RELATIONSHIP OF THE REDUCED FOLATE CARRIER GENE POLYMORPHISM G80A TO METHOTREXATE TOXICITY AND PLASMA LEVELS IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA

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Background

Methotrexate (MTX) is a key agent in the chemotherapeutic regiments used in the treatment of childhood acute lymphoblastic leukemia (ALL), the most frequent malignancy of the pediatric population. MTX inhibits RNA and DNA synthesis in the cell by blocking the turnover of folate. It is transported into the cell by the reduced folate carrier encoded by the gene RFC1. A common polymorphism at position 80 in exon 2 of the RFC1 gene has been identified. Recently higher MTX plasma levels were described with AA genotype.

Aim

To investigate whether common polymorphism G80A in the RFC gene is associated with a higher incidence of MTX toxicity and higher MTX plasma levels in children and adolescents with ALL. If this was the case, determination of RFC1 G80A polymorphism prior to the start of the chemotherapeutic regiment could be used to individualize the MTX dosage in ALL patients with the aim of reducing its toxicity.

Methods

Sixty-two individuals of Slovenian ethnicity (37 females and 25 males) were included in the study. Average age at the time of diagnose was 6.6 ± 5.0 years. During chemotherapeutic regiment subjects on 4 separate occasions received a high dose of MTX (5 g/m^2). MTX toxicity was determined by collecting data on MTX and leucovorin administration, leukocyte and platelet counts, severity of mucositis, severity of neurotoxicity and survival status. Plasma MTX concentrations were measured 24, 36 and 42 hours after the MTX administration by the fluorescence polarization immunoassay following manufactures instructions (TDX, Abbott). DNA was isolated from peripheral leukocytes obtained from blood draws using standard procedures. Genotypes of the individuals for G80A polymorphism were determined using the previously described PCR-RFLP test. Simple descriptive statistics, the Chi-square test, analysis of variance (ANOVA), Kruskal-Wallis test and T-test were used as appropriate. For statistical analysis statistical software SPSS version 10.0 (SPSS Inc., Chicago, II) was used. $P < 0.05$ was considered statistically significant, and all P-values were based upon two-tailed tests.

Results

There was no difference between three immunophenotype groups according to gender distribution, age at diagnosis, body weight and height.

	RFC1 polymorphism		
	AA	AG	GG
RFC1 frequency (%)	26.4	41.4	32.1
Delay* (No)	7	2	9
Mucositis (No)	6	14	11
Neurotoxic episodes (No)	5	3	8
Other complicatios (No)	7	6	13
Relaps (No)	4	0	3
Fatal outcome (No)	0	1	1
AUC_{MTX1} (mmol*h /L)	1486 ± 572	1629 ± 571	1352 ± 503
AUC_{MTX2} (mmol*h /L)	1414 ± 639	1605 ± 588	1378 ± 566
AUC_{MTX3} ± SD (mmol*h /L)	1358 ± 452	1470 ± 348	1420 ± 809
AUC_{MTX4} ± SD (mmol*h /L)	1354 ± 461	1415 ± 283	1309 ± 607

Legend: * delay of next MTX application; AUC_{MTX} – average MTX concentration under the curve ± SD after first (1), second (2), third (3) and fourth (4) application respectively.

The distribution of RFC genotypes in investigated ALL patients was similar to the distribution in the general Slovene population. No correlation between any of the RFC1 genotypes

and MTX toxicity on the bone marrow, need for delays of continuation of chemotherapy, oral mucositis, neurotoxicity, disease outcome or MTX concentration (area under the curve value) was detected after any of the MTX applications.

Conclusions *No correlation could be found between any of the RFC1 polymorphisms and any of the investigated MTX toxicities and MTX concentration. Determination of RFC1 G80A polymorphism alone can not be used to individualize MTX dosage in ALL patients with the aim of reducing its toxicity.*