REVIEWS

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The Influence of Genetic RFC1, MS and MTRR Polymorphisms on the Risk of Acute Lymphoblastic Leukemia Relapse in Children and the Adverse Effects of Methotrexate

Wpływ polimorfizmów genetycznych RFC1, MS, MTRR na ryzyko nawrotu ostrej białaczki limfoblastycznej u dzieci i nasilenie działań niepożądanych po zastosowaniu metotreksatu

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Abstract

Polymorphism in genes coding drug-metabolizing enzymes may cause individual differences in the effectiveness and toxicity of many medications, including cytostatics. Although in recent years intensive treatment has positively influenced the prognosis in leukemias, many adverse effects resulting from nonspecific actions and the narrow therapeutic index of anti-cancer drugs are still observed during therapy. Determining selected gene polymorphisms may increase both the safety and the efficacy of treatment, and might help in developing individual therapies (**Adv Clin Exp Med 2013, 22, 4, 579–584**).

Key words: polymorphism, methotrexate, acute lymphoblastic leukemia, adverse effects.

Streszczenie

Polimorfizm z zakresu genów kodujących enzymy metabolizujące leki może stanowić podłoże indywidualnych różnic skuteczności i toksyczności wielu preparatów, w tym cytostatyków. Chociaż w ostatnich latach zastosowanie intensywnych metod leczenia wpłynęło na poprawę rokowania w białaczkach, nadal obserwuje się dużą liczbę działań niepożądanych podczas samej terapii, związaną z nieswoistością działania i wąskim współczynnikiem terapeutycznym leków przeciwnowotworowych. Oznaczenie wybranych polimorfizmów genetycznych może pomóc zindywidualizować terapię i wpłynąć na poprawę bezpieczeństwa oraz skuteczność leczenia (**Adv Clin Exp Med 2013, 22, 4, 579–584**).

Słowa kluczowe: polimorfizm, metotreksat, ostra białaczka limfoblastyczna, działania niepożądane.

Methotrexate (MTX) is an important cytostatic used in chemotherapy of neoplasms in children. Its efficacy is limited by a number of possible side effects. It is important to look for factors that could permit an individual approach in methotrexate treatment and reduce the toxicity of the treatment. Determining selected gene polymorphisms may increase treatment safety and efficacy, and might contribute to the development of individual therapies.

The aim of the study was to analyze the influence of genetic polymorphisms in RFC1 (reduced

folate carrier), MS (methionine synthase) and MTRR (methionine synthase reductase) on the occurrence of adverse effects from MTX therapy, such as hematological disorders, hepatotoxicity and nephrotoxicity.

Methotrexate is transported by the RFC1 protein through the cell membrane. Intracellular methotrexate is converted into methotrexate polyglutamate (MTXPG) – an "active" form of the drug – with the participation of the enzyme FPGS (folylpolyglutamyl synthetase) [1, 2]. Methotrexate affects intracellular folate metabolism by inhibiting dihydrofolate reductase (DHFR) and thymidylate synthase (TYMS). Furthermore, MTXPG has a longer half-life and is not so quickly transported outside the cell.

The RFC1 gene is located on the long arm of chromosome 21 (21q22.2-22.3) and encodes a membrane protein called the reduced folate carrier [3]. Single nucleotide polymorphism (SNP) consists in the substitution of adenine for guanine in the 80th position. Histidine replaces arginine in the structure of the protein. The impact of the exchange of amino acids on the function of this protein is unknown. Probably the transport protein has less affinity with the reduced form of folate, including methotrexate.

The methionine synthase gene (MS) is located on chromosome 1(1q43) [4]. Methionine synthase is an important enzyme involved in intracellular folate metabolism. The enzyme causes the disconnection of a methyl group from 5-CH3-THF (5-methyltetrahydrofolate), which results in tetrahydrofolate (THF). The methyl group is incorporated into homocysteine, which gives rise to methionine. MS polymorphism is associated with an exchange to G at position 2756A, which causes the conversion of Asp instead of Gly. As a result, the enzyme activity is reduced, which leads to increased levels of homocysteine and reduced DNA methylation processes.

The MTRR gene is located on 5p15.3-p15.2 [5]. Methionine synthase reductase catalyzes the methylation of cobalamin (methionine synthase enzyme cofactor). The methyl group donor is S-adenozylomethionine (SAM).

Adverse Reactions After the Use of Methotrexate

Among the most frequently reported adverse reactions to the use of methotrexate (including both high-dose MTX and MTX in remission maintenance therapy) are ulcerative stomatitis, nausea, abdominal distress, fever and leukopenia. Other adverse reactions that have been reported after MTX administration are listed below by organ system [6, 7]:

The alimentary system: gingivitis, pharyngitis, stomatitis, anorexia, nausea, vomiting, diarrhea, melena, gastrointestinal ulceration and bleeding, enteritis, pancreatitis;

Adverse hematologic reactions: pancytopenia: leukopenia, neutropenia and/or thrombocytopenia, anemia;

The cardiovascular system: pericarditis, pericardial effusion, hypotension, thromboembolic events;

The central nervous system: headaches, drowsiness, blurred vision, transient blindness, speech impairment including dysarthria and aphasia, hemiparesis;

The hepatobiliary system: hepatotoxicity, acute hepatitis, chronic fibrosis and cirrhosis, liver enzyme elevations;

The pulmonary system: respiratory fibrosis, respiratory failure, interstitial pneumonitis;

The skin: erythematous rashes, pruritus, urticaria, photosensitivity, pigmentary changes, alopecia, ecchymosis, telangiectasia, erythema multiforme, toxic epidermal necrolysis, Stevens-Johnson Syndrome, skin necrosis, skin ulceration, exfoliative dermatitis;

The urogenital system: renal failure, cystitis, hematuria, transient oligospermia, menstrual dysfunction, gynecomastia, infertility, miscarriage, fetal defects.

Polymorphisms in RFC1, MS, MTRR

Chang et al. (2000) were amongst the first researchers whose study took into account the role of the RFC1 80G>A polymorphism in the intracellular transport of the reduced form of folic acid [8]. The replacement of arginine with histidine at position 27 of the RFC1 protein resulted in decreased receptor affinity and changes in the transmembrane transport of folic acid antimetabolites. In *ex vivo* studies the folate concentrations in serum were higher in the 80AA genotype than the allele G variant: 19 mmol/L vs 15 mmol/L, respectively. However, other researchers, including Whestine et al., lowered the enthusiasm regarding the role of the reduced folate carrier in carcinogenesis [9]; their study indicated that the effect of functional RFC1 was marginal. It was shown that most forms of folic acid are transported by the folic acid receptors and not by RFC1. The tests were conducted *in vitro* and require further expansion.

A study by Laverdiere et al. also dealt with the role of RFC1 polymorphisms in the metabolism

of folic acid. The results were encouraging, particularly with respect to the treatment of patients suffering from hematologic diseases [10]. The researchers analyzed the relationship between the RFC 80A polymorphism and the risk of relapse of acute lymphoblastic leukemia (ALL) in children. In a group of 204 patients with ALL, the RFC 80AA variant was associated with higher concentrations of methotrexate in the serum. It was anticipated that the polymorphism may reduce the affinity of RFC1 to methotrexate.

Subsequent studies have also shown the role of RFC in methotrexate metabolism and methotrexate treatment efficacy. Japanese researchers have shown increased liver toxicity in homozygous 80A, and increased side effects in the form of severe vomiting in the 80G group homozygotes [11, 12]. Also, Ge et al. examined 91 patients with ALL and found an association between the level of hRFC transcript and the risk of leukemia relapse (p=0.0052) [13]. Yates and Lucock confirmed earlier reports of increased extracellular folate concentrations in patients with the RFC 80G>A polymorphism [14], caused by impaired transport across the cell membrane.

Decreased methylation of homocysteine leads to lower levels of methionine and reduced DNA methylation. Abnormalities in folate metabolism may affect the risk of developing the disease through inappropriate incorporation of uracil into the DNA, increasing the risk of chromosome fractures, abnormal methylation of proto-oncogenes or tumor suppressor genes. Methylation appears to be an important factor in the regulation of gene transcription. Its role in carcinogenesis is still the subject of many studies. Disturbances in the methylation process may include so-called epigenetic changes. These are changes in gene expression that do not result from abnormalities in the DNA structure. Abnormal methylation may be a source of stimulation or suppression of the process of carcinogenesis [15].

Other studies have shown no correlation between 80G>A polymorphism and the risk of Hodgkin's lymphoma, non-Hodgkin lymphoma (NHL), neural tube defects or colorectal cancer [16–19].

De Jonge et al. have repeatedly investigated the role of reduced folate carrier in the metabolism of folates. In one of their first reports (2005) they did not find any associations between RFC1 polymorphism and methotrexate [20]. The examined group consisted of 157 patients with ALL. The study was conducted *ex vivo*. The cells were incubated in an appropriate concentration of the drug over a period of either 3 or 21 hours, and then the level of the obtained substrates was measured. In 2009, de Jonge et al. published a new study evaluating the role of selected polymorphisms in genes involved in folate metabolism [21], including MTHFR, methionine synthase (MTR), thymidylate synthase (TS) and RFC1. The study group consisted of 245 patients with ALL and 500 patients in the control group. The frequency of the RFC 80G>A polymorphism in the group of healthy subjects was 38%. These results were similar to those reported in previous studies conducted on the French population (169 adults – 36%) and the British population (602 children – 41%). A relationship between the RFC1 80G>A polymorphism and an increased risk of ALL was also shown. In patients with the variant RFC1 80AA, the risk of ALL increased 2.1-fold, while in patients with the variants RFC1 80GG and NNMT IVS-151 + TT, the risk increased 4.2-fold. However, the functional effect of 80 G>A polymorphic substitution remains unknown.

A recent study confirmed that RFC1 polymorphisms may influence the effects of treatment of acute leukemia in children [22]. In a study group consisting of 500 children it was found that the RFC 80AA variant correlated with a lower risk of relapse (a 50% increase in the chances of successful therapy) compared to patients with variants GG and GA (p = 0.046). In patients receiving high-dose methotrexate and with the variant AA vs. GA/GG, increased toxicity of the treatment in the form of hematological abnormalities was also observed: 73 vs 99/105x109/L PLT (p = 0.004), Hb 5.6 vs 5.9/6.0 mmol/L (p = 0.004). A greater increase in liver enzymes was observed in children with the GG variant (p = 0.05). In 200 patients from the control group, there were no differences in the incidence of allelic variants of RFC1 in comparison to the hematological patients. Better treatment results were also obtained in patients with extra copies of chromosome 21 in leukemic cells. Similar relationships were reported by Belkov et al. and Zhang et al. [23, 24], who found that extra copies of chromosome 21 (RFC1 gene) are associated with increased expression of RFC1 mRNA and reflect elevated capacities for methotrexate transport.

In a multicenter study of a Polish group published in 2009 [25], the examined group consisted of 289 patients being treated for acute leukemia. Polymorphisms were determined for the following genes: MTHFR, TPMT, GSTT1, GSTM1, GSTP1 and TS. The study showed a significant relationship between genotype 677C > T and an increased death rate (OR: 4.09, 95% CI, p=0.028). Eight of the 31 patients whose death occurred during treatment had homozygous 677TT (26%), while in the remaining group homozygous 677TT patients represented only 8% (28 of 358 children – a significantly lower percentage). There were no statistically

significant differences in the frequencies of genotypes 677C> G in the population of children treated for ALL compared to the healthy group. There was also no significant correlation between the 677 C> G genotype and the risk of ALL relapse.

No studies regarding the prevalence of RFC1 polymorphisms in the Polish group have been published as yet. Reduced folate carrier's role in the metabolism and efficacy of methotrexate is not known. There are few data in the literature regarding the impact of specific polymorphisms, including RFC1, MS and MTRR, on the effectiveness of treatment during ALL maintenance therapy. The RFC 80G/A and MTHFR 677C/T polymorphisms may be associated with the severity of adverse events during maintenance therapy. In patients with polymorphic variants, treatment is frequently interrupted because the criteria for hematologic and biochemical therapy were not met [26].

Huang et al. studied a group of 81 patients with ALL and showed that in patients with polymorphisms in MTRR 66A> G, stomatitis (mucositis) occurred more frequently than in the control group ($p = 0.018$) [27]. Gemmati et al. evaluated the role of polymorphisms in folate metabolism and methylation with regard to the risk of ALL and NHL in adults [28]. They studied 120 patients with ALL, 200 with NHL and 257 healthy individuals. In patients with polymorphisms in both the MTHFR 677TT and MTRR 66AG genes, the risk of developing a proliferative process was 4.2 times lower compared to the rest of the group. Demonstration of a polymorphism in MS 2756GG decreased the risk of ALL five times. Also, Skibola et al. demonstrated the protective effects of mutations (a lower risk of ALL) [29]. Matsuo et al. pointed out that MS polymorphisms increase the risk of NHL [30].

Research conducted by Świerkot et al. offered interesting results in patients receiving methotrexate for rheumatoid arthritis [31]. The examined group consisted of 273 patients who received methotrexate for a minimum period of six months in weekly doses of 15–25 mg. The aim of the study was to determine the effect of MTHFR polymorphisms C677T and A1298C on the efficacy of the therapy and on the side effects. They demonstrated that the MTHFR 677CC polymorphism was associated with fewer adverse effects in the form of liver toxicity ($p = 0.02$). However, there were no statistically significant differences in efficacy depending on the investigated polymorphisms.

Conclusions

Polymorphisms in genes coding drug-metabolizing enzymes may cause individual differences in the effectiveness and toxicity of many medications, including cytostatics. The role of genetic changes affecting enzymes and methotrexate metabolites transporters is not yet fully understood. Researchers are hopeful that more detailed knowledge of the phenomenon of genetic polymorphism might improve the safety of methotrexate treatment.

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