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The effect of 677C>T and 1298A>C *MTHFR* polymorphisms on sulfasalazine treatment outcome in rheumatoid arthritis

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Abstract

Despite the availability of several new agents for the treatment of rheumatoid arthritis (RA), sulfasalazine remains the mainstay because of both cost and experience with its use. Methylenetetrahydrofolate reductase (*MTHFR*) is involved in folate metabolism and several polymorphisms have been described in the *MTHFR* gene. Of these, the 677C>T and 1298A>C polymorphisms have been associated with altered enzyme activity. To examine the association between 677C>T and 1298A>C *MTHFR* polymorphisms and sulfasalazine efficacy for the treatment of RA, a total of 117 RA patients treated with sulfasalazine (1 g daily; duration of treatment 17 ± 5 months) were analyzed. The 677C>T and 1298 A>C polymorphisms were detected using a PCR-RFLP method. RA was diagnosed according to the criteria of the American College of Rheumatology (ACR). The remission of RA symptoms was evaluated according to the ACR 20% response criteria. Allele and genotype frequencies were compared by the two-sided Fisher exact test. The frequency of remission was 47.2% and 44.6% in carriers of 677T and 1298C alleles, compared to 40.7% and 42.0% in carriers of 677C and 1298A alleles, respectively. These differences were statistically non-significant. When the multivariate analysis was additionally adjusted for patients' age, gender and RA duration, the association of the *MTHFR* 677T allele with increased frequency of remission was statistically significant. Although RA remission rate in carriers of the *MTHFR* 677T and 1298C alleles was more frequently observed, it does not seem that 677C>T and 1298A>C *MTHFR* polymorphisms have a major influence on treatment outcome in RA patients treated with sulfasalazine.

Key words: MTHFR; Polymorphisms; Sulfasalazine; Rheumatoid arthritis

Introduction

Rheumatoid arthritis (RA) is a disease of complex pathogenesis and its treatment is mainly based on drugs modulating its course, e.g., methotrexate and sulfasalazine (1). It is well known that methotrexate inhibits dihydrofolate reductase (DHFR) and folate-dependent enzymes (2). Studies have shown that the anti-inflammatory action of sulfasalazine is also associated with inhibition of folate metabolism (3).

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. The C677T polymorphism, first described in the mid 1990's, results in an alanine by valine substitution of a C by T at nucleotide 677 of the MTHFR enzyme. This leads to the thermolabile variant of MTHFR with decreased enzyme activity and subsequent increased plasma homocysteine levels (4). The homozygous 677T variant, with about 30% of wild-type activity, is present in about 8-10% of the Caucasian population. Heterozygotes have about 60% activity and form approximately 40% of the population (5). The 677C>T polymorphism has been shown to be associated with a decreased risk for acute lymphoblastic leukemia (6) and colorectal neoplasia (7), and an increased risk for neural tube defects (8) and cardiovascular disease (5). It has also been shown to influence the clinical effects of drugs such

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as anticonvulsants (9), levodopa (10), estrogens (11), and cholestyramine (12).

A second common polymorphism in the *MTHFR* gene is a 1298A>C transition that results in a glutamate to alanine substitution within a presumed regulatory domain of *MTHFR*. The 1298C allele has been reported to lead to decreased enzyme activity, although not to the same extent as the 677T allele (8).

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 treated with sulfasalazine.

Material and Methods

Patients

The study was approved by the local Ethics Committee and written informed consent was obtained from all subjects.

The study was carried out on 117 patients [92 women and 25 men aged 21-70 (61.0 ± 11.9) years, RA duration 1-35 (10.3 ± 8.2) years] diagnosed with RA and treated with 1 g sulfasalazine daily. RA was diagnosed according to the criteria of the American College of Rheumatology (ACR). All patients underwent a monthly evaluation for one year based on the 1995 ACR preliminary definition of improvement in RA (20% response criteria). The ACR core set of variables include: number of swollen joints, 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, and functional status of the patient 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 (13). A patient was classified as a good responder when both tender joint count and swollen joint count showed ≥20% improvement from baseline and at least 3 of the following criteria were met: ≥20% improvement in visual analog scale, in erythrocyte sedimentation rate, in physician's global assessment of disease activity, in patient's global assessment of disease activity, and in HAQ. The "good responders" group included patients in remission for at least 6 months (14,15).

Genotyping

Genomic DNA was extracted manually by precipitation with trimethylammonium bromide salts from leukocytes contained in 450 µL of venous blood collected with ethylenediaminetetraacetic acid as an anticoagulant. DNA was then precipitated in 95% ethanol, dissolved in distilled water and stored at -20°C until analysis. The 677C>T and 1298A>C polymorphisms were detected using a PCR-RFLP method as previously described (16).

Statistical analysis

Allele and genotype frequencies were compared by the two-sided Fisher exact test. Odds ratios (OR) and their 95% confidence intervals (95%CI) were calculated for the chance to respond to sulfasalazine treatment.

Univariate and multivariate logistic regression models were used to analyze the influence of 677C>T and 1298A>C polymorphisms on sulfasalazine treatment response. The independent variables in these models were numbers of 677T and 1298C alleles (0, 1, or 2) or haplotypes for each patient. A multivariate model additionally adjusted for patients' age, gender and RA duration was also analyzed.

A P value level of less than 0.05 was considered statistically significant. Calculations were performed using the Statistica 6.1 software package (USA).

Results

The clinical features of the patients before treatment are presented in Table 1.

Distribution of both MTHFR genotypes was consistent with Hardy-Weinberg equilibrium (P > 0.40). The efficacy of RA therapy with sulfasalazine is presented in Table 2. Under sulfasalazine therapy, remission of RA symptoms was achieved in 9 of 11 MTHFR 677TT genotype carriers, in 33 of 50 subjects with the 677CT genotype, and in 33 of 56 patients with the 677CC genotype. The frequency of the T allele among sulfasalazine responders was not statistically different from the group of poor responders (OR = 1.55, 95%CI = 0.85-2.81, P = 0.184).

The remission of RA symptoms was observed in 5 of 7 MTHFR 1298CC genotype carriers, in 34 of 51 subjects with the 1298AC genotype, and in 36 of 59 patients with the 1298AA genotype. The frequency of the C allele among sulfasalazine responders was 29%, compared to 25% for a

Table 1. Clinical features of 117 patients with rheumatoid arthritis before treatment.

Parameter	
Erythrocyte sedimentation rate (mm/h)	47.65 ± 21.24
Swollen joints (N)	6.32 ± 2.12
Tender joints (N)	7.75 ± 2.78
Visual analog scale (mm)	52.6 ± 17.1
C-reactive protein (mg/dL)	35.48 ± 25.42
Disease duration (years)	10.3 ± 8.2

Data are reported as mean ± SD.

Table 2. 677C>T and 1298A>C genotypes and rheumatoid arthritis patients' response to treatment.

	Responders (N = 75)	Non-responders (N = 42)	OR (95%CI)
677C>T genotype			
TT	9 (12%)	2 (5%)	3.14 (0.62-15.88)
CT	33 (44%)	17 (40%)	1.35 (0.61-2.98)
CC	33 (44%)	23 (55%)	-
677C>T allele			
T	51 (34%)	21 (25%)	1.55 (0.85-2.81)
C	99 (66%)	63 (75%)	-
1298A>C genotype			
CC	5 (7%)	2 (5%)	1.60 (0.29-8.93)
AC	34 (45%)	17 (40%)	1.28 (0.58-2.80)
AA	36 (48%)	23 (55%)	-
1298A>C allele			
C	44 (29%)	21 (25%)	1.25 (0.68-2.28)
A	106 (71%)	63 (75%)	-

Data are reported as number and percent. P > 0.05 when the numbers of homozygotes 677TT or 1298CC and 677CT or 1298AC heterozygotes were compared with homozygotes, 677CC or 1298AA, by the Fisher exact test.

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

Logistic regression model	OR (95%CI)	
	Number of 677T alleles	Number of 1298C alleles
Univariate	1.55 (0.84-2.87)	1.27 (0.67-2.42)
Multivariate (677T + 1298C)	1.89 (0.96-3.69)	1.67 (0.82-3.39)
Multivariate (677T + 1298C + age + gender + RA duration)	2.28 (1.09-4.79)*	1.76 (0.84-3.66)

Odds ratios calculated for presence of one copy of the indicated allele. RA = rheumatoid arthritis. *P = 0.027.

Table 4. Univariate logistic regression models predicting odds ratios for patients' response to treatment in relation to 677-1298 haplotypes.

Haplotype	OR (95%CI) for positive response
677C-1298A	0.56 (0.31-0.99)*
677C-1298C	1.27 (0.67-2.42)
677T-1298A	1.55 (0.84-2.87)

Odds ratios calculated for presence of one copy of the indicated haplotype. *P < 0.05.

group of poor sulfasalazine responders (OR = 1.25, 95%CI = 0.68-2.28, P = 0.544).

Univariate regression analysis revealed that the *MTHFR* 677T allele was associated with a 1.5-fold higher frequency of remission. In the multivariate regression analysis, taking into account the combined effect of the *MTHFR* 677T and 1298C alleles, the numbers of both alleles appeared to be associated with increased frequency of remission, but this association was not statistically significant (Table 3). However, when the multivariate analysis was additionally adjusted for patient age, gender and RA duration, the association of the *MTHFR* 677T allele with increased frequency of remission became statistically significant (Table 3).

In the haplotype analysis, three of four theoretically possible haplotypes of two loci were found in our patients (Table 4). The higher number of 677C-1298A haplotypes was associated with a decreased frequency of remission (OR = 0.56, 95%CI = 0.31-0.99, P = 0.046). This association was even more significant when the logistic regression model was adjusted for patient age, gender and RA duration (OR = 0.50, 95%CI = 0.27-0.92, P = 0.025).

Discussion

Although sulfasalazine has been used for many years in the management of patients with RA, its mechanism of action is not known. With respect to its potential immunomodulatory effects, several *in vitro* studies have shown that sulfasalazine and its metabolites inhibit the release of cytokines produced by various cell types. Among the cytokines affected by sulfasalazine are T-cell cytokines such as interleukin 2 (IL-2) (17) and those produced by monocytes or macrophages, including IL-1, IL-6, IL-12 (17-19) and tumor necrosis factor alpha (TNF-α) (17). How sulfasalazine inhibits the release of cytokines has not been fully elucidated. Some studies have shown, for example, that sulfasalazine inhibits TNF-α expression in macrophages by inducing apoptosis (20). Cytokines mediate and regulate a number of cellular responses, including T-cell proliferation, natural killer cell activity and activation of B cells. Sulfasalazine and, to a lesser extent, its metabolites sulfapyridine and mesalazine have been shown to inhibit these cellular responses in a number of different systems *in vitro* (21).

Previous studies have suggested that sulfasalazine also has antifolate properties (3). Sulfasalazine was found to influence folate concentrations, possibly by interfering with folate absorption. The influence of sulfasalazine on serum folic acid concentrations in RA patients has been disputed. A direct influence on folate metabolism *in vivo* has not been investigated and the influence of folates on the clinical impact of sulfasalazine has never been studied.

In the present study, we examined the effect of 677C>T and 1298A>C *MTHFR* polymorphisms on the treatment outcome of RA patients treated with sulfasalazine. In the genotype analysis, there was no statistically significant association of the genotypes studied with response to treatment. In the multivariate analysis additionally adjusted for patient age, gender and RA duration, the *MTHFR* 677T allele was significantly associated with increased frequency of remission.

The increased frequency of remission of RA symptoms in patients treated with sulfasalazine carrying the *MTHFR* 677T allele, which is associated with lower MTHFR activity, suggests that decreased *MTHFR* expression might be associated with a better response to treatment. This effect may 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. S-adenosylmethionine is the main donor of a methyl group in several biochemical pathways and reactions of DNA methylation (22). A limited availability of S-adenosylmethionine may affect the expression of genes involved in the inflammatory response in RA patients.

Moreover, *MTHFR* polymorphism may influence plasma homocysteine concentrations in RA patients treated with methotrexate and sulfasalazine. Haagsma et al. (3) showed that the patients homozygous for the *MTHFR* gene mutation C677T had significantly higher baseline homocysteine, and that the heterozygous individuals presented significantly higher plasma homocysteine after therapy. No correlation was found between clinical efficacy variables and homocysteine. Patients with gastrointestinal toxicity had a significantly higher increase in homocysteine. A persistent increase in plasma homocysteine concentrations was observed in patients treated with methotrexate alone and more pronounced in combination with sulfasalazine, in contrast with sulfasalazine alone.

The results cited above indirectly suggest that the anti-inflammatory action of sulfasalazine in patients with RA might also be associated with influence on folate and purine synthesis in cells involved in the inflammatory response. Nevertheless, this hypothesis requires further investigation. Although RA remission was observed more frequently in carriers of the *MTHFR* 677T and 1298C alleles, it does not seem that 677C>T and 1298A>C *MTHFR* polymorphisms have a significant influence on treatment outcome in RA patients treated with sulfasalazine.

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