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Effect of Cyclosporine A on the expression of *GSTO2* metabolizing enzyme in Jurkat cell line

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A B S T R A C T

Cyclosporine A (CsA), a cyclic polypeptide metabolite extracted from the fungus, is used clinically to combat organ graft rejection in transplant subjects. Previous studies have shown that CsA exposure enhances the production of reactive oxygen species (ROS) and lipid peroxidation, which are directly involved in CsA toxicity. To protect cells and organs against ROS, the human body has evolved a highly antioxidant protection system to neutralize free radicals. The aim of this study was to investigate the effect of CsA on mRNA expression of anti-oxidant *GSTO2*. To do this, Jurkat cells were incubated for 24 h with different doses of CsA, ranging from 1-80 µg/ml, and the IC50 of CsA was calculated to be 40 µg/ml. Subsequently, Jurkat cells were treated with 3 µg/ml CsA for 24 h and the gene expression of *GSTO2* was quantified by quantitative Real-time PCR. Results showed that the mean (SD) expression of the *GSTO2* gene in CsA treated cells was 1.10 (0.07) (when assuming an expression level in untreated cells of 1.0). However, statistical analyses showed that the alterations were not significant (t=2.29, df=2, P=0.149). These findings suggest that at this concentration of CsA, other antioxidant enzymes are up-regulated in Jurkat cell lines to detoxify free radicals induced by CsA.

Key words: Cyclosporine A, *GSTO2*, Gene expression, Real time PCR

INTRODUCTION

Cyclosporine A (CsA), a cyclic undecapeptide, was derived from extracts of Topocladium inflatum gams, a member of the Fungi imperfecti family [1]. CsA is a powerful immunosuppressive agent that inhibits T helper lymphocyte proliferation and hence depresses both cellular and humoral immune response. It can be used to inhibit the inflammatory reaction in the prevention and treatment of organ transplant rejection episodes [2-4]. Apart from transplant medicine, CsA is also used in a number of autoimmune diseases: dematological-psoriasis; severe atopic dermatitis; pyoderma gangrenosum; rheumatologic-rheumatoid arthritis and gastrointestinal such as ulcerative colitis and Crohn's disease [5-9]. Also, for the treatment of severe dry eye disease, CsA, which has an anti-inflammatory effect, is frequently used [10]. However, despite its wide spectrum of clinical cases, CsA is known to cause several side effects

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including nephrotoxicity, hepatotoxicity and neurotoxicity, among which nephrotoxicity is one of the most important [11, 12]. The cellular/biochemical mechanisms by which CsA causes toxicity is poorly understood. However, numerous studies support the hypothesis that CsA induced toxicity may be the consequence of oxidative stress [13-16]. The protective role of antioxidants was found in CsA-induced nephrotoxicity and hepatotoxicity upon decreased lipid peroxidation and reactive oxygen species (ROS) [17].

The presence of oxidative stress can alter normal cellular homeostasis by modifying proteins involved in DNA repair and anti-oxidant enzymes; activating signal transduction pathways involved in cell survival, apoptosis and inflammation [18, 19]. The Glutathione S-transferases (GSTs; EC 2.15.1.18) superfamily is one of the great anti-oxidant enzymes involved in phase II of the detoxification and biotransformation of exogenous and endogenous compounds [20]. In human beings, this superfamily comprises several classes including class omega (*GSTO*). The GSTO has some different structural and functional characteristics from other GSTs. They have a cysteine residue in their active site in contrast to serine or tyrosine that exist in active sites of other subfamilies. The GSTO class has two members, *GSTO1* and *GSTO2* [21]. The aim of the present study was to evaluate the effect of CsA on the expression of GSTO2 detoxification enzymes in the Jurkat cell line.

MATERIALS AND METHODS

Cell culture: Jurkat cells (from ATCC) were grown in a suspension in RPMI-1640 supplemented with 10% heat-inactivated fetal calf serum and 0.1 mg/ml streptomycin and 1000 IU/ml penicillin (Gibco, Invitrogen) in a humidified atmosphere containing 5% CO_2 at $37^{\circ}C$ with passage every 2-3 days. For experimental use, cells were plated into 10 cm dishes at 3×10^6 cells per dish and treated with an indicated concentration of CsA for 24h.

MTT assay: Cell viability was assessed with MTT assay. The cells (3×10^4) were placed in 200 µL of media with or without indicated concentrations of CsA in each well of a 96-well flat-bottomed microtiter plates in triplicate cultures for 24 h and treated with an MTT reagent [3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyl-tetrazolium bromide] for 4 h. The precipitated formazan was dissolved with SDS and the optical density was measured at 570 nm using an ELISA reader (Bio-rad 550, USA). The living cells were expressed as a percentage of absorbance in cells with CsA treatments to that in the control cells and the IC50 was determined.

RNA extraction and cDNA synthesis: The total RNA was extracted from the cells using RNX-plus™ solution (Cinnagen, Iran) according to the manufacturer's protocol. RNA concentration was measured for each sample at a wavelength of 260 nm (A_{260}). The purity of extracted total RNA was determined by the A_{260}/A_{280} ratio. The A_{260}/A_{280} ratio was between 1.8 and 2.1. Synthesis of cDNA was done with a Primescript RT reagent kit perfect real time (Takara Biotechnology Dalian CO., Ltd.).

Real time PCR: Relative quantitative polymerase chain reaction was performed on a Rotor-gene 6000 real-time PCR system (Corbett Life Science) using SYBR green PCR master mix (Takara Biotechnology Dalian CO., Ltd.). The sequences of PCR primers for *GSTO2* and glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) are listed as Table 1.

Table 1: Nucleotide sequence of primers for Real-time PCR amplification

Primer	Sequence
GSTO2	F: 5' CCGCATCTACAGCATGAGG 3'
	R: 5' CCCACATCTCAACGCTACC 3'
GAPDH	F: 5' CGTGGAAGGACTCATGACC 3'
	R: 5' CCAGGGGTCTTACTCCTT 3'

The *GAPDH* gene was used as a reference gene to normalize target gene expression. Specific primers were designed using the sequences obtained in GeneBank, through the Blast program (http://www.ncbi.nlm.nih.gov/blast/blast.cgi). PCR reactions were performed for all genes: 30 s of pre-incubation at 95 °C followed by 40 cycles for 15 s at 95 °C and 30 s at 65 °C and 20 s 72 °C. Specificity of PCR products was tested according to the dissociation curves.

Relative values of transcripts were calculated using the equation: $2^{-\Delta Ct}$, where ΔCt is equal to the difference in threshold cycles for the target and reference. All reactions were performed in triplicates.

Statistical analysis: The data were expressed as fold-change relative to untreated cells, after normalizing to *GAPDH*. Results were expressed as mean ± standard deviation (SD) from three independent experiments. A one sample Student *t*-test was applied to detect differences of the expression level between the two groups. SPSS 16.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analyses. The $P<0.05$ level was considered as statistically significant.

RESULTS AND DISCUSSION

The inhibitory effect of CsA on cell viability was determined by MTT assay. As indicated in Fig. 1, CsA reduced viable cell numbers in a dose dependent manner and concentration range of 1-80 µg/ml. According to our data, the concentration of CsA around 40 µg/ml caused approximately 50% loss of cell viability (IC50) at 24 h.
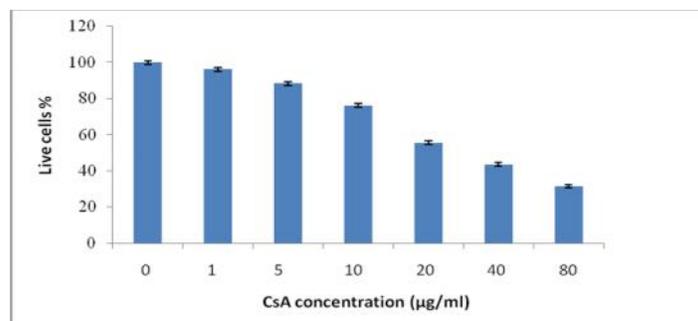

Figure 1: Inhibition of Jurkat cells viability by CsA treatment. Cells were incubated with indicated concentrations of CsA for 24 h. The relative number of living cells was determined by MTT assay. Values were the means ± SD of three independent experiments.

To investigate the effect of CsA on the expression of *GSTO2*, a concentration of CsA (3 µg/ml) exhibiting low toxicity in the MTT assay was used. By employing the real-time PCR analysis, we found that the mean (SD) expression of the *GSTO2* gene in CsA treated cells was 1.10 (0.07) (when assuming the expression level in untreated cells to be 1.0). However, statistical analysis showed that the alterations were not significant (t=2.29, df=2, P=0.149).

CsA immune-suppression is associated with many side effects including nephrotoxicity, hepatotoxicity and neurotoxicity [11, 12]. Numerous studies provide insight into the mechanism of CsA side effects due to the over production of ROS [13-16]. Both enzymatic and non-enzymatic antioxidants function synergistically to neutralize free radicals in the body as a defense system [22]. Oxidative stress usually changes the expression of anti oxidative enzymes. However, the present work does not support the idea that exposure to CsA is associated with any significant induction of the *GSTO2* metabolizing enzyme in Jurkat cell lines. Our results suggest that at this concentration and exposure time, other antioxidant enzymes are upregulated and detoxify free radicals induced by CsA.

Several limitations of the present study are worth mentioning. Here we study the effect of CsA on the mRNA level of *GSTO2* in the Jurkat cell line. Since the GST superfamily contains eight classes including alpha, mu, pi, theta, sigma, omega and kappa, further studies are required to investigate the effect of CsA on the expression of other GSTs and other antioxidant enzymes.

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CONFLICTS OF INTEREST: None

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