



Case Report

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Correlation of SLC19A1 A80G and MTHFR C677T Polymorphisms with Methotrexate-Induced Hepatotoxicity in a Paediatric Acute Lymphoblastic Leukaemia Patient: A Case Report

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Abstract

Background: High-dose methotrexate is a crucial component of acute lymphoblastic leukaemia (ALL) treatment regimens. Adherence and tolerance to the treatment plan are critical factors to achieve the prognostic goals, therefore, any signs of toxicity should be taken into consideration.

Observations: This study describes the clinical case of an ALL paediatric patient who developed hepatotoxicity after methotrexate treatment. SLC19A1 and MTHFR variants were identified suggesting an association between these polymorphisms and the increase in methotrexate toxicity.

Conclusions: The present study highlights the potential of polymorphisms analysis to improve outcome predictions and treatment personalization for paediatric patients with ALL.

Keywords

Methotrexate, Hepatotoxicity, Paediatric All, Slc19a1, Mthfr

Introduction

Acute lymphoblastic leukaemia (ALL) represents 25-35% of paediatric cancer diagnoses and it is, therefore, the most frequent neoplastic process in the paediatric population. The treatment of ALL is based on various chemotherapy regimens among which methotrexate (MTX) plays a key role [1]. MTX antitumour effect is due to its double action: it exerts a direct inhibition on thymidylate synthetase (TS) on one hand, and, on the other hand, it blocks the action of dihydrofolate reductase in a competitive manner. This mechanism results in an intracellular tetrahydrofolate deficiency which leads to purine and thymidine synthesis deficit, thereby blocking DNA replication and inducing cell death.

Usually, MTX efficacy correlates with its plasma concentration, nevertheless, the intracellular concentration is the key determinant of its efficacy. MTX intracellular concentration mainly depends on the reduced folate

transporter protein (RFC-1) activity. MTX molecules undergo a polyglutamylation process in the cell cytoplasm, in the same way as the folates, which increases MTX retention rate inside the cell and thus enhances its cytotoxic action [2].

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As in many other cancerous processes, treatment resistance develops relatively frequently among ALL paediatric patients. Additionally, congenital primary resistances derived from alterations in the activity of the RFC protein can be detected. In particular, the change in the SLC19A1 gene (A80G polymorphism) that results in the substitution of histidine for arginine at residue 27 of the protein sequence has been identified. The A80G polymorphism impairs the transport capacity of RFC-1 protein and, consequently, carriers develop greater toxicity to high-dose MTX. On the other hand, the most studied alterations are those affecting MTHFR enzymatic activity, which is responsible for the conversion of 5,10-methylenetetrahydrofolate (5,10-CH₂-THF) to 5-methylenetetrahydrofolate (5-CH₁-THF) in the folic acid cycle. The C677T polymorphism in the MTHFR gene results in an alanine to valine substitution which leads to reduced MTHFR enzymatic activity (35% and 65% reduction for the heterozygotes and homozygotes respectively) and

increases the risk of hepato- and gastrointestinal toxicity [3].

This study describes the case of a paediatric patient diagnosed with ALL, homozygous carrier of A80G mutation in the SLC19A1 gene and heterozygous carrier of C677T mutation in the MTHFR gene, who developed hepatotoxicity upon administration of MTX.

Case Presentation

An 8-year-old male patient was diagnosed in September 2017 with standard-risk B-lymphoblastic leukaemia and treated according to the SEHOP-PETHEMA/13 scheme [4]. After completion of the treatment, total remission was confirmed through a bone marrow aspirate and subsequent tissue analysis (October 2019). Throughout that initial first-line treatment, MTX-derived hepatotoxicity was observed in the treatment consolidation phase (Table 1).

In July 2020, relapse was identified after 9 months of

Table 1: Hepatotoxicity and MTX plasma concentrations after high-dose MTX administration.

Date	MTX Dose (mg/m ²)	ALT (U/L)	GGT (U/L)	MTX PC (µmol/L)
ALL treatment at debut SEHOP-PETHEMA/13				
20-12-2017	5			
21-12-2017		47	21	
22-12-2017		467	18	0.54
28-12-2017		641		
02-01-2018		166	30	
08-01-2018	5			
10-01-2018		113	70	0.68
11-01-2018		126	69	
17-01-2018		46	58	
21-01-2018		27	40	
25-01-2018	5			
27-01-2018		338	45	0.56
28-01-2018		318	42	
07-02-2018		51	29	
08-02-2018	5			
10-02-2018		40		0.64
20-02-2018		32	19	
26-02-2018		24	15	
ALL treatment at relapse SEHOP-PETHEMA/15				
16-07-2020	1			
18-07-2020		191	23	1.11
19-07-2020		279	27	
20-07-2020		483	30	
21-07-2020		456	36	
20-10-2020	1			
22-10-2020		121	45	7.04
25-10-2020		87	40	
27-10-2020		74	40	

12-11-2020	1			
14-11-2020		224	60	0.66
15-11-2020		288	61	
16-11-2020		435	66	
17-11-2020		411	73	
04-12-2020	1			
05-12-2020		305	121	
06-12-2020		432	125	1.08
07-12-2020		498	127	
08-12-2020		531	135	

MTX: (methotrexate) Dose in mg/m²; ALT: Alanine Amino Transferase; GGT: Gamma Glutamyl Transferase; PC: Plasma Concentrations at 36 hours after methotrexate administration in µmol/L

Table 2: Genotyping of genetic variants associated with response to MTX.

Gene	Rsnumber	Variation	Genotype	Interpretation
MTHFR	rs1801133	C677T	CT	HET
	rs1801131	A1298C	AA	WT
GGH	rs11545078	-T401C	CC	WT
SLC19A1	rs1051266	A80G	GG	MUT
SLCO1B1	rs11045879	g.103492T>C	TT	WT
DHFR	rs1105525	g.5293G>C	GG	WT
SHMT1	rs1979277	c.1303C>T	CC	WT
ABCC2	rs717620	-C24T	CT	HET
ABCB1	rs1045642	c.3434C>T	CC	WT
ITPase	rs1127354	c.94C>A/G	CC	WT
TYMS	rs34743033	Indel (28 pb 2R o 3R)	3R3R	MUT

*WT: Wild Type; HET: Heterozygous; MUT: Homozygous Mutant; R: Repeats

treatment and the patient was treated according to the SEHOP-PETHEMA/15 [5] recommendations. During the consolidation period, after the administration of methotrexate (1,000 mg/m²), the patient showed liver toxicity once again (Table 1), which led to a delay in the administration of cytarabine (ARA-C).

Toxicity was not due to a delayed elimination of MTX in any of the cases, since plasma level showed expected values for this treatment at 36 h (Table 1). Causal relationship between drug administration and toxicity was assessed using Naranjo algorithm [6] and adverse drug reaction was classified as definite with a final score of 9 (Supplemental Digital Content).

We decided to analyze the presence of certain germ line mutations in genes involved in the pharmacodynamics of MTX (Table 2). Genomic DNA was extracted from a dried blood spot test using an alkaline lysis extraction method [7]. Genetic analysis was performed by Sanger sequencing (*Macrogen, Spain*), after DNA amplification by conventional PCR on a T100TM thermal cycler (*Bio-Rad*) by using specific primers corresponding to each variant. The size and yield of the amplicons were tested by electrophoresis and further purified before analysis. The electropherograms obtained were compared with the reference sequences deposited in

Gen Bank after alignment by *MEGA* software [8].

The analysis showed that the patient carried mutant alleles for some of the mutations tested. More precisely, he showed to be homozygous for A80G (SLC19A1) and C677T (MTHFR) mutations.

Discussion

In this study, we have described the case of a paediatric patient diagnosed with ALL who developed liver toxicity after MTX treatment, despite not showing signs of delayed elimination of the drug. Multiple studies regarding MTX pharmacogenetics have linked protein activity defects during MTX pharmacodynamics with drug tolerance.

The patient was a homozygous carrier of the A80G mutation in the SLC19A1 gene and a heterozygous mutant C677T in the MTHFR gene. Both variants have been identified as conditioning factors for MTX toxicity. Gregers, et al. [9] reported that patients with the 80GG genotype had lower plasma concentrations of MTX and higher toxicity. Greger's team also observed lower folate and higher homocysteine plasma levels in the healthy population. Chango, et al. [10] reported the same phenomenon in patients similar to that described in our report (GG in SLC19A1 and CT in MTHFR).

Concerning the C677T marker of the MTHFR gene, it has been correlated on multiple occasions with MTX toxicity: Ongaro, et al. [3] confirmed that the presence of the T allele implies a higher risk of liver and gastrointestinal toxicity in the adult population, which was also described in pediatrics by Kantar, et al. [11]. Years later, Noha M [12]. Established a similar correlation, being able to estimate a relapse probability based on the T allele presence itself (50% TT, 28.57% CT and 13.64% CC). In the same line, the D'Angelo group quantified a 12-fold increased risk of experiencing an adverse event among TT patients. Nevertheless, the clinical relevance of C677T as a marker is still controversial, as contradictory results have also been reported, likely due to the heterogeneity of the patients. Thus, another set of studies as those carried out by Aplenc [13], a robust study involving 520 ALL patients; Shimasaki's [14] with 15 ALL patients; and Frikha's involving 35 young patients, failed to establish the same correlation [15]. Furthermore, López-López in a letter to the editor discusses D'Angelo work results to the point of recalculating the statistical values in order to provide evidence that refutes their findings [16]. Further studies have tried to correlate MTHFR gene mutations with the risk of developing ALL. Kałużna, et al. found an association between the presence of the T polymorphism and the risk of ALL in subjects under 18 years of age [17]. However, a more recent meta-analysis conducted by Li Yu's team revealed that there was no significant correlation between the MTHFR polymorphism and the risk of ALL in any of the populations studied [18].

Although no MTX dose adjustment is currently recommended when mutations in SLC19A1 and MTHFR genes are identified, the present case report aims to provide evidence that being aware of their presence in advance, could help the clinical team to consider potential treatment-derived toxicity and allow a more personalized care approach for ALL patients.

Patient Consent for Publication

Written informed consent was obtained from patient legal representatives to publish case-relevant information in the present report.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest concerning the research, authorship, and/or publication of this case report.

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