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Lack of association between CXCL9 and CXCL10 gene polymorphisms and the outcome of rheumatoid arthritis treatment with methotrexate

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Abstract. – OBJECTIVE: Methotrexate (MTX) in low doses is used in the therapy of rheumatoid arthritis (RA). The aim of many studies is to identify factors predicting the outcome of treatment with methotrexate in rheumatoid arthritis. The action of MTX in RA is associated with the inhibition of inflammatory mediators synthesis. CXCL9 and CXCL10 chemokines play the important role in inflammatory response in RA patients. The aim of this study was to examine the association between CXCL9/10 gene polymorphisms and response to therapy of RA patients with MTX.

PATIENTS AND METHODS: The study included 422 patients diagnosed with rheumatoid arthritis, treated with MTX in doses 20 mg weekly. Good responders were defined as patients who were receiving MTX and had a DAS28 of ≤ 2.5 at 6 months of therapy. Poor-responders were defined as patients who were receiving MTX and had a DAS28 of > 2.5.

RESULTS: There were not statistically significant associations between studied polymorphisms and the outcome of rheumatoid arthritis treatment with methotrexate.

CONCLUSIONS: The results of this study suggest lack of associations between the polymorphisms in CXCL9 and CXCL10 genes and the response to MTX in RA patients.

Key Words:
Chemokines, Methotrexate, Polymorphism, Rheumatoid arthritis.

Introduction

Rheumatoid arthritis (RA) is a multifactorial disease and its treatment is mainly based on disease modifying antirheumatic drugs (DMARDs)[1]. The optimal strategy in RA therapy is to use (DMARDs) in an early stage of disease in order to reduce disease activity and to prevent joint destruction. This treatment goal is frequently achieved by methotrexate (MTX)[2]. MTX is a foliate antagonist used in the therapy of malignant disorders and low doses of methotrexate were introduced for the treatment of rheumatoid arthritis because of its antiproliferative, immunosuppressive and antiinflammatory properties[3,4]. MTX is however not effective in all patients and a significant proportion of patients stop the therapy because of inefficacy or adverse events. Identification of genetic determinants of drug efficacy and toxicity will be valuable because they may help in the individualization of the therapy in the patient before initiation of treatment. Previous studies have revealed that therapy with MTX causes the decreased production of mediators which are involved in the inflammatory process such as numerous cytokines and chemokines[5,6].

Chemokines have been classified into four families. One of these are the CXC chemokines. To these chemokines belong CXCL9 and CXCL10, secreted by various cell types, such as, neutrophils, monocytes, endothelial cells, fibroblasts, keratinocytes[7]. The high level of CXCL9/10 in peripheral liquids is a marker of immune response, especially involving Th1-cells. In tissues Th1 lymphocytes may be responsible for increased IFN-γ and TNF-α production, which in turn stimulates CXCL9/10 secretion[8-10]. CXCL9 and CXCL10 have been detected in sera, synovial fluid, and synovial tissue in RA patients[11-13]. In *CXCL9/10* genes several polymorphisms have been detected and correlated with various diseases[14-16]. CXCL9 and CXCL10 are the important mediators determining RA activity, the anti-inflammatory action of MTX is associated with the influence on chemokine synthe-

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Table I. Association between CXCL9 and CXCL10 gene polymorphisms and response to therapy of RA patients with MTX.

	Poor responders		Good responders					
	n	%	n	%	p^a		p^b	OR (95%) CI)
CXCL9 rs3733236 genotype								
GG	218	59.73%	147	40.27%	0.31	AA+GA vs GG	0.47	1.24 (0.71-2.18)
GA	29	52.73%	26	47.27%		AA vs GA+GG	0.52	-
AA	2	100.00%	0	0.00%		AA vs GG	0.52	-
						GA vs GG	0.38	1.33 (0.75-2.35)
						AA vs GA	0.49	-
CXCL9 rs3733236 allele								
G	465	93.37%	320	92.49%				
A	33	6.63%	26	7.51%		A vs G	0.68	1.14 (0.67-1.95)
CXCL10 rs8878 genotype								
GG	84	60.43%	55	39.57%	0.46	AA+AG vs GG	0.75	1.09 (0.72-1.65)
AG	120	56.34%	93	43.66%		AA vs AG+GG	0.35	0.77 (0.45-1.30)
AA	45	64.29%	25	35.71%		AA vs GG	0.65	0.85 (0.47-1.54)
						AG vs GG	0.51	1.18 (0.77-1.83)
						AA vs AG	0.27	0.72 (0.41-1.25)
CXCL10 rs8878 allele								
G	288	57.83%	203	58.67%				
A	210	42.17%	143	41.33%		A vs G	0.83	0.97 (0.73-1.28)

[a]χ^2 test; [b]Fisher exact test

sis and therefore the aim of this study was to examine the association between *CXCL9/10* gene polymorphisms and response to therapy of RA patients with MTX.

Patients and Methods

The study included 422 patients (340 female, 82 male, mean age 57.5 ± 12.4 years) diagnosed with rheumatoid arthritis, treated with MTX in doses 20 mg weekly. RA was diagnosed according to the ACR/EULAR criteria[17].

Evaluation of clinical efficacy

Good responders were defined as patients who were receiving MTX and had a DAS28 of ≤2.5 at 6 months of therapy (patients with remission of disease symptoms). Poor-responders were defined as patients who were receiving MTX and had a DAS28 of >2.5[18,19]. The study was approved by the local Ethics Committee and written informed consent was obtained from all subjects.

Genotyping

DNA was extracted from 200 µL of whole blood samples using a GeneMATRIX Quick Blood DNA Purification Kit (EURx, Poland). SNPs within the *CXCL9* (rs3733236 G>A) and *CXCL10* (rs8878 A>G) genes were genotyped using TaqMan genotyping assays from Life Technologies Genomic. Fluorescence data were captured using a 7500 FAST Real-Time PCR System (Applied Biosystems, Waltham, MA, USA).

Statistical Analysis

Chi-square or Fisher exact tests were used to compare genotype and allele frequencies between the study groups. $p < 0.05$ was considered statistically significant.

Results

The efficacy of RA therapy with MTX is presented in Table I. Under MTX therapy remis-

sion of RA symptoms was achieved in 40.27% of *CXCL9 GG* genotype carriers and in 47.27% of subjects with GA genotype. The differences were not statistically significant (Table I).

The remission of RA symptoms was observed in 39.57% of *CXCL10* GG genotype carriers, in 43.66% of subjects with AG genotype and in 35.71% with AA genotype. The differences were not statistically significant (Table I).

Discussion

MTX is the drug commonly used in the therapy of RA. The mechanisms by which MTX at low doses modulates inflammation in RA are still not fully explained. Many pharmacological mechanisms have been suggested, including inhibition of purine synthesis, promotion of adenosine release, inhibition of production of proinflammatory cytokines and chemokines, suppression of lymphocyte proliferation[3-6]. In this study we analyzed the *CXCL9* and *CXCL10* gene polymorphisms as the factors predisposing to the achieving of RA remission during therapy with MTX. Our results indicate that these polymorphisms are not the factors associated with the response to the therapy in RA patients treated with MTX. Many previous studies revealed the significant role of CXCL9 and CXC10 in the pathogenesis and inflammatory process in RA. Kuan et al[11] examined the association between CXCL9, CXCL10 serum levels and disease activity in RA. The authors observed that the CXCL9 and CXCL10 serum concentrations were significantly increased in patients with active RA compared to controls. The improvement in clinical activity in patients with RA was accompanied by a significant reduction in the serum concentration of these chemokines. Yoshida et al[12] analyzed gene expression in the microdissected synovial lining cells of RA patients, using those of OA patients as the control. Expression levels of the chemokines *CXCL9* and *CXCL10* were significantly higher in the synovium of RA than in that of OA. Ruschpler et al[20] investigated gene expression patterns in synovial tissue from RA and OA patients. These authors observed that *CXCR3* mRNAs, as well as *CXCL9* and *CXCL10* mRNAs, were significantly increased in RA as compared with OA. Elevated CXCR3 protein was found to be especially expressed on mast cells within synovial tissue from RA patients. These findings suggest that substantial expression of CXCR3 protein on mast cells within synovial tissue from RA patients plays a significant role in the pathophysiology of RA, accompanied by elevated levels of the chemokines CXCL9 and CXCL10. Above studies indicate that chemokines CXCL9 and CXCL10 are the important mediators associated with disease activity in RA patients. Many studies revealed that disease activity is the important factor determining the response of RA patients to MTX[21]. In our previous study we have detect the association between *CXCL10* (rs8878) G allele and RA[22]. In this study we examined whether *CXCL9* and *CXCL10* gene polymorphisms are associated with the response to the therapy in RA patients treated with MTX.

Conclusions

Although CXCL9 and CXCL10 play the important role in the RA pathogenesis, the results of this study suggest lack of associations between the polymorphisms in *CXCL9* and *CXCL10* genes and the response to MTX in RA patients. Nevertheless this hypothesis requires further investigations.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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