Cytochrome P450 as A Predictor of Ovarian Toxicity and Clinical Response to Pulse Cyclophosphamide in Systemic Lupus Erythematosus Patients

T H E S I S

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Abstract

Systemic lupus erythematosus is a chronic, multisystem, inflammatory autoimmune disease of unknown origin characterized by the production of nonorgan-specific auto-antibodies and a broad spectrum of clinical and immunological manifestations. This study was conducted to assess the association of cytochrome P450 polymorphism with ovarian toxicity and clinical response in patients with systemic lupus erythematosus treated with intravenous pulse cyclophosphamide. Forty seven SLE patients and twenty control subjects were included in this study. The association between cytochrome P450 (CYP2C19) genotypes and the incidence of ovarian toxicity in SLE patients was significant. Also, the association between cytochrome P450 genotypes of SLE patients and their hormonal levels was highly significant.

Key words:

- **Systemic lupus Enythematosus,**
- **Cytochrome P450.**
- **Polymorphism.**
- **Ovarian toxicity.**

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LIST OF ABBREVIATIONS

ACR	American College of Rheumatology
ADR	Adverse Drug Reactions
ALT	Alanine Transaminase
ANA	Antineuclear Antibody
AST	Aspartate Transaminase
BMT	Bone Marrow Transplant
CNS	Central Nervous System
CrCl	Creatinine Clearance
CYC	Cyclophosphamide
CYP	Cytochrome P 450
E	Estrogen
E2	Estradiol
EBV	Ebstein-Barr Virus
ECG	Electrocardiogram
ESR	Erythrocyte Sedimentation Rate
ESRD	End Stage Renal Disease
Fig	Figure
FSH	Follicle Stimulating Hormone
GERD	Gastro-Esophageal Reflux Disease
GnRH	Gonadotropin Releasing Hormone
GnRHα	Gonadotropin Releasing Hormone Agonist
Hb	Hemoglobin
Hct	Hematocrite
HLA	Human Leukocytic Antigen
hr	hour
HT	Hormone Replacement
Inh A	Inhibin A

Inh B	Inhibin B
IUD	Intra uterine Device
IVF	Invitro Fertilization
IVM	Invitro Maturation
L	Litre
LH	Leutinizing Hormone
Max	Maximum
mg	milligram
Min	Minimum
ml	Milliliter
N	Number
P	Progesterone
PCR	Polymerase Chain Reaction
RBC	Red Blood Cell
RFLP	Restriction Fragment Length Polymorphism
SD	Standard Deviation
SLAM	Systemic Lupus Activity Measure
SLE	Systemic Lupus Erythematosus
SNP	Single nucleotide Polymorphism
TBA	Total Body Area
TIA	Transient ischemic attack
TLC	Total Leukocytic Count
TRM	Transplant Related Mortality
UV	Ultraviolet
WBC	White Blood cell
wt	wild type
yrs	years

Introduction

Systemic lupus erythematosus (SLE) is a chronic usually life-long, autoimmune disease, characterized by exacerbations and remissions. The hallmark of the disease is the immune system deregulation resulting in the production of nonorgan specific autoantibody (ANA), generation of circulating immune complexes and activation of the complement system ***(Katsifis and Tzioufas, 2004).***

Women and minorities are disproportionately affected and SLE is most common in women of childbearing age ***(Boumpas et al., 1993).***

Therapy with cyclophosphamide (CYC) is required in 15-20% of patients with SLE with major organ disease ***(Singh et al., 2007).*** Pulsed intravenous CYC is considered as a standard therapy for lupus nephritis and several other manifestations of SLE. While the response rate to intravenous cyclophosphamide is substantial, concern has arisen about its toxicity. In addition to increased susceptibility to infections, bone marrow suppression, alopecia, hemorrhagic cystitis and malignancy, ovarian failure is an important side effect associated with the use of cyclophosphamide ***(Ioanidis et al., 2002).***

Cyclophosphamide is an oxaphospharine alkylating agent with cytotoxic and immunosuppressive effects. Cyclophosphamide itself is not cytotoxic but it is enzymatically converted by hepatic microsomal enzymes cytochrome P450 (CYP) to multiple metabolites ***(Tsokos, 1987).***

Several CYP are implicated in metabolism of CYC. The enzymes CYP2B6 and CYP2C19 are genetically polymorphic and are known to have variant alleles, resulting in wide interpatient variability on exposure to cyclophosphamide ***(Lang et al., 2001).***

Ovarian toxicity is of major concern in premenopausal women receiving CYC, and 12-59% develop premature ovarian failure ***(Huoang et al., 2002).*** Younger patients and lower cumulative dose of CYC are associated with lower risk of ovarian failure ***(McDermott and Powell, 1996).*** Genotyping, therefore, may be used to identify patients who are at higher risk of premature ovarian failure ***(Takada et al., 2004).*** Co-treatment with gonadotropin-releasing hormone agonists may preserve future fertility and ovarian function in these young women. Ovarian banking before administration of cyclophosphamide should be considered in selected patients ***(Katsifis and Tzioufas, 2004).***

Aim of the work

The aim of the work is to study the association of cytochrome P450 (CYP2C19) genetic polymorphism with ovarian toxicity and clinical response in patients with systemic lupus erythematosus (SLE) treated with intravenous pulse cyclophosphamide.

Systemic Lupus Erythematosus (SLE)

Systemic lupus erythematosus is a prototypic autoimmune disease characterized by the production of auto-antibodies to components of the cell nucleus in association with diverse clinical manifestations encompassing all organ systems ***(Pisetsky, 2008).***

SLE primarily is a disease of young women, with a peak incidence between the ages of 15 and 40 and a female: male ratio of 6 to 10 : 1 ***(Pisetsky, 2008).***

SLE affects approximately 1 in 1000 to 2500 in the general population, but disease incidence in African American and Latino women is much higher (up to one in 250 in African American women between the ages of 18 to 65 years). The 5-year survival after diagnosis is 90% ***(Gravel et al., 2007).***

The extreme heterogeneity of the disease has led some investigators to propose that SLE represents a syndrome rather than a single disease ***(Tassiulas and Boumpas, 2009).***

Pathogenesis:

The pathogenesis of systemic lupus erythematosus (SLE) is complex, as shown in **Fig. (1).**

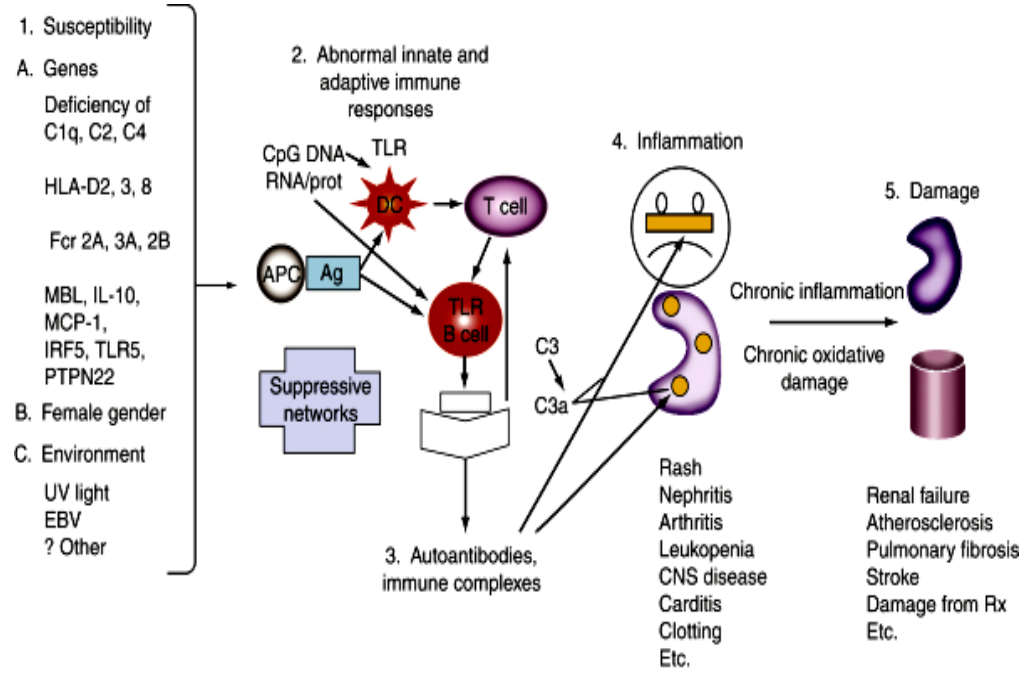

Fig. (1): Overview of the stages and pathogenesis of systemic lupus erythematosus (SLE). The immune abnormalities that characterize SLE are pictured in five phases. In phase 1, an individual is susceptible to SLE because of genetics, gender, and the external environment that influences antigen presentation and other immune responses. In an individual with adequate numbers of predisposing factors, stage 2 develops, consisting of abnormal persistence of antigens, including hypomethylated DNA (CpG DNA) in DNA/anti-DNA complexes, and other DNA/protein and RNA/protein self-antigens. These antigens activate cells of innate (dendritic cells [DC]) and adaptive (B-cells) immune systems via Toll-like receptors (TLR); the activated cells then activate T lymphocytes. The adaptive system is working at the same time, where antigen presenting cells (APC) present self-antigens to T-lymphocytes and B lymphocytes; the B cells mature to plasma cells and secrete autoantibodies. At the same time, suppressive networks (regulatory and inhibitory T cells, phagocytic cells, idiotypic networks) are in place to dampen the harmful immune responses. Phase 3 begins with a clinically healthy individual having positive tests for autoantibodies in the serum. Immune complexes form. Phase 4, following phase 3 by a mean of 3 years, is clinical disease. The result of complement activation and other proinflammatory responses from tissue attacked by autoantibodies and immune complexes causes symptoms and signs of disease, which can include the features listed in the figure. Phase 5 occurs after months and years of chronic inflammation and chronic oxidative damage, which promote scarring of tissue such as kidney and lung, plaque deposition in arteries, and clotting. Irreversible tissue damage occurs. It is likely that at each of these phases, some individuals progress to the next phase, and others do not. CNS, central nervous system; EBV, Epstein-Barr virus; UV, ultraviolet (Hahn and Tsao, 2009).