677C > T and 1298A > C MTHFR polymorphisms affect arechin treatment outcome in rheumatoid arthritis

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Abstract: Despite the availability of several new agents for the treatment of rheumatoid arthritis (RA), arechin (hydroxychloroquine) remains the mainstay because of both cost-effectiveness and experience with its use. However, there is considerable variation in response to this drug, with toxicity limiting treatment in some patients. Methylenetetrahydrofolate reductase (MTHFR) is involved in the folate metabolism and has been shown to be polymorphic what affects the enzyme activity. To examine the association between 677C > T and 1298A > C MTHFR polymorphisms and arechin efficacy in the treatment of RA, a total of 50 RA patients, treated with arechin were analyzed.

In univariate regression analysis model, MTHFR 677T allele was associated with significantly higher frequency of remission, whereas 1298C allele carriers showed a tendency to higher remission rate. In univariate regression analysis model, the presence of MTHFR 677T allele was associated with 2.3-fold higher frequency of remission. Multivariate regression analysis taking into the account the combined effect of MTHFR 677T and 1298C alleles revealed that both alleles were independent factors associated with increased frequency of remission. The results of our study suggest that 677T and 1298C alleles are independent factors associated with increased frequency of remission and the evaluation of C677C > T and A1298A > C MTHFR polymorphisms may be a useful tool to predict arechin treatment outcome in RA patients.

Key words: MTHFR, polymorphism, arechin, rheumatoid arthritis

Introduction

Pharmacogenetics focuses on the genetic variations responsible for drug metabolism, drug transport and drug targets to determine how these variations result in inherited alterations in medication outcomes [14, 15, 20]. 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 [1, 3, 13].

Despite the availability of several new agents for the treatment of rheumatoid arthritis (RA), arechin remains the mainstay because of both cost-effectiveness and experience with its use. However, there is considerable variation in response to this drug, with toxicity limiting treatment in some patients [21]. Recent studies have shown that mechanisms of resistance of malaria parasites to arechin are associated with folate metabolism [10]. Several polymorphisms have been described in the methylenetetrahydrofolate reductase (MTHFR) gene. Of these, the 677C > T and 1298A > C polymorphisms have been associated with altered phenotypes and adverse drug events [19]. The 677C > T polymorphism, first described in the mid 1990s, re-
results in an alanine to valine substitution in polypeptide chain. It leads to the thermolabile variant of MTHFR with decreased enzymatic activity, and subsequently increased plasma homocysteine levels. The homozygous 677TT variant, with about 30% of wide-type activity, is present in about 8–10% of the general population [12]. Heterozygotes have about 60% activity and constitute approximately 40% of the population. The 677C > T polymorphism has been shown to be associated with decreased risk of acute lymphoblastic leukemia (ALL) and colorectal neoplasm, as well as with increased risk of neural tube defects and cardiovascular disease [8, 16, 22]. It has also been shown to influence the clinical effects of drugs, such as methotrexate, estrogen, anticonvulsants, levodopa, and cholestyramine [2, 23–25, 30]. Urano et al. showed that patients with 1298C allele were administered significantly lower MTX doses, while a higher rate of MTX side effects was observed in patients with 677T allele [24]. Moreover, the presence of 1298C allele, correlated with the improvement of RA symptoms. Women with the 677TT genotype did not show decreased homocysteine in response to hormone replacement therapy as demonstrated for women with the 677CC genotype and may receive decreased cardiovascular benefits from hormone replacement therapy [2]. Yasui et al. found that the levels of homocysteine, a possible risk factor for vascular disease, were elevated by 60% in levodopa-treated patients with Parkinson’s disease, with the most marked elevation occurring in patients with the 677TT genotype [30]. In children with familial hypercholesterolemia, heterozygosity and homozygosity for the 677T allele was associated with low serum folate and increased susceptibility to elevation of plasma total homocysteine during cholestyramine treatment [23].

The aim of the present study was to examine the effect of 677C > T and 1298A > C MTHFR polymorphisms on treatment outcome in patients with RA administered with arechin.

**Materials and Methods**

**Patients**

The study was carried out on 50 patients from Pomeranian region of Poland (41 women, 9 men, aged 21–70 years, mean age, 53.3 ± 10.7 years) diagnosed with rheumatoid arthritis, treated with arechin 250 mg/day. The rheumatoid arthritis was diagnosed according to the criteria of American College of Rheumatology (ACR). All patients underwent a monthly evaluation for one year, applying the 1995 ACR preliminary definition of improvement in rheumatoid arthritis. Clinical improvement was evaluated according to the ACR 20% response criteria. The ACR core set of variables included: the number of swollen joints, the number of tender joints, physician’s global assessment of disease activity on a 0–10 scale, patient’s global assessment of disease activity on a 0–10 scale, patient’s assessment of pain on a 100-mm visual analog scale (VAS), functional status of patient’s using the Health Assessment Questionnaire (HAQ) scored on a 0–3 scale. A 28-joint count (including the metacarpophalangeal joints, the proximal interphalangeal joints, wrists and elbows) was used [4, 29]. A patient was classified as a good responder when both the tender joint count and the swollen joint count were ≥ 20% improved from baseline and at least 3 of the following criteria were met: ≥ 20% improvement in VAS, in ESR (erythrocyte sedimentation rate), in physician’s global assessment of disease activity, in patient’s global assessment of disease activity, and in HAQ. The group “good responders” included patients in remission for at least 6 months [5, 18]. The study was approved by the local ethics committee and written informed consent was obtained from all subjects.

**Genotyping**

Genomic DNA was extracted manually (precipitation with trimethylammonium bromide salts from leukocytes contained in 450 µl of venous blood collected with ethylenediaminetetraacetic acid as an anticoagulant) [11]. DNA was then precipitated in 95% ethanol, dissolved in distilled water and stored at −20°C until analysis. The 677C > T (rs1801133) and 1298A > C (rs1801131) polymorphisms were detected using a PCR-RFLP method, as previously described [24]. The genotyping has been carried out without knowing the participants’ treatment response.

**Statistical analysis**

Allele and genotype frequencies were compared using two-sided Fisher exact test. Odds ratios (OR) and their 95% confidence intervals (95%CI) were calcu-
lated for the chance of response to arechin treatment. Univariate and multivariate logistic regression models were used to analyze the influence of 677C > T and 1298A > C polymorphisms on the response to arechin treatment. The independent variables in these models were the numbers of 677T and 1298C alleles (0, 1 or 2) for each patient. A p level of less than 0.05 was considered statistically significant. Calculations were performed using Statistica 6.1 software package.

Results

The efficacy of RA therapy with arechin is presented in Tables 1 and 2. Under arechin therapy remission of RA symptoms was achieved in 100% of MTHFR 677TT genotype carriers (1 of 1), in 61.5% of subjects with 677CT genotype (16 of 26), and in 43.4% of patients with 677CC genotype (10 of 23, Fig. 1). The probability of remission of RA symptoms was 2-fold higher in carriers of 677CT genotype as compared with patients with 677CC genotype (OR = 2.08, 95%CI: 0.66–6.52, p = 0.258). The frequency of 677T allele among arechin responders was 33.3%, compared to 21.7% in a group of poor arechin responders (OR = 1.80, 95%CI: 0.73–4.43, p = 0.265) (Tab. 1).

The remission of RA symptoms was observed in 100% of MTHFR 1298CC genotype carriers (4 of 4), in 42.3% of subjects with 1298AC genotype (11 of 26), and in 60.0% of 1298AA homozygotes (12 of 20). The frequency of 1298C allele among arechin responders was 35.2%, compared to 32.6% in the group of poor arechin responders (OR = 1.12, 95%CI: 0.49–2.58, p = 0.835) (Tab. 2).

In univariate regression analysis model, the presence of MTHFR 677T allele was associated with 2.3-fold higher frequency of remission (Tab. 3). In multivariate regression analysis taking into account the combined effect of MTHFR 677T and 1298C alleles, it was found that both alleles were independent factors associated with increased frequency of remission (Tab. 4). In haplotype analysis, 677C-1298A haplotype was associated with decreased frequency of remission (OR = 0.37; 95%CI: 0.12–1.16, p = 0.079).

As shown in Table 5, there were no significant differences in age, disease duration, erosive disease, RF positivity, baseline DAS28 between patients in remission and non-responders treatment.

Discussion

In contrast to the well-documented safety and efficacy of arechin, its mechanism of action is poorly understood. It has been proposed that the marked accumulation of arechin in the lysosomal compartment is responsible for the therapeutic effect of these drugs.
Arechin is a weak base, so high concentration of arechin can elevate the pH within lysosomes, which results in inactivation of acid proteases. Arechin was shown to interfere with inhibition of receptor function, to inhibit intracellular processing and secretion of proteins, to decrease lymphocyte proliferation and to interfere with natural killer T-cell activity [17].

In the present study, we have examined the effect of 677C > T and 1298A > C MTHFR polymorphisms on treatment outcome in RA patients administered arechin. In genotype analysis, the probability of remission of RA symptoms was higher in carriers of 677T and 1298C alleles, but this effect did not reach statistical significance.

In univariate regression analysis model, the higher number of MTHFR 677T alleles was associated with significantly higher rate of remission, whereas in multivariate regression analysis, taking into account the combined effect of MTHFR 677T and 1298C alleles, both these alleles were associated with increased fre-

**Fig. 1.** Efficacy of RA therapy with arechin in relation to MTHFR 677C > T (A) and 1298A > C (B) polymorphisms.

**Tab. 3.** Univariate and multivariate logistic regression models predicting odds ratios for patients' response to treatment in relation to MTHFR 677C > T and 1298A > C genotypes.

<table>
<thead>
<tr>
<th>Logistic regression model</th>
<th>Number of 677T alleles</th>
<th>Number of 1298C alleles</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Univariate</td>
<td>2.30 (0.76–7.01)</td>
<td>1.15 (0.45–2.91)</td>
<td></td>
</tr>
<tr>
<td>Multivariate (677T + 1298C)</td>
<td>4.02 (0.96–16.91)*</td>
<td>2.27 (0.68–7.60)</td>
<td></td>
</tr>
</tbody>
</table>

* p = 0.05; Odds ratios calculated for the presence of one copy of the indicated allele.

**Tab. 4.** Univariate logistic regression models predicting odds ratios for patients' response to treatment in relation to MTHFR 677T-1298C haplotypes.

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>OR (95% CI) for positive response</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>677C-1298A</td>
<td>0.37 (0.12–1.16)</td>
<td>0.079</td>
</tr>
<tr>
<td>677C-1298C</td>
<td>1.15 (0.45–2.91)</td>
<td>0.768</td>
</tr>
<tr>
<td>677T-1298A</td>
<td>2.30 (0.76–7.01)</td>
<td>0.132</td>
</tr>
</tbody>
</table>

Odds ratios calculated for the presence of one copy of the indicated haplotype.

**Tab. 5.** Characteristics of patients in relation to response to treatment.

<table>
<thead>
<tr>
<th></th>
<th>Responders</th>
<th>Non-responders</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (years)</td>
<td>53.4 ± 11.0</td>
<td>53.1 ± 9.8</td>
<td>0.921</td>
</tr>
<tr>
<td>Disease duration (years)</td>
<td>6.7 ± 3.8</td>
<td>6.5 ± 3.4</td>
<td>0.847</td>
</tr>
<tr>
<td>Erosive disease (%)</td>
<td>81.4</td>
<td>78.3</td>
<td>1.0</td>
</tr>
<tr>
<td>RF positive RA (%)</td>
<td>70.4</td>
<td>73.9</td>
<td>1.0</td>
</tr>
<tr>
<td>DAS28 baseline</td>
<td>5.7 ± 2.4</td>
<td>5.9 ± 1.8</td>
<td>0.871</td>
</tr>
</tbody>
</table>
frequency of remission. The haplotype analysis showed the decreased frequency of remission in carriers of 677C-1298A haplotype.

In our study, the increased frequency of remission of RA symptoms in patients treated with arechin carrying MTHFR 677T and 1298C alleles, previously associated with lower MTHFR activity, suggests that decreased MTHFR activity (independently of polymorphism) might be associated with better response to treatment. It might involve more efficient down-regulation of 5-methyl-THF synthesis through MTHFR, and subsequently reduced methionine production from homocysteine and 5-methyl-THF through methionine synthase and S-adenosylmethionine [6]. S-adenosylmethionine is the main donor of methyl group in several biochemical pathways and reactions of DNA methylation. The limited availability of S-adenosylmethionine may affect expression of genes involved in inflammatory response in RA patients.

An increased availability of 5-methylene-THF might be another mechanism of better response to arechin treatment in carriers of alleles associated with lower MTHFR activity. 5-Methylene-THF is a substrate in the alternative de novo pyrimidine biosynthetic pathway, where this compound is metabolized by thymidylate synthase (TYMS) to dihydrofolate (DHF) [7]. 5-Methylene-THF is subsequently metabolized to active pool of tetrahydrofolate (THF) derivatives through dihydrofolate reductase (DHFR). Impaired reduction of 5-methylene-THF to 5-methyl-THF associated with lower MTHFR activity produces depletion of THF pool through TYMS pathway and subsequently enhances decreased availability of THF derivatives for many biochemical pathways in cells involved in inflammatory reactions [26].

Hyperhomocysteinemia is an independent risk factor of coronary artery disease. In our study, the plasma levels of homocysteine were not measured because the considerable part of patients was treated with drugs influencing the circulatory systems. As shown previously, these drugs may affect homocysteine plasma levels [9, 28]. This fact may be one of the limitations of our study as well as the number of patients, insufficient to draw the ultimate conclusion about the influence of MTHFR polymorphisms on efficacy of arechin treatment in RA and its importance in clinical practice.

The aforementioned results suggest indirectly that anti-inflammatory action of arechin in patients with RA is associated with the influence on folate and pyridoxine synthesis in cells involved in inflammatory response, and the evaluation of 677C > T and 1298A > C MTHFR polymorphisms might be a useful tool to predict arechin treatment outcome in RA patients. Nevertheless, this hypothesis requires further investigations.

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References:


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