GENETIC POLYMORPHISMS IN FOLATE METABOLIC PATHWAY INFLUENCE THE RISK FOR DEVELOPING ADVERSE EFFECTS DURING METHOTREXATE TREATMENT IN RHEUMATOID ARTHRITIS

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Introduction

Methotrexate (MTX) is the most widely prescribed disease-modifying antirheumatic drug (DMARD) for the treatment of rheumatoid arthritis (RA). MTX enters the cells through reduce folate carrier (RFC1) and directly inhibits several enzymes of the folate metabolic pathway. Intracellular folate metabolism is complex and involves enzymes such as 5,10-methylenetetrahydrofolate reductase (MTHFR), thymidylate synthase (TS), methionine synthase (MS) and methionine synthase reductase (MTRR) that channel the methyl group of tetrahydrofolate (THF) into the direction of the DNA synthesis or methylation. MTHFR is the central enzyme of the folate metabolic pathway that defines the ratio between the two forms of THF. Two common polymorphisms C677T and A1298C are known in the MTHFR gene resulting in a lower enzyme activity. The promoter enhancer region of the TS gene contains a double (2R) or a triple (3R) 28-base pairs (bp) tandem repeat polymorphism. 3R is associated with a higher TS gene expression and therefore higher conversion rate of dUMP to dTMP. MS and MTRR are enzymes crucial for the efficient methionine synthesis and methylation reactions. A2756G polymorphism in the MS gene results in a lower enzyme activity, while the function of MTRR A66G polymorphism is not yet understood. RFC1 gene also contains a genetic polymorphism (A80G) that may have an impact on affinity of the transporter for the folate. Common genetic polymorphisms in RFC1 and in enzymes in the folate metabolic pathway alter enzymatic activity and thus may modify MTX uptake or influence MTX metabolic effect.

Aim

The aim of our study was to analyse if genetic polymorphisms in RFC1 and the folate metabolic pathway influence the efficacy and/or toxicity of the RA treatment.

Methods

Study population consisted of the group of 214 consecutive, unrelated RA patients, 26 male (12.1 %) and 188 female (87.9 %), median age 60.5 (range 51–69) years. At the entry into the study 157 out of 214 patients were treated with methotrexate, while the remaining 57 patients were treated with other disease modifying antirheumatic drugs. A Genotyping approach using both restriction fragment length polymorphism (RFLP) and/or TaqMan genotyping assays (Applied Biosystems) was used to determine RFC1 A80G, MTHFR C677T/A1298C, MS A2756G, MTRR A66G and TS 2R/3R polymorphisms in 214 RA patients. Binary logistic regression analysis with the addition of independent variables, such as sex, disease and treatment duration, RF seropositivity and the amount of the folate in the erythrocytes was utilized to determine the risk for inefficacy of the treatment and/or developing adverse effects. The risk was expressed as odds ratio (OR) with 95 % confidence intervals (CI).

Results

MTX treatment was well tolerated by 157 patients (73.3 %), 58 of them (27.1 %) were co-treated with additional disease-modifying anti-rheumatic drug (DMARD). Efficient MTX treatment was achieved in 136 patients (63.6 %), 36 patients (16.8 %) had intermediate MTX treatment efficacy, while 17 (7.9 %) and 25 (11.7 %) patients experienced poor and inefficient MTX treatment, respectively. Adverse effects were present in 148 patients (61.8 %), among them 38 (17.7 %) developed severe adverse effects and were discontinued from the MTX treatment. No significant association between genetic polymorphisms in the folate metabolic pathway and the efficacy of the RA treatment was observed however, specific genetic polymorphisms influenced the risk for developing adverse effects. Carriers of RFC1

80GG and TS 3R/3R genotype had a 2.65-fold and a 2.46-fold higher risk for developing MTX adverse effects than carriers of at least one RFC1 80A allele and TS 2R allele, respectively (P = 0.021 and P = 0.025, respectively; 95 % CI = 1.160–6.033 and 95 % CI = 1.120–5.396, respectively). Furthermore, patients with RFC1 80GG genotype had an 11.17-fold higher risk for developing infections than patients with RFC1 80AA/AG genotype (P = 0.006, 95 % CI = 2.030–61.486). A combination of at least one MTHFR 677T or 1298C polymorphic allele and MS 2756AG/GG and MTRR 66AG/GG genotypes, which may lead to lower methylation capacity reduced the risk for developing adverse effects 3.175-fold (P = 0.032, OR = 0.315, 95 % CI = 0.109–0.907).

Conclusion *Our results suggest that genetic polymorphisms in RFC1 and the folate metabolic pathway do not modify the MTX efficacy of RA treatment but influence the risk for developing MTX adverse effects.*