S-transferase GSTA1 [2].

In the present study, we explained the influence of GSTA1 gene polymorphism on CTX therapy in LN patients according to Pharmacokinetics.

Methods and Results

Clinic data and blood samples were collected from patients (n=77) histologically confirmed LN (Classes III-V) in the Nephrology Department of Huashan Hospital affiliated Fudan University. All patients received monthly intravenous CTX (0.5 g/m^2) therapy for 6 months. Some patients are not included if (1) they are pregnant or breast-feeding, (2) they had serious chronic heart or haematological or lung disease not due to SLE complication, (3) AST or ALT is exceed 3 times the upper limits of normal, (4) they are taking or used allopurinol, busulphan, chloramphenicol or ciprofloxacin within 2 months as these drugs may restrain CTX metabolism, (5) they are lost to follow up during the period of clinical observation. This study was approved by the Ethics Committee of Fudan University for Clinical Studies. Written informed consent was obtained from all participants.

A complete remission (CR) was defined as a value for urinary protein excretion that was less than 0.3 g per 24 h, with normal urinary sediment and serum albumin (ALB) concentration, and values for both serum creatinine (sCr) and creatinine clearance that were 15% or less above the baseline values. A partial remission (PR) was defined as a value for urinary protein excretion that was 0.3-2.0 g per 24 h, with an ALB concentration of at least 3.0 g/dL, and stable renal function. Complete remission and partial remission were defined as effective remission.

None-remission was defined as a value for urinary protein excretion that remained at or above 3 g per 24 h, or a value of 0.3-2.9 g per 24 h but with an ALB concentration of less than 3.0 g/dL, an increase in the sCr concentration greater than or equal to 0.6 mg/dL (50 μmol/L), or a value for creatinine clearance that was more than 15% above the baseline value, or the discontinuation of treatment due to side effects [3].

The study patients were 37 (25, 47.5) years of age, included 70 females and 7 males, and the remission to CTX therapy occurred in 62 patients (80.5%, 57 females and 7 males). No statistically significant difference about the baseline levels of laboratory parameters was noted between remission group and none-remission group. The values about urinary protein, ALB and serum creatinine in remission group were markedly improved compared to none-remission group after CTX therapy for 6 months (Table1).

Genotype of GSTA1(C>T, rs3957356) was performed in all subjects using SNaPshot as described previously [4]. We observed that GSTA1 CT variants had a significant risk of none-response compared to wild-type GSTA1 gene (P=0.005, Figure 1).

	Remission group, N=62	None-remission group, N=15	P
Age (year)	38 (25,47.3)	30 (22,49)	0.19
Gender			0.42
Female	57 (91.9)	13 (86.7)	
Male	5 (8.1)	2 (13.3)	
Body surface area(m^2)	1.51 (1.43,1.61)	1.65 (1.45,1.88)	0.2
Pathology			0.9
III	2	1	
IV	47	12	
V	2	0	
III+V	6	1	
IV+V	5	1	
Urinary protein (g/24h)			
Pre-treatment	2.66 (1.7,4.82)	3.74 (2,6.03)	0.36
Post-treatment	0.35 (0.13,1.24)	3.66 (1.48,4.46)	0.0006
ALB (g/L)			
Pre-treatment	29 (24.5,33.3)	28 (17,29.6)	0.07
Post-treatment	38(35,40)	27 (20,30)	0.0001
Serum creatinine (μmol/L)			
Pre-treatment	71(56,88)	80(44,237)	0.12
Post-treatment	63(52.5,72.5)	73(66,128.3)	0.01

Table 1: Demographic data of the study patients according to therapeutic response. Data were described as median (inter-quartile range, IQR) for continuous variables or number (frequency) for categorical variables; ALB, serum albumin concentration. No significant differences with respect to the characteristics of study population in baseline were observed between remission group and none-remission group. The values about urinary protein, ALB and serum creatinine in remission group were markedly improved compared to none-remission group after CTX therapy for 6 months. P values were determined using Fisher's exact test.

Pharmacokinetics study was completed in 22 randomly from 77 patients. Blood samples were collected at 0, 0.5, 1, 3, 6, 10 and 22 h after CTX-infusion initiation according the method reported [5]. The concentrations of CTX and 4-OH-CTX were measured by HPLC-coupled with electrospray ionization tandem mass spectrometry (LC-MS/MS) at seven time points. Non-compartmental pharmacokinetic estimates of CTX and 4-OH-CTX were obtained using Winnonlin v4.1. The area under the curve (AUC), one of primary parameters which could typically reflect drug pharmacokinetics, is commonly used to describe the exposure to drug metabolites. In this part, we aimed to analyze the interplay between gene, pharmacokinetics and clinical efficacy of CTX.

On analyzing the single genotypes of GSTA1 to parent compound CTX and active metabolic 4-OH-CTX pharmacokinetics, we found that the exposure to active 4-OH-CTX, not CTX, was significantly affected by GSTA1 variant (P=0.023, Figure 2A). Patients carrying the GSTA1 heterozygous variant had statistical lower exposure to 4-OH-CTX compared to wild-type patients.

As expected, the remission patients had a significant higher exposure to 4-OH-CTX compared to none-response patients (P =0.038, Figure 2B). However, pharmacokinetics of pro-drug CTX did not statistically influence therapeutic efficacy in our study.

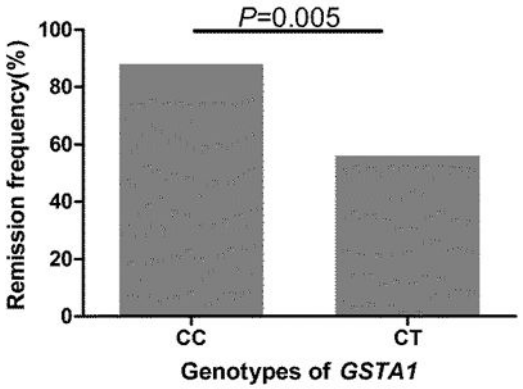

Figure 1: Relationships between Genotypes of GSTA1 and therapeutic efficacy in 77 LN patients- Patients with GSTA1 CT variants had a significant risk of none-response compared to wild-type GSTA1 (P=0.005). GSTs, glutathione-S-transferase gene; P value was determined by Fisher's exact test.

Adverse drug reactions (ADRs) of CTX, such as GI symptoms, myelotoxicity and amenorrhea based on the National Cancer Institute Common Toxicity Criteria (CTC) [6], were observed during 6 months follow-up. Almost 20% patients happened ADRs, whereas no relation to different genotypes of GSTA1 or pharmacokinetic of active 4-OH-CTX.

Discussion

Inter-patient variability with respect to CTX therapy outcomes has not been fully elucidated, especially the interplay between pharmacogenomics, pharmacokinetics and pharmacodynamics of CTX. Our study attempts to explain the influence of pharmacogenomics on CTX therapy in LN patients. We find that patients carrying the GSTA1 CT genotype were much poor responding to CTX therapy compared to wild-type patients.

GSTA1, as the highest catalytic activity for GSH conjugation of CTX, has been reported to detoxify the alkylating cytotoxic drugs by conjugation of glutathione (GSH) [2]. Because of the importance of the GSTA1 enzyme in CTX metabolism, reducing GSTA1 activity toward drug substrates, usually due to polymorphisms [7], can theoretically increase systemic exposure to CTX and improve therapeutic effects. However, studies of GSTA1 gene mutations on treatment efficacy to supporting this speculation are limited. To our knowledge, Carol's group [8] was the first to report that breast cancer patients homozygous for the GSTA1 allele, had greater overall survival during the first 5 years after diagnosis. Several clinical conditions, particularly

immunological diseases, such as SLE, have been associated with reduced GSH [9], which could hamper conjugation reactions with CTX metabolites. This, to some extent, may explain the discrepancies in reported effects of GST gene polymorphisms on CTX efficacy.

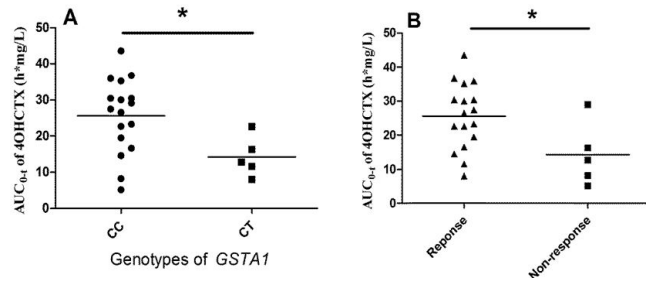

Figure 2: The interplay between genotype, pharmacokinetics and clinical efficacy of 4-OH-CTX. **Figure A:** The differences of the median area under the curve (AUC) of 4-hydroxycyclophosphamide (4OHCTX) according to the genotypes in 22 lupus nephritis (LN) patients; GSTA1*A heterozygous variant had statistical lower exposure to 4OHCTX compared to wild-type patients (AUC4OHCTX: 12.8(9.8, 19.5) vs.27.5 (18.1, 32.8) h*mg/L, P =0.023).GSTsglutathione-S-transferases gene. **Figure B:** Association between AUC of 4OHCTX and therapeutic outcome to cyclophosphamide (CTX) treatment in 22 lupus nephritis (LN) patients. Clinical remission was significantly related to higher exposure to 4OHCTX (AUC4OHCTX: 26.5 (18.1, 32.8) vs.12.8 (6.7, 22.7) h*mg/L, P =0.038).

We focused our hypothesis on modification of CTX pharmacokinetics as the most likely explanation of associations between the GSTA1 CT genotype and worse response to CTX therapy. We sought to identify GSTA1 gene variant factors that altered the disposition of CTX and activate metabolite, 4-OH-CTX. The GSTA1 SNP was significantly correlated with exposure to 4OHCTX, but not CTX. There was still in arguing with the influence of GSTA1 SNPs on the pharmacokinetics of CTX [10-12]. Therefore, more studies are needed to clarify relationships between GSTA1 variants and CTX pharmacokinetics.

Our data further show that changes in the disposition of 4-OHCTX more greatly affect the clinical response to CTX therapy than modifications to the disposition of CTX. 4OHCTX, only active metabolite, is the crucial determinant of delivery of phosphoramide mustard to cells, and measurements of exposure to 4OHCTX may be more predictive of CTX efficacy than measurements of CTX [13].

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