European Journal of Pharmacology 769 (2015) 93–99

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Pharmacogenomics

Functional variants of gene encoding folate metabolizing enzyme and methotrexate-related toxicity in children with acute lymphoblastic leukemia

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ARTICLE INFO

Article history:
Received 24 June 2015
Received in revised form
11 October 2015
Accepted 30 October 2015
Available online 1 November 2015

Keywords:
MTHFR polymorphism
Acute lymphoblastic leukemia
Folate metabolism

ABSTRACT

Methotrexate (MTX) is commonly used agent in therapy of malignancies, including acute lymphoblastic leukemia (ALL). Based on the literature data it is known that MTX elimination and toxicity can be affected by polymorphisms in genes encoding enzymes involved in MTX metabolism.

The aim of our study was to investigate the influence of C677T and A1298C polymorphisms in methylenetetrahydrofolate reductase (*MTHFR*) gene on MTX-induced toxicity during treatment of children with ALL. We also tried to answer the question whether simultaneous occurrence of these two polymorphisms has a clinical significance.

MTHFR polymorphisms were assessed in 47 pediatric ALL patients, treated according to *intensive chemotherapy* for childhood ALL, ALL IC BFM 2009.

Prolonged MTX elimination and higher incidence of toxicity were observed for patients with 677T-1298A haplotype. On the other hand, occurrence of 677C-1298A haplotype had protective effect on MTX clearance and toxicity, that was not observed in carriers of 677C-1298C haplotype. In patients with co-existence of studied variants 677CT/1298AC heterozygotes as well as in 677TT/1298AA homozygotes more frequently toxicity incidents were noted. The obtained results suggest that occurrence of 677T allele and coexistence of 677T and 1298C alleles may be associated with lower MTX clearance and elevated risk of adverse effects during MTX-treatment of pediatric ALL patients.

1. Introduction

The 5,10-methylenetetrahydrofolate reductase (MTHFR) is the pivotal enzyme in folate metabolism, essential in DNA synthesis and cellular methylation reactions, as well as in methylcobalamin regeneration. The MTHFR irreversibly catalyzes the reduction of 5,10-methylenetetrahydrofolate into 5-methyltetrahydrofolate a cosubstrate for homocysteine remethylation to methionine (Wiemels et al., 2001). MTHFR is encoded by *MTHFR* gene, located on short arm of distal region of chromosome 1 (1p36.3), which contains 11 exons and 10 introns and spins region of 2.2 kbp. Single nucleotide polymorphisms (SNPs) in *MTHFR* gene may have significant effect on enzyme activity, homocysteine accumulation as well on some drugs metabolism and toxicity (e.g. folic acid antagonists). It was found that SNPs in this gene are associated with occurrence of several pathological conditions such as: leukemia (Dong et al., 2008), cardiovascular diseases (Cortese and Motti, 2001), psychiatric disorders (Gilbody et al., 2007), colorectal and gastric cancer (Dong et al., 2008), migraine, allodynia and fatigue (Bahadir et al., 2013), epilepsy (Scher et al., 2011), spontaneous embryo loss (Callejón et al., 2007). There are two the most common polymorphisms in *MTHFR* gene, that is C677T (rs1801133)

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http://dx.doi.org/10.1016/j.ejphar.2015.10.058
0014-2999/

and A1298C (rs1801131). These polymorphism have been identified as factors causing decrease in MTHFR activity, which results in folate metabolism and DNA methylation disturbances (Schwan and Rozen, 2001). C677T polymorphism leads to change of alanine to valine in codon 222 (Ala222Val), that result in less stable, thermolabile variant of MTHFR with decreased activity. Protein isoform in 677TT homozygotes has only 30% of enzymatic activity in comparison to that in homozygotes of wild type (677CC), while heterozygotes (677CT) have approximately 60% of enzyme activity. Polymorphism A1298C leads to change of glutamic acid to alanine in codon 429 (Glu429Ala), that in homozygotes (1298CC) results in decreased enzyme activity by 40%. It was also shown that homozygous genotype of A1298C polymorphism (1298CC) may be associated with higher risk of adverse effects during methotrexate (MTX) treatment of chronic inflammatory bowel disease and nonmalignant gastrointestinal diseases in comparison to wild type genotype (1298AA) (Herrlinger et al., 2005; Saito and Camilleri, 2006).

Due to, the fact that MTHFR is an important drug-metabolizing enzyme, genetic variability in *MTHFR* gene may influence efficacy and toxicity of therapy with MTX. MTX is an effective antifolate chemotherapeutic agent used for treatment of a number of cancers, including ALL. However therapy with MTX is associated with many adverse effects, including hepatotoxicity, renal and pulmonary, hematological and neurological toxicity, which may lead even to discontinuation of treatment (Cortese and Motti, 2001; Gilbody et al., 2007; Yang et al., 2012). Understanding of the association between SNPs in *MTHFR* gene and MTX toxicity may help optimizing and individualizing the MTX treatment as well as minimize the patient's exposure to its toxicity. The aim of our study was to investigate the influence of *MTHFR* C677T and A1298C polymorphisms on MTX elimination and MTX-induced toxicity during treatment of children with ALL. We also try to answer the question whether simultaneous occurrence of these polymorphisms has a clinical significance.

2. Material and methods

The study group included forty seven pediatric patients diagnosed with acute lymphoblastic leukemia (ALL) and treated in the Department of Pediatric Oncology, Hematology and Bone Marrow Transplantation of University of Medical Sciences Poznan, Poland. Median age at diagnosis and at treatment was 5 years (range: 2–17), all patients were of Caucasian ancestry. The diagnosis of leukemia was made according to the French–American–British criteria, after conventional immunophenotyping surface-marker analysis. Inclusion criteria for participation in the study were age at diagnosis younger than 18 years, absence of other malignancy, qualification for the treatment according to i*ntensive chemotherapy scheme* for childhood acute lymphoblastic leukemia, ALL IC BFM 2009. All children were from the group IR BCP-ALL (Intermediate Risk group B-precursor ALL), according to BFM protocol randomization. In consolidation, methotrexate at 5 g/m^2 was administered for all patients, four times in 2 weeks intervals. Plasma MTX concentration was measured in 24th, 36th, 42th, 48th, 54th h after drug administration. In the case of detectable MTX level in 54th h, the concentration was further measured up to 2 weeks.

Data on patient age, gender, ethnicity, toxicity, were obtained from patients' medical records. In all patients, following parameters were analyzed and compared: the frequency of toxicity incidents (hepatotoxicity, myelotoxicity, nephrotoxicity, and occurrence of infections) according to World Health Organization (WHO) toxicity scale, MTX concentration at 24th h, 54th h, and after 54 h of its administration. All biochemical analyzes were performed as a part of standard treatment monitoring. Aminotransferases activity (> 120 U/l) and bilirubin (> 20.5 μmol/l) concentration in serum were monitored as a measure of a drug hepatotoxicity; level of platelets (< 100 G/l), neutropenia (< 1.0 and < 0.5 G/l) and hemoglobin (< 6.2 mmol/l) as a measure of myelotoxicity; creatinine (> 61.9 mmol/l) and cystatin C as a measure of nephrotoxicity.

For analysis of *C677T* and *A1298C* polymorphisms first DNA was extracted from 5 million cells, obtained after lysis of peripheral whole blood, using DNA extraction kit (DNA Extraction Kit, Stratagene) according to the manufacturer's protocol. Then C677T and A1298C polymorphisms were assessed by amplification of region of interest by PCR using commercial available kit (MTHFR C677T and MTHFR A1298C; GeneProof). Briefly, 2 μl of DNA template was added to 18 μl of PCR master mix containing three probes labeled with JOE fluorophore (for mutant genotype), FAM fluorophore (for wild type genotype) and both JOE and FAM fluorophores (for heterozygous genotype). Amplification conditions involved initial denaturation for 10 min at 95 °C, followed by 40 cycles of 10 s at 95 °C, 20 s at 64 °C and 20 s at 72 °C. The study has been performed according to the World Medical Association Declaration of Helsinki and was approved by the local ethical committee of the University of Medical Sciences in Poznan, Poland.

MTX concentration was determined with use of Architect Methotrexate Reagent Kit (ARK Diagnostics, Inc.) and Abbott Architect i100SR Immunology Analyzer. This immunoassay is based on competition between MTX molecule in the analyzed sample and MTX labeled with glucose-6-phosphate dehydrogenase (G6PDH) for binding with antibody. The presence of endogenous MTX leads to increased enzyme activity, which is directly proportional to the MTX concentration. Active G6PDH converts coenzyme adenine dinucleotide to the its reduced form that is measured spectrophotometrically.

2.1. Statistical analysis

Genotype frequencies were tested for Hardy–Weinberg equilibrium (HWE) using the χ^2 test (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl), while linkage disequilibrium between the *MTHFR* SNPs and haplotype frequencies using Haploview software (Barrett et al., 2005). Kolmogorow–Smirnov test was performed to assess normality of data ($P > 0.05$). In univariate analyses, continuous variables, that were not normally distributed were logarithmically transformed for normalization and analyzed using parametric tests (Anova and *t*-test). The results were also confirmed using non-parametric tests (the Kruskal–Wallis test and the Mann–Whitney *U* test, respectively). As the results of both types of analyses were consistent, the significance levels obtained in the parametrical analyses are shown in tables. Categorical data were analyzed using chi square (χ^2) test. For categorical risk factors, including genotypes and alleles, the odds ratios (ORs) were estimated with 95% confidence intervals (CI). The differences were considered significant if the value of probability (P) did not exceed 0.05.

Independent factors for MTX elimination were selected using linear regression analysis, while independent factors for MTX toxicity were determined using logistic regression. Clinical factors evaluated in both analyses are: age (for the effect of 1 year), time period from first diagnosis (for the effect of 1 year), male sex, round of drug administration (protocol M, one to four), incidence of hepatotoxicity, myelotoxicity, nephrotoxicity and infections (logistic regression only). In linear regression analysis of MTX levels were logarithmically transformed for normalization (ln-MTX). Genetic factors evaluated in both analyses are the effects of *MTHFR* 677/1298: CC/AA, CT/AC and TT/AA genotypes and the effects of 677T (677T-1298A haplotype) and 1298C (677C-1298C haplotype) alleles: dominant, recessive and dose of allele (haplotype). All statistical analyses were performed using Statistica v. 10.