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Effect of allograft inflammatory factor-1 gene polymorphisms on rheumatoid arthritis treatment with methotrexate

Wpływ polimorfizmu genów allograft inflammatory factor-1 na leczenie reumatoidalnego zapalenia stawów metotreksatem

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Summary

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. AIF-1 is a cytokine playing a role in chronic inflammatory processes. The levels of AIF-1 were significantly increased in synovial fluid from patients with RA. The aim of this study was to investigate the association between AIF1 gene polymorphisms (rs2269475:C>T, rs2736182:G>A, rs2259571:A>C) and response to treatment of RA patients with MTX.

Material/Methods: The study was carried out on 221 patients diagnosed with active rheumatoid arthritis, treated with MTX. Good responders were defined as patients who were receiving MTX and had a DAS28 of ≤2.4 at 6 months.

Results: With regard to the *AIF1* rs2259571 polymorphism the remission of RA symptoms was observed in 52.99% of AA genotype carriers, in 45.25% of subjects with AC genotype, and in 32.84% with CC. The differences were statistically significant. CC vs AA p=0.03,OR 0.41, 95%CI (0.18-0.92).

Conclusions: The results of this study suggest that the patients with the rs2259571 *CC AIF1* genotype have a poorer response to therapy with MTX.

Keywords: **allograft inflammatory factor-1 • methotrexate • polymorphisms • rheumatoid arthritis**

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Introduction

Rheumatoid arthritis (RA) is a multifactorial disease and its treatment is mainly based on drugs modulating its course [14]. The optimal strategy in treatment of RA is to use effective disease-modifying antirheumatic drugs (DMARDs) in an early stage of disease in order to reduce disease activity and to prevent destructive arthropathy. This treatment goal is frequently achieved by methotrexate (MTX) [4]. 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 presumed antiproliferative, immunosuppressive and antiinflammatory properties [5,6]. 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 can be ascertained in the individual patient before initiation of therapy. Previous studies have revealed that therapy with MTX causes the decreased production of mediators which are involved in the inflammatory process [7]. Allograft inflammatory factor-1 (AIF-1) is a cytoplasmic, inflammation-responsive protein that has been implicated in the regulation of inflammation. AIF-1 was first identified by Utans et al. [23] in 1995 in rat cardiac allografts with chronic rejection, in which the inflammatory reaction played a key role. Autieri et al. [3] demonstrated that expression of AIF-1 was correlated with the severity of cardiac rejection in clinical heart transplantation. Kimura et al. [13] identified AIF-1 in synovial tissues and fluid derived from RA patients.

Several single nucleotide polymorphisms (SNPs) have been identified in the *AIF1* gene [2,18,19,20]. In our previous studies we detected the association between *AIF-1* gene polymorphism and rheumatoid arthritis [19,20]. In this study we examined the association between rheumatoid arthritis and *AIF1* gene polymorphisms (rs2269475:C>T, rs2736182:G>A and rs2259571:A>C) and response to treatment of RA patients with MTX.

Patients

The study was carried out on 221 RA patients (179 women, 42 men) diagnosed with active rheumatoid arthritis, treated with MTX in a dose of 15 mg weekly and glucocorticosteroids. RA was diagnosed according to the criteria of the American College of Rheumatology (ACR).

Evaluation of clinical efficacy and toxicity

Good responders were defined as patients who were receiving MTX and had a DAS28 of ≤2.4 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 >2.4 [8,12]. The study was approved by the local ethics committee and written informed consent was obtained from all subjects.

Genotyping

SNPs within the *AIF1* gene were genotyped using Taqman genotyping assays. Genomic DNA was extracted from 200 µL of whole blood samples using GeneMATRIX Quick Blood DNA Purification Kit (EURx, Poland). Pre-validated allelic discrimination TaqMan real-time PCR assays were used for detection of rs2269475:C>T, rs2736182:G>A and rs2259571:A>C SNPs. (respective assay IDs: C__15876778_10, C__16286882_10, and C___3274002_10, Applied Biosystems, USA). Fluorescence data were captured using the 7500 FAST Real-Time PCR System (Applied Biosystems, USA), after 40 cycles of PCR.

Statistical analysis

Chi-squared or Fisher's exact test was used for qualitative variables, while the Mann-Whitney test was used for the quantitative ones. P-value < 0.05 was considered statistically significant.

Results

The efficacy of RA therapy with MTX is presented in table 1. Under MTX therapy remission of RA symptoms was achieved in 45.63% of *AIF1* rs2269475 CC genotype carriers, in 46.77% of subjects with CT genotype and in 60.00% of patients with TT genotype. The differences were not statistically significant (table 1).

The remission of RA symptoms was observed in 46.33% of *AIF1* rs2736182 GG genotype carriers, and in 66.67%

Table 1. The associations between AIF-1 gene polymorphisms and response to treatment of RA patients after 6 months of MTX therapy

	Patients with disease remission N=103		Patients with active disease N=118			p-valuea	OR (95% CI)
	n	%	n	%			
AIF1 rs2269475 genotype							
CC	68	45.63	81	54.37	TT+CT vs CC	0.77	0.89 (0.50-1.56)
CT	29	46.77	33	53.23	TT vs CT+CC	0.52	1.76 (0.48-6.43)
TT	6	60.00	4	40.00	TT vs CC	0.52	1.79 (0.48-6.59)
AIF1 rs2269475 allele							
C	165	45.80	195	54.20			
T	41	50.00	41	50.00	T vs C	0.54	0.84 (0.52-1.37)
AIF1 rs2736182 genotype							
GG	101	46.33	117	53.66	AA+GA vs GG	0.6	2.32 (0.21-25.93)
GA	2	66.67	1	33.33	AA vs GA+GG	1.0	-
AA	0	0.00	0	0.00	AA vs GG	1.0	-
AIF1 rs2736182 allele							
G	204	46.5	235	53.5			
A	2	66.7	1	33.3	A vs G	0.6	2.30 (0.21-25.59)
AIF1 rs2259571 genotype							
AA	42	52.99	37	47.01	CC+AC vs AA	0.16	0.66 (0.38-1.15)
AC	49	45.25	55	54.75	CC vs AC+AA	0.049	0.47 (0.22-0.98)
CC	12	32.84	26	67.16	CC vs AA	0.03	0.41 (0.18-0.92)
AIF1 rs2259571 allele							
A	133	50.80	129	49.20			
C	73	47.40	107	52.60	C vs A	0.04	0.66 (0.45-0.97)

[a] Fisher exact test

of subjects with GA genotype. The differences were not statistically significant.

With regard to the *AIF1* rs2259571 polymorphism the remission of RA symptoms was observed in 52.99% of AA genotype carriers in 45.25% of subjects with AC genotype and in 32.84% with CC. The differences were statistically significant. CC vs AA p=0.03 OR 0.41 95% CI (0.18-0.92) (table 1).

The remission of RA symptoms was achieved in 50.8% of A allele carriers and in 47.4% of patients with C allele. The differences were statistically significant; p=0.04 OR 0.66, 95% CI (0.45-0.97) (table 1).

Additionally we analyzed the association between *AIF-1* gene polymorphisms and selected clinical parameters of RA. As shown in table 2 there were no statistically significant associations between *AIF-1* gene polymorphisms and clinical parameters of RA (table 2).

Discussion

AIF-1 is a cytokine playing an important role in chronic inflammatory processes, especially involving macrophages. AIF-1 can affect macrophage functional state [16]. Immunohistochemical staining showed that this protein was strongly expressed in infiltrating mononuclear cells and synovial fibroblasts in RA compared with osteoarthritis. It was also demonstrated that AIF-1 induced proliferation of cultured synovial cells in a dose-dependent manner and increased IL-6 production in synovial fibroblasts and peripheral blood mononuclear cells [13]. Macrophages stimulated with AIF-1 produced significan-

Table 2. Analysis of clinical parameters in relation to *AIF1* genotypes

Genotype	Age at onset [years]			Rheumatoid factor positive		Erosive RA		Extra-articular manifestations	
	n	Mean ± SD	p [a]	(%)	p [b]	(%)	p [b]	(%)	p [b]
AIF1 rs2269475 genotype									
CC	149	48.71 ± 12.47		70.5		59.7		18.8	
CT	62	47.59 ± 13.67	0.72	72.6	0.92	62.9	0.89	19.3	0.42
TT	10	48.74 ± 12.92		70.0		60.0		30.0	
AIF1 rs2736182 genotype									
GG	218	47.64 ± 12.79	0.83	70.6	0.99	61.0	0.71	19.3	0.84
GA+AA	3	46.84 ± 14.97		100.0		66.7		33.3	
AIF1 rs2259571 genotype									
AA	79	47.59 ± 12.53		73.4		60.7		19.00	
AC	104	46.75 ± 12.38	0.69	69.2	0.57	62.5	0.52	15.4	0.25
CC	38	47.23 ± 13.78		71.0		57.9		21.1	

[a] Mann-Whitney test
[b] χ^2 test

tly increased amounts of IL-6, IL-10 and IL-12p40 compared with control cells [24]. The above results suggest that AIF-1 plays an important role in cells of the monocyte/macrophage lineage upon stimulation with inflammatory stimuli. It was shown that the macrophage cell line expressed a certain level of endogenous AIF-1 which could be enhanced by pro-inflammatory cytokines IL-1β or TNF-α [27]. The inflammatory mediators released by macrophages play an important role in the pathogenesis of RA and in the maintenance of inflammation in joints [15]. Moreover, the enhancement of macrophage apoptosis by MTX is an important mechanism of anti-inflammatory action in RA [17].

MTX is a 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 suppression of lymphocyte proliferation [10]. MTX inhibits a number of important intracellular enzymes in the folate pathway including dihydrofolate reductase and thymidylate synthase. Inhibition of 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR) transformylase results in accumulation of AICAR and increased adenosine release into the circulation. Extracellular adenosine increases cAMP, which inhibits production of proinflammatory cytokines including TNF-α, IL-6 and IL- 1, which are important in the inflammatory process in RA [26].

So far *AIF-1* gene polymorphisms have not been widely investigated. In our previous reports we described the association of the *AIF1* rs2269475 T allele with increased risk of RA development as well as the rs2259571 CC genotype with active form of RA [19,20]. The significant role of AIF-1 and *AIF-1* gene polymorphisms in RA pathogenesis was also confirmed by Harney et al., who studied the *AIF-1* gene rs2259571 and rs2269475 polymorphisms in RA patients [9].

The results of this study suggest that the patients with the rs2259571 *CC AIF1* genotype have a poorer response to therapy with MTX. The reasons for this association may be composed. The worse response to therapy in these patients is probably due to increased disease activity before therapy. However, studies examining disease activity as a predictor of MTX response are inconsistent, with some studies suggesting that patients with low disease activity are less likely to respond to therapy [1,21,22,25]. Nevertheless, in a study published by Hoekstra et al., patients with low disease activity at baseline were more likely to respond to treatment with MTX [11].

In this study we analyzed the association between *AIF-1* gene polymorphisms and the achievement of total

remission in RA patients because in our previous report we revealed that the rs2259571 CC genotype was associated with active form of RA [19].

Previous studies examined various clinical factors predisposing to disease remission in RA patients treated with MTX. To analyze whether *AIF1* gene rs2259571 polymorphism is associated with response to MTX therapy independently of clinical RA parameters, in this study we analyzed the association between selected RA parameters and *AIF-1* genotypes. Our analysis did not reveal statistically significant associations. However, the mechanism by which *AIF1* gene rs2259571 polymorphism modulates the response to MTX therapy in RA patients requires further investigations.

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