

SHORT PAPER

Th1, Th2 and Th17 Cytokine Profile in Patients with Multiple Sclerosis Following Treatment with Rapamycin

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ABSTRACT

Background: Management of multiple sclerosis (MS) is based on the usage of immunosuppressive and immune-modulating medications. Cytokines play an important role in the pathogenesis of MS. **Objective:** To evaluate the effects of rapamycin on the concentrations of Th1/Th2/Th17 serum cytokines in patients with MS. **Methods:** Six patients with relapsing remitting MS as a case group and 6 healthy individuals as a control group were enrolled. The patients have been receiving 2 mg rapamycin daily for 6 months. The individuals in control group received nothing during 6 months of the experiment. Enzyme linked immunosorbent assay (Simultaneous Multi-Analyte ELISA) technique was used for determination of serum concentrations of IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, IL-13, IL-17, IFN- γ , TNF- α , G-CSF and TGF- β before and after therapy with rapamycin. **Results:** The mean absorbance of 10 out of the 12 studied cytokines showed reduction after the therapy with rapamycin including IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, IL-13, IL-17, IFN- γ and TNF- α . The only statistically significant reduction was observed in the absorbance of IFN- γ ($p=0.028$). Two cytokines illustrated increase in the patients sera after the therapy, including G-CSF and TGF- β , but only increase in TGF- β was statistically significant ($p=0.046$). None of the studied cytokines in the control group varied significantly after 6 months. **Conclusion:** Based on the findings of this study, rapamycin has some immunosuppressive effects, such as decreasing IFN- γ , which can improve the quality of life of the patients with multiple sclerosis. Also the increased level of TGF- β may also have benefits on the disease, which needs further clinical studies.

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Keywords: Cytokine Profile, Multiple Sclerosis, Rapamycin

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INTRODUCTION

Multiple sclerosis (MS) is a degenerative chronic inflammatory disease that affects the central nervous system (CNS) of human. It is primarily an autoimmune disorder, in which infiltration of lymphocytes to inflammatory foci cause demyelination, axons injury and loss of neurological function of CNS. MS is not curable and the cause of the disease is not known (1,2). MS is a T cell mediated disease, and these cells are believed to play a major role in the pathogenesis of the disease. Among them, Th1, Th2 and Th17 (T) cells are the candidate T cell subsets which may play a role in the pathogenesis of MS (3-5). Management of the disease is based on the administration of immunosuppressive and immune-modulating medications, which have been used for several decades (6). Today, therapy of MS is often disease-modifying, which reduces the attack rate of the disease and delays its progression (6). However, some patients do not respond to the routinely used medications such as interferon-beta 1a or glatiramer acetate (7). Rapamycin, a novel immunosuppressive agent, has been practiced for successful phase I, II, and III clinical trials and is approved to be used for prevention of transplant rejection (8). Treatment with this drug causes negligible nephrotoxicity and it has got tolerable side-effect profile which is easily tolerated by the patients (6,9,10). Rapamycin induces apoptosis in the lymphocytes, whether they are activated or not (11). It also exerts its effect by preventing the activation of B and T cells through inhibition of their response to interleukin-2 (12). Cytokines are very important components of the inflammatory process and also contribute in axonal degeneration, oligodendrocyte cell death, and neuronal dysfunction. These characteristics are involved in the pathogenesis of MS (13). Pathogenesis of MS is mostly linked to autoimmunity mediated by Th1/17 (T) cells, but Th2 activation may help management of the disease (14).

Since rapamycin has not been used for management of MS in other clinical trials and because of the importance of cytokines in the pathogenesis of MS, this study planned to evaluate the immunological effects of rapamycin on the level of Th1/Th2/Th17 serum cytokines in patients with MS.

MATERIALS AND METHODS

In this clinical trial study (IRCT2012092510936N1), six patients with MS, including two males and four females with mean age of 25.83 ± 5.5 years, referred to Ayatollah Kashani Teaching Hospital were chosen and were enrolled in the study. The patients had MS for 1 to 10 years (mean= 4.83 ± 3 year). All six patients had the following conditions: relapsing remitting MS defined by McDonald criteria (15), MRI records with evidence of demyelination, 0-6 expanded disability status scale (EDSS) and lack of response to interferon beta-1a or glatiramer acetate. The patients stopped using any medication specially interferon-beta 1a or glatiramer acetate one month before the experiment.

The patients with the following conditions were not included in the study; primary progressive MS, having history of steroid and immunosuppressive therapy, sign of any infection or cancer, high serum cholesterol, hematological and cardiovascular disorders, history of hepatitis C and B, hepatic cirrhosis or other treatment-required liver diseases, treatment-required renal diseases, lung diseases, diabetes, CMV infection, HIV

infection, hyperthyroidism, tuberculosis, alcohol misuse during last 6 months and lactating and pregnant women.

All six patients received rapamycin (Rapacan, Biocon, India), two tablets (1 mg) each day for six months (16), initiated in May 2013. To ensure the safety of rapamycin on the patients, their clinical symptoms were monitored on days 0, 30, 60, 90, 120, 150 and 180. Also adverse side effects of the medication and the patients' blood parameters were monitored during the study. For control, 6 healthy individuals enrolled to the study, but none had received the experimented drug or other medications.

Serum samples of the patients and healthy controls were taken before and after the therapy with rapamycin and they were kept frozen at -80°C until the experiments. Enzyme linked immunosorbent assay (Multi-AnalyteELISArray, QIAGEN[®]) method was used for determination of serum levels of interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-6 (IL-6), interleukin-10 (IL-10), interleukin-12 (IL-12), interleukin-13 (IL-13), interleukin-17 (IL-17), interferon gamma (IFN- γ), tumor necrosis factor-alpha (TNF- α), granulocyte-colony stimulating factor (G-CSF) and transforming growth factor-beta (TGF- β) before and after therapy with rapamycin. The ELISA procedure carried out according to the instruction of the manufacturer and absorbance at 450 nm was reported as the result of the assay.

Data were analyzed by SPSS software (version 16.2, SPSS Inc., Chicago, IL, USA) through Wilcoxon test.

RESULTS AND DISCUSSION

During and after the treatment, no remarkable clinical side effects were observed. The mean absorbance of 10 (83.33%) out of the 12 studied cytokines showed reduction after the therapy with rapamycin including that of IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, IL-13, IL-17, IFN- γ and TNF- α . The only statistically significant reduction was observed in the absorbance of IFN- γ ($p=0.028$), but not for other studied cytokines (Table 1). In the control group, none of the studied cytokines varied significantly after the six months of the experiment (Table 2). Two cytokines illustrated an increase in the sera of patients after the therapy, including G-CSF and TGF- β . This increase was significant only for TGF- β ($p=0.046$). Mean absorbance of the studied cytokines before and after treatment with rapamycin are illustrated in Figure 1.

IL-17 is produced by Th17 (T) cells and its production is under influence of TGF- β and some other cytokines in mice (5). TGF- β is an immunosuppressive factor which inhibits proliferation of IL-1-dependent lymphocytes (17). A pleiotrophic cytokine, TGF- β , has numerous functions. Its role in the Th17 cell-differentiation that can be involved in pathological outcomes in central nervous system has been controversial. TGF- β in the presence of IL-6 enhances the development of myelin-specific Th17 cells. Th17 cells reported to be not encephalitogenic and reduce the expression of molecules associated with encephalitogenic T cells (18). In the present study, TGF- β has been increased significantly after treatment with rapamycin, which may favor management of MS.

TGF- β possesses variety of functions in systemic immunity such as antibody generation, T cell differentiation, suppression of immune system, and tolerance induction.

Table 1. Mean absorbance of the studied serum cytokines before and after 6 months of therapy with rapamycin (n=6).

	Cytokines											
	IL-2	IL-4	IL-5	IL-6	IL-10	IL-12	IL-13	IL-17	IFN- γ	TNF- α	GCSF	TGF- β
Mean Absorbance												
Before	.478	.566	.513	.896	.860	.213	.346	.138	.893	.188	.736	1.756
After	.403	.438	.483	.763	.78	.196	.283	.128	.553	.158	.748	2.165
Z	-1.89	-1.36	-.31	-1.78	-.84	-.42	-.94	-.31	-2.20	-.31	-.31	-1.99
P Value	.058	.173	.753	.074	.400	.674	.345	.752	.028	.753	.753	.046

Table 2. P value estimated for studied cytokines among controls in day 0 compared to day 60 (the controls did not get the drug).

	IL-2	IL-4	IL-5	IL-6	IL-10	IL-12	IL-13	IL-17	IFN- γ	TNF- α	GCSF	TGF- β
Z	-1.36	-.12	-1.81	-1.76	-1.78	-.11	-.10	-.31	-.13	-1.16	-.10	-.73
P value	.173	.927	.097	.073	.075	.919	.917	.752	.931	.244	.917	.463

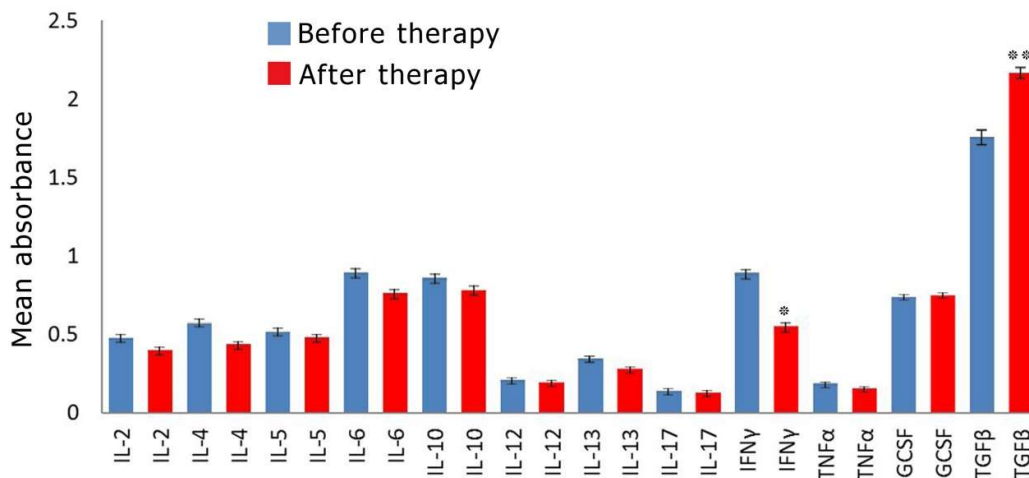


Figure 1. the level of studied cytokines before and after therapy with rapamycin.
 * $P=0.028$
 ** $P=0.046$

It is also essential for the differentiation and development of FoxP3⁺ regulatory T cells (19). TGF- β is well known for possessing inhibitory effects on the T cell responses and inducing differentiation of FoxP3⁺ T cells.

These effects show conflicting roles of TGF- β , as an inducer of pro-inflammatory Th17 cells and anti-inflammatory Tregs (20). We observed a significant reduction in serum IFN- γ , which is a pro-inflammatory cytokine and this reduction may benefit the treatment of the disease. IFN- γ is also reported to be in association with increased risk of relapse in patients with MS (21). In a study conducted by Jorgensen *et al.* (2001), the effects of rapamycin, tacrolimus and cyclosporine A on the production of cytokines on human whole blood have been studied. They reported a strong inhibitory effect of rapamycin on IL-10 secretion induced by bacterial products. On the contrary, cyclosporine A and tacrolimus reduced the TNF- α production in response to bacterial lipopolysaccharides (LPS) (22). In the present study, production of IL-10 decreased by rapamycin, but it was not significant ($p=0.400$). Mahon *et al.* (2003) studied cytokine profile of the patients with MS supplemented with vitamin D for six months. They reported increase in TGF- β 1 levels, but variable and inconclusive data for IFN- γ , IL-2, TNF- α and IL-13. Similar to their findings, we found that TGF- β was increased after six months of therapy with rapamycin (23). Regulatory T cells produce TGF- β 1, which slows down the development of EAE in mice (24). The increased TGF- β following treatment with rapamycin indicates that rapamycin could potentially ameliorate the symptoms of MS patients. In mice, rapamycin has been reported to exert inhibitory effects on relapsing–remitting experimental autoimmune encephalomyelitis (RR-EAE) (25).

In control group none of the studied cytokines was varied significantly after the six months of the experiment (Table 2). Two cytokines illustrated an increase in the sera of the studied patients after the therapy, including G-CSF and TGF- β . This increase was

significant only for TGF- β ($p=0.046$). Mean absorbance of the studied cytokines before and after treatment with rapamycin are illustrated in Figure 1.

Our report indicated that rapamycin could be a potent candidate for the treatment of MS. Thus based on the results of the present study, rapamycin increase an immunosuppressive agent, TGF- β , and decreases the pro-inflammatory cytokine, IFN- γ , after six months of treatment and it may be a potential treatment choice for management of MS. Based on the findings of the present study, rapamycin has some immunosuppressive effects, such as decreasing IFN- γ , which can improve the life quality of the patients with multiple sclerosis. The increased level of TGF- β may also have benefits on the disease, but needs further clinical studies. For better understanding of the effect of rapamycin on cytokine production in patients with MS and evaluate its therapeutic potentials, further studies with the bigger sample size, can be suggested. Also another clinical trial on the clinical effects of the drug on MS patients seems to be necessary to be carried out in order to determine its benefits and harms for these patients.

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