Leukemia Research 39 (2015) 1048–1054

Contents lists available at ScienceDirect

Leukemia Research

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

Frequency of *ITPA* gene polymorphisms in Iranian patients with acute lymphoblastic leukemia and prediction of its myelosuppressive effects

Fatemeh Azimi[a], Yousef Mortazavi[a,**], Samin Alavi[b], Mitra Khalili[a], Ali Ramazani[c,d,*]

[a] *Molecular Medicine & Genetics Departments, Faculty of Medicine, Zanjan University of Medical Sciences, Zanjan, Iran*
[b] *Pediatric Congenital Hematologic Disorders Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
[c] *Cancer Gene Therapy Research Center, Zanjan University of Medical Sciences, Zanjan, Iran*
[d] *Biotechnology Department, School of Pharmacy, Zanjan University of Medical Sciences, Zanjan, Iran*

ARTICLE INFO

Article history:
Received 20 February 2015
Received in revised form 23 June 2015
Accepted 29 June 2015
Available online 4 July 2015

Keywords:
Acute lymphoblastic leukemia
Polymorphism
ITPA
6-MP
Iran

ABSTRACT

6-Mercaptopurine (6-MP) plays an important role in treatment of childhood acute lymphoblastic leukemia (ALL). Inosine triphosphate pyrophosphohydrolase (ITPA) is an enzyme involved in 6-MP metabolic pathway that convert the inosine triphosphate (ITP) to inosine monophosphate (IMP) and prevents the accumulation of the toxic metabolite ITP. Our objective was to evaluate the *ITPA 94C>A*, *IVS2+21A>C* polymorphisms in patients with ALL treated with 6-MP and prediction of its clinical outcomes. Our study population consisted of 70 patients diagnosed with ALL in the Division of Hematology–Oncology of Tehran Mofid Hospital. PCR was carried out to amplify exon 2, exon 3, intron 2, and intron 3 of *ITPA* gene then, all the amplified fragments were subjected to directional sequencing and then association between genotype and 6-MP toxicity was studied. In this study two exonic variants including *94C>A* and *138G>A* showed a prevalence of 8.5% and 36.4%, respectively. Two intronic variants, *IVS2+21A>C* and *IVS3+101G>A* were found in 13.5% and 7% of the samples, respectively. The rate of myelosuppression in the presence of mutant homozygote and heterozygous alleles (*94C>A*, *138G>A*, *IVS2+21A>C* and *IVS3+101G>A*) was higher than that of wild type alleles during the use of 6-MP. Hepatotoxicity in patients with mutant homozygous and heterozygous *94C>A* and *IVS3+101G>A* during the treatment 6-MP was higher than before treatment with 6-MP. Our results showed that patients with aberrant ITPase genotype (mutant homozygous or heterozygous), more likely to be myelosuppressed and show liver toxicity after treatment with 6-MP. Our results suggest that pre-therapeutic screening of patients for *ITPA 94C>A*, *IVS2+21A>C* and *IVS3+101G>A* can help in minimizing the adverse effects of 6-MP in ALL patients.

© 2015 Elsevier Ltd. All rights reserved.

1. Introduction

Acute lymphoblastic leukemia (ALL), the most common cancer among children, can be treated with combination chemotherapy and routine maintenance therapy with 6-mercaptopurine (6-MP), a purine nucleoside analog [1]. The cytotoxic effects of 6-MP can be life threatening, primarily due to the myelosuppression that is associated with 6-thioguanine nucleotides (6-TGNs) incorporation into the DNA of leukocytes and the resultant treatment failure and increased risk of relapse [2–5]. Germ-line polymorphisms can modify drug-metabolizing enzymes, drug transporters, or the drug target and, thereby, influence the pharmacodynamic and pharmacokinetic effects and, thus, the efficacy or toxicity of anti-leukemic therapy [6–8]. Three enzymes are central to 6-MP metabolism: xanthine oxidase (XO) and thiopurine S-methyl transferase (TPMT) are catabolic, whereas hypoxanthine guanine phosphoribosyl transferase (HGPRT) mediates the anabolic pathway. Xanthine oxidase metabolizes 6-MP to an inactive thiouric acid (TU), whereas TPMT methylates 6-MP to an inactive metabolite, 6-methyl mercaptopurine (meMP). 6-M ercaptopurine is metabolized by initial conversion to 6-thioinosine-5′-monophosphate (6-TIMP) further multi-step metabolism to the active 6-TGNs [2], which is incorporated into DNA/RNA or with phosphate in 6-thioinosine triphosphate (TITP), a step reversible by inosine triphosphate pyrophosphatase (ITPA) [9].

Polymorphisms of the *TPMT* gene have been reported to affect 6-MP dose reduction and therapy interruption [10–14] as well

* Corresponding author at: Cancer Gene Therapy Research Center, Zanjan University of Medical Sciences, Zanjan, Iran.
** Corresponding author.
E-mail addresses: youmort@yahoo.com (Y. Mortazavi), ramazania@zums.ac.ir (A. Ramazani).

http://dx.doi.org/10.1016/j.leukres.2015.06.016
0145-2126/© 2015 Elsevier Ltd. All rights reserved.

Table 1
Primer sequences and PCR conditions for target amplifications.

Primer name	Sequence (5′–3′)	Product length (bp)	PCR conditions
ITPA F1	TAGGAGATGGGCAGCAGAGT	500	ID: 95 °C/5 min (1 cycle) D: 94 °C/1 min (35 cycles) A: 62 °C/45 s (35 cycles) E: 72 °C/1 min (35 cycles) FE: 72 °C/5 min (1 cycle)
ITPA R1	GTGTATGGTGGTGGTCTGGG		

ID, initial denaturation; D, denaturation; A, annealing; E, extension; FE, final extension.

as a decrease in leukocyte and neutrophil counts during maintenance therapy [12,15–17]. Stocco et al. [18] studied the effects of genetic polymorphisms of *TPMT* and *ITPA* on 6-MP toxicities in ALL patients, and reported that febrile neutropenia was significantly higher in patients with the *ITPA* variant alleles. Variant *ITPA* alleles increased the risk of febrile neutropenia in Caucasian patients whose 6-MP dosages were adjusted by *TPMT* genotype. Thus, 6-MP toxicity effects are not caused by the *TPMT* polymorphisms alone.

Inosine triphosphate pyrophosphatase is an important enzyme in thiopurine metabolism that converts inosine triphosphate (ITP) back to inosine monophosphate (IMP). *ITPA* gene defects cause the accumulation of non-canonical nucleotides in cells and their incorporation into nucleic acids [19]. The most common polymorphisms identified to be associated with ITPA deficiency are *ITPA 94C>A* and *IVS2+21A>C*. The *ITPA 94C>A* allele frequency ranges between 0.01 and 0.19 across various ethnic groups worldwide and is 0.06 among Caucasians [20,21]. Subjects homozygous for this polymorphism do not have erythrocyte ITPA activity, whereas heterozygous subjects have an average ITPA activity 22.5% of the normal. The *IVS2+21A>C* polymorphism influences splicing efficiency and appears at a frequency of 0.13 in Caucasian populations. ITPA activity in heterozygous individuals with the intron 2 (*A>C*) polymorphism averages 60% of the control mean, whereas those homozygous for this polymorphism have activity similar to that of heterozygous individuals with the *ITPA 94C>A* polymorphism. Individuals with the compound heterozygous polymorphisms *94C>A* and *IVS2+21A>C* have 10% of the normal mean ITPA activity [22]. Previous studies have shown significant association between the *ITPA 94C>A* polymorphism and the onset of adverse events, especially flu-like symptoms, pancreatitis, hepatotoxicity, fever, or rash [23]. Patients with an *ITPA 94C>A* polymorphism, in comparison with those without or with a variant *94C>A*, have significantly earlier onset of adverse events [24]. An ITPA deficiency might predict the likelihood of adverse events to 6-MP therapy and its prodrug azathioprine (AZA). However, the effects of *ITPA* polymorphisms on 6-MP toxicity have not been studied in Iranian subjects.

This is the first study to investigate *ITPA 94C>A* and *IVS2+21A>C* polymorphisms in ALL patients treated with 6-MP and the prediction of its myelosuppressive effects. We selected only two exons and two introns in the *ITPA* gene with an aim to focus on the mutants that are usually associated with hematologic toxicity in other ethnic groups and populations.

2. Materials and methods

2.1. Patients and treatment

Our study population comprised 70 patients with ALL (36 girls and 34 boys; age at diagnosis: 1–9 years) and subjects were assigned to the standard risk group if their leukocyte count was less than 50×10^9/L. The standard risk group included patients who had received chemotherapy in accordance with the Children's Cancer Group (CCG) protocols, modified from the CCG-1881, -1891, or -1952 between 2012 and 2014. The CCG protocol specifies a standard daily dose of 50 mg/m^2 6-MP for maintenance therapy in ALL. In this study, the 6-MP dose was adjusted to maintain a white blood cell (WBC) count of $2–3 \times 10^9$/L and to avoid adverse events. After 6 weeks of 6-MP maintenance therapy, the average decrease in total leukocyte and neutrophil counts and increase in hepatic enzyme concentrations during the therapy was considered a measure of toxicity.

The study protocol was approved by the institutional ethics committee of Zanjan University of Medical Sciences (Zanjan, Iran). Informed consent was obtained from the parents or guardians of all patients who participated in the study. All patients were of Iranian ethnicity.

2.2. DNA isolation and PCR amplification

2 ml of blood samples were collected from all the patients in EDTA anticoagulant tubes. Genotyping was performed during or after completion of maintenance therapy. Genomic DNA was extracted from whole blood using AccuPrep^k Genomic DNA Extraction kit (Bioneer, South Korea). Isolated DNA was stored at −20 °C until use. The exon 2, exon 3, intron 2 and intron 3 of ITPA gene fragments were amplified using gene specific oligonucleotide primers. The primer sequences and PCR conditions are shown in Table 1.

PCR was performed in 50 μL mixture containing 200 ng of genomic DNA, 10 pM of each primer, 25 μl of 2 × Master Mix (Fermentas, USA) containing Taq DNA polymerase, dNTPs, $MgCl_2$, and reaction buffers. PCR products were analyzed in 2 % agarose gel electrophoresis and visualized under UV light.

Then 50 μl of PCR product with forward primer for direct sequencing of ITPA (exon 2, exon 3, intron 2 and intron 3) was sent to South Korea. Electropherograms were analyzed in sense directions for the presence of polymorphisms (Fig. 1). Sequencing was performed by the Sanger method on an ABI 3730 sequencer (Bioneer, South Korea). The resulting sequences were analyzed using BLAST, Clustal X2, Chromas lite, and Bio Edite softwares and submitted to the GeneBank database (Accession numbers: KP144803–KP144871).

2.3. Statistical analysis

The statistical significance of the differences in genotype frequencies was assessed using a logistic regression ($P < 0.05$ was considered to be statistically significant), and odds ratios (ORs) were calculated along with their 95% confidence intervals (CI) with the SPSS (version 16.0). The linkage disequilibrium measured by means of D, D' and r^2.

3. Results

Seventy children (34 boys and 36 girls; age, 1–9 years) with ALL were included in the study. There was no statistically significant difference between the sexes [$P = 0.86$, OR (95% CI) = 1 (0.6–2)].

By analyzing *ITPA* sequences in addition to polymorphisms *94C>A* and *IVS2+21A>C*, two other polymorphisms *138G>A* and *IVS3+101G>A* were identified in the study population. Sequencing results in homozygous and heterozygous mutants are shown in Fig. 1.

Genotyping of *ITPA* was done in all patients. Allelic variant *ITPA* genes and their frequencies are shown in Table 2. Because of the bidirectional sequencing of two exonic and two intronic *ITPA* genes, we identified a *138G>A* in exon 3 and a novel mutation *IVS3+101G>A* in intron 3. Two exonic variations were observed in the *ITPA* gene [ITPA exon 2 (*94C>A*) and exon 3 of *ITPA* (*138G>A*); variant allele frequencies: 8.5% and 36.4%, respectively] and two intronic variants [*ITPA Int2* (*A>C*) and *Int3* (*G>A*); variant allele frequencies: 13.5% and 7%, respectively].

In the present study, the allele frequencies of compound heterozygous *94C>A* and *138G>A*, *94C>A* and *IVS3+101G>A*, and *IVS2+21A>C* and *138G>A* were 14.3%, 12.9%, and 4.3%, respectively.

Download English Version:

https://daneshyari.com/en/article/2136498

Download Persian Version:

https://daneshyari.com/article/2136498

Daneshyari.com