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Research paper

Association between a microRNA binding site polymorphism in *SLCO1A2* and the risk of delayed methotrexate elimination in Chinese children with acute lymphoblastic leukemia

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ABSTRACT

Organic anion-transporting polypeptide 1A2 (OATP1A2) is involved in the cellular uptake of methotrexate (MTX). Genetic variation in solute carrier organic anion transporter family member 1A2 (*SLCO1A2*, the coding gene of OATP1A2) has important implications for the elimination of MTX. We investigated the association between a microRNA (miRNA) binding site polymorphism (rs4149009 G > A) in the 3′-untranslated region (3′-UTR) of *SLCO1A2* with the serum MTX concentrations in Chinese children with acute lymphoblastic leukemia (ALL). Genotyping for *SLCO1A2* rs4149009 G > A in 141 children with ALL was performed using the Sequenom MassARRAY system. Serum MTX concentrations were determined by fluorescence polarization immunoassay. The percentages of MTX level ≥1 μmol/L at 42 h were compared among the AA, GA, and GG genotypes. The minor allele frequency observed in this study (33.0%) was significantly lower than that in the African samples reported in the 1000 Genomes Project (57.4%, $P = 0.00$). The incidence rate of delayed MTX elimination was significantly higher in patients with the GG genotype (23.1%) compared with the AA genotype (0.0%, $P = 0.03$). Bioinformatics tools predicted that the rs4149009 A allele would disrupt the putative binding sites of hsa-miR-324-3p and hsa-miR-1913. These results indicate that the rs4149009 G > A polymorphism might affect MTX pharmacokinetics by interfering with the function of miRNAs.

1. Introduction

Methotrexate (MTX) is a folate analogue widely used in high dose (HD) in consolidation and maintenance therapy for childhood acute lymphoblastic leukemia (ALL) [1,2]. The antileukemic effect of MTX is attributed to its competitive inhibition of dihydrofolate reductase (DHFR). However, patients with delayed elimination of MTX develop severe toxicities, including hepatotoxicity and myeloid suppression, which often lead to the interruption or discontinuation of chemotherapy, which may thereby increase relapse risk [3–5]. About 20% of pediatric ALL patients do not achieve satisfactory efficacy with MTX therapy [6]. There is a clear association between MTX exposure and response. Therefore, MTX concentrations are monitored in routine clinical practice to reduce toxicities caused by excessive exposure to the drug. Therapeutic drug monitoring has been demonstrated as a good strategy for personalized therapy with HD-MTX [7].

The pharmacokinetics of MTX exhibit large interindividual variability [8]. Identifying the predictive markers of MTX exposure is important for maintaining the balance between efficacy and toxicity. To some extent, the genetic variations in transporters, drug-metabolizing enzymes, and targets account for the inter-patient differences in MTX pharmacokinetics [9]. MTX enters the cells through active transport mediated by reduced folate carrier 1 (RFC1) [10]. Folylpolyglutamate synthase (FPGS) and gamma-glutamyl hydrolase (GGH) are the two main metabolic enzymes involved in the intracellular polyglutamylation of MTX [11,12]. Methylenetetrahydrofolate reductase (MTHFR) is a target in the folate cycle that is blocked by MTX and its metabolites [13]. Laverdière et al. showed that rs1051266 G > A polymorphism in the coding gene of RFC1 was associated with the plasma MTX levels and treatment outcome in patients with ALL [14]. Previously, we found that the *FPGS* rs1544105 G > A, *GGH* rs3758149C > T, and *MTHFR* rs1801133C > T genetic polymorphisms contributed to the variability

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of MTX pharmacokinetics [15].

Organic anion-transporting polypeptide 1A2 (OATP1A2) belongs to the OATP transporter family that is expressed in a variety of tissues and organs important for drug elimination. OATP1A2 plays a role in the drug intestinal absorption, tubular reabsorption from urine, secretion into bile, as well as uptake into the brain [16,17]. Previous studies have indicated that MTX is also a substrate of OATP1A2. Badagnani and colleagues confirmed that OATP1A2 participates in the active tubular reabsorption of MTX [18]. Several studies have shown that certain OATP1A2 variants may result in the impaired cellular uptake and pharmacokinetics of some drugs. Badagnani et al. identified four non-synonymous variants in solute carrier organic anion transporter family member 1A2 (*SLCO1A2*, the coding gene of OATP1A2) that exhibited altered transport of MTX [18]. Therefore, this transporter is an important determinant of MTX elimination and toxicity.

So far, pharmacogenetic studies of OATP1A2 have focused on single-nucleotide polymorphisms (SNPs) in the coding and 5′-regulatory regions of *SLCO1A2*. Recent studies have suggested that SNPs within the microRNA (miRNA) binding sites of the 3′-untranslated region (3′-UTR) are associated with disease risk and drug responses [19,20]. Mishra et al. identified a miR-24 binding site polymorphism in the 3′-UTR of *DHFR* that led to the loss of miR-24 function and resulted in *DHFR* overexpression and MTX resistance [21]. Considering the important role of OATP1A2 played in MTX transport, whether polymorphisms in the 3′-UTR of *SLCO1A2* would affect the disposition of MTX in pediatric patients with ALL remained poorly understood. Therefore, in the present study, we investigated the distribution of a novel miRNA binding site polymorphism (rs4149009 G > A) in the 3′-UTR of *SLCO1A2*, and its association with the risk of delayed MTX elimination in Chinese children with ALL.

2. Materials and methods

2.1. Study population

Blood samples were obtained from 141 patients with ALL who were treated at the Pediatric Department of Beijing Shijitan Hospital, Capital Medical University, Beijing, China, from May 2009 to November 2013. All patients received HD-MTX, followed by leucovorin rescue treatment. All patients were in complete remission prior to MTX administration. The patients were assigned to a high risk or non-high risk group according to their clinical characteristics, leukemic cell biological profile, and early response to induction treatment. The patients received 0.5–3 g/m^2 MTX (non-high risk group) or 3–5 g/m^2 MTX (high risk group). The first dose (1/6 of the full dose; no more than 500 mg) was intravenously transfused within 30 min. The remaining dose was infused during the following 23.5 h. Leucovorin rescue (15 mg/m^2) was started 36 h after HD-MTX and continued for at least six doses every 6 h until the serum MTX concentration was < 0.1 μmol/L. The patients received intravenous hydration and alkalization according to standard protocols.

2.2. MTX determination

All blood samples were centrifuged (3000*g*, 5 min) at room temperature to obtain the serum. The serum concentrations of MTX were measured using fluorescence polarization immunoassay on a TDxFLx analyzer (Abbott Laboratories, Abbott Park, IL, USA). The serum MTX concentrations were determined at 24 h and 42 h after the start of infusion (MTX C_{24h} and C_{42h}). The lowest measurable concentration was 0.01 μmol/L. The measurements of quality control samples with low, medium, and high concentrations were between 90% and 110% of the stated sample concentrations. The intra- and inter-day variations were all within 10% of the mean concentrations.

2.3. Genotyping

Genomic DNA was extracted from frozen blood samples using a QIAamp DNA Blood Mini Kit (Qiagen, Valencia, CA, USA) in accordance with the manufacturer's directions. DNA concentration and purity were determined by the absorbance at 260 nm and 280 nm. DNA samples were diluted to working concentrations of 30–50 ng/mL before genotyping. Approximately 20 ng genomic DNA was used to genotype each sample. The rs4149009 G > A polymorphism was genotyped using the Sequenom MassARRAY system (Sequenom iPLEX assay, San Diego, CA, USA). Locus-specific PCR and detection primers were designed using MassARRAY Assay Design 3.1 software (Sequenom). Allele detection was performed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. To evaluate the genotyping accuracy for the investigated polymorphism, 10 samples were genotyped repeatedly, and the results were 100% consistent. The genotyping call rates for rs4149009 G > A were > 95%.

2.4. Statistical analyses

Data were analyzed using GraphPad Prism Software version 4.00 (www.graphpad.com). The distributions of genotypes and alleles were assessed for deviation using the Hardy–Weinberg equilibrium, and the percentage of patients with delayed MTX elimination was compared based on genotype using the chi-square test or Fisher's exact test. The associations between covariates and the risk of delayed MTX elimination were evaluated using relative risks (RR). The Kruskal–Wallis H test was used for comparisons among three non-normally distributed groups. $P < 0.05$ was considered statistically significant.

3. Results

3.1. General characteristics of study population

A total of 141 Chinese children with ALL (male/female, 86/55; B-cell/T-cell/mixed-lineage ALL, 112/14/15) were included in the study. The mean age (± SD) of the participants was 7.35 (± 3.97) years. The median MTX dosage was 2.50 g/m^2. The mean MTX C_{24h} and C_{42h} were 32.68 (± 14.34) and 0.64 (± 1.29) μmol/L, respectively. There was delayed MTX elimination ($C_{42h} \geq 1$ μmol/L) in 27 patients (19.1%).

3.2. Genotype and allele frequencies of SLCO1A2 rs4149009 G > A

Table 1 summarizes the genotype and allele frequencies of the *SLCO1A2* rs4149009 G > A polymorphism. No significant deviations from Hardy–Weinberg equilibrium were observed for the investigated polymorphism ($P > 0.05$). Overall, 17 (12.1%), 59 (41.8%), and 65 participants (46.1%) had the AA, CT, and TT genotype, respectively. The minor allele frequency (MAF) observed in the present study (33.0%) were similar to those in the HCB (Han Chinese in Beijing, China; 35.0%), JPT (Japanese in Tokyo, Japan; 31.3%), and CEU (Utah residents with northern and western Europe ancestry; 30.3%) samples of the 1000 Genomes Project. However, it was significantly lower than that in the YRI (Yoruba in Ibadan, Nigeria; 57.4%) samples ($P < 0.01$), indicating marked ethnic differences in the distributions of the *SLCO1A2* rs4149009 G > A polymorphism.

3.3. Effects of SLCO1A2 rs4149009 G > A polymorphism on the risk of delayed MTX elimination

Table 2 presents the association between the *SLCO1A2* rs4149009 genotype and the risk of delayed MTX elimination. There were no statistical differences in MTX doses given for the three genotype groups with AA, AG, and GG. The mean MTX dosages for three genotype groups were 2.35, 2.36 and 2.45 g/m^2, respectively. And the median MTX dosages for three genotype groups were all 2.50 g/m^2. There were