

Case Report

High-Dose Methotrexate, A Tried-and-True Treatment with Genomic Delicacies: A Case Report

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Received: 17 Sep 2020

Accepted: 28 Sep 2020

Published: 30 Sep 2020

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1. Abstract

The prediction of methotrexate (MTX) adverse events has been a challenge to the clinician; here we present a case of toxicity after the previous exposition with the development of hepatic, renal, and hematologic toxicity. The development of methotrexate toxicity has been previously analyzed in our institution, where a significant percentage of the patients present polymorphisms in the reduced folate carrier and the methylene-tetrahydrofolate reductase, which several groups have associated with toxicity and treatment efficacy. The clinical applicability of this knowledge in the prevention and dose adjustment is still debatable; a genomic screening for all patients that will be exposed to methotrexate (especially in high doses) is warranted.

2. Keywords: Methotrexate; Liver toxicity; Renal toxicity; Single nucleotide polymorphisms; Reduced folate carrier

3. Case Report

Methotrexate (MTX) is one of the oldest drugs that is still used in standard treatments. The potential toxicity is difficult to predict, and current guidelines are not available in this aspect. The identification of polymorphisms could pave the way. Here, we present the case of a patient who developed toxicity after a second exposition to the drug. The pharmacogenomics associated with the apparently idiosyncratic adverse events of methotrexate have been extensively studied, and multiple single nucleotide polymorphisms have been identified to protect [4, 6, 11] or predispose [4-9] certain toxicities, although an important quantity of information is published in regard to pharmacogenomics and toxicity risk, the clinical applications are scarce and no clear consensus in which patients should be tested and which mutations [18, 19].

We present a 73-year-old patient with a diagnosis of stage IV diffuse large B cell lymphoma (DLBCL) in 2014, was treated with six cycles of R-CHOP and radiotherapy to the neck. The patient had a complete remission and was assigned to continue under surveillance. In July 2019, the patient developed weakness of the lower limb, sixth cranial nerve paralysis, and uncoordinated movements of the upper limb. A relapse of DLBCL with central nervous system (CNS) infiltration was documented by both the head magnetic resonance and the spinal fluid analysis. The patient was treated with rituximab (375 mg/m², 4 hour IV infusion in day+1) and high-dose MTX (3 g/m², 24 hour IV infusion on day +1) plus folinic acid rescues.

The patient received her first cycle in July 2019 without any complications; a second chemotherapy cycle was assigned for August 2019. During the second cycle, the patient was pre-hydrated, and the urine alkalization therapy reached its upper pH7.5 goal. After-

ward, the chemotherapy infusion started and was completed without adverse events; the folinic acid rescues began after the methotrexate infusion. The 24-hour control laboratories showed an elevation of creatinine (2.0 mg/dl, previous 0.7 mg/dl), a urinary catheter was inserted, hydration optimized, kidney ultrasound showed no structural anomalies, and MTX levels obtained (1.4 mmol/l). The folinic acid rescues doses were adjusted according to the MTX blood levels. Even with the treatment modifications, the patient developed grade 1 oral mucositis, elevated creatinine persisted (2.3 mg/dl) after 96 hours of finishing the chemotherapy infusion.

A new MTX blood level was obtained (0.6 mmol/l), and folinic acid rescue treatment was readjusted. After five days of initial MTX infusion, the patient developed grade 3 neutropenia, with the persistence of acute renal lesion and elevations of liver enzymes (AST 582 IU/l, ALT 403 IU/l, LDH 815 IU/l, GGT 148 IU/l, ALP 80 IU/l, total bilirubin 0.65 mg/dl, direct bilirubin 0 mg/dl). After 11 days of treatment, the creatinine blood concentration decreased, neutropenia ceased, and liver enzymes normalized. The patient was discharged with normal creatinine clearance, and leucocyte levels and the MTX level were <0.1 mmol/l. In previous studies conducted in our institution, the presence of single nucleotide polymorphism (SNP) in the reduced folate carrier (RFC) (SLC19A1) G80A and methylene-tetrahydrofolate reductase (MTHFR) C677T have been reported, the testing for these polymorphisms was also positive in this particular case.

4. Discussion

The MTX action mechanism and pharmacogenetic considerations have been described in depth. The extremely similar structure of this drug compared with folic acid allows it to act as a competitive inhibitor of the enzyme that utilizes folate with a 1000-fold increased affinity [1]. MTX enters the cell using the solute carrier family 19 (SLC19A1), also named reduce folate carrier 1 (RFC-1). After entering, it will convert into its active form, MTX polyglutamate. This process is catalyzed by the folylpolyglutamate synthetase; using its MTX-PG form, the drug can inhibit the dihydrofolate reductase (DHFR) [2]. Along with the DHFR, the thymidylate synthase (TYMS) is inhibited, suppressing the conversion of dUMP to dTMP, which provides an alternate pathway to suppress the DNA and RNA synthesis [3]. As any other drug, the MTX needs to be metabolized to prevent sustained effects and damage; the efflux MTX transporter ABC sub-family C (ABCB), mainly ABCG2 and ABCB1, is required to remove it.

Mutations in the access way and in the metabolism process have been involved with an increased risk of resistance or toxicity. Polymorphisms of the solute carriers organic anion transporter 1B1 (SLC01B1), MTX carrier predominantly located on human hepatocytes, in rs11045879 and rs4149081 have been correlated with increased activity and transport to the liver, where it then passes to the digestive system where it generates increased gastrointestinal toxicity [4]. The single nucleotide polymorphisms (SNP) in SLC01B1 have been

studied in extensive, and many have been identified, although only a few are known to have clinical significance: SLC01B1 rs4149056 variant is associated with decreased transport in vitro [5], SLC01b1 rs2306283/rs11045879/rs4149056 are associated with clearance of MTX [6]. The SLC19A1 (RFC-1) has also been associated with toxicity-related polymorphisms. The G80A has demonstrated to increase overall toxicity, with growing evidence [7-9]. In the population of our institution, predominantly of Hispanic and Mesoamerican indigenous ancestry, we have identified a frequency of 40-50% [20], which is bears similarity to European reports with a frequency of 36-41% [21]. In a previous study, we weren't able to correlate the polymorphism with toxicity, but in the multivariate analysis, a reduction in overall survival and relapse-free-survival was observed, a similar decrease in treatment efficacy has been observed in other populations [22].

Previous studies in SNP of the genes encoding for transporter MTX genes suggest that these changes have an influence in the treatment response. In a study by Liu and colleagues, the pharmacogenomics of 322 patients was evaluated after the infusion of HD-MTX. The patients with the genotype ABCB1 rs1128503 C allele presented longer-hospitalization duration and worse outcome compared with the TT and TC allele genotype. The presence of oral mucositis was not statistically significant, with ABCB1 rs 1128503C TC or CC allele [6].

The presence of oral mucositis is a side effect commonly reported in patients who receive methotrexate, especially those with high-doses. Certain polymorphisms have been associated with an increase or decrease in the risk of developing mucositis, with controversial results. The groups of Zheng et al. found an increase in developing mucositis in pediatric patients with SLC01B1 rs4149056 [10], whereas in several other studies, it was associated with a lower risk of developing mucositis [4, 6, 11]. In a series by Lopez-Lopez et al., the presence of SLC01B1 rs11045879 with CC genotype alleles had a 100% risk of higher methotrexate concentrations in blood [12].

The risk of side effects is in close relation to the blood concentration of MTX. A common definition of high concentration is if MTX levels are over one $\mu\text{mol/l}$ at 48 hours or over 0.2 $\mu\text{mol/l}$ at 72 hours [12]. Several polymorphisms of SLC01B1 have been identified in relation to the MTX plasma levels, being the rs11045879 one of the most studied [12, 13] and particularly related to MTX toxicity when CC genotype is carried (OR 1.872 (1.099-3.011)) [14].

Also, the elimination transporters have SNP associated with MTX toxicity. The polymorphisms in ABCC4 (elimination transporter located in kidney cells) and ABCC2 (elimination transporter in the liver cell) are associated with the diverse response for ABCC4 rs9516519/rs2619312/rs1678392/rs9302061 and were associated a decrease in MTX plasma levels, whereas ABCC4 rs7317112 GG genotype was associated with high MTX plasma concentrations after 72 hours [12].

One of the most studied genes studied is the MTHFR, which pro-

duces an enzyme with the homologous name that is in charge of the folate-homocysteine cycle. The most studied SNP are 667C>T and 1298A>C, both decreasing the activity in vitro, but the clinical impact the importance has been controversial. Different studies have reported a higher risk of relapse and non-specified toxicity [9, 15, 16-17]. In our previous study, a frequency of MTHFR 667C>T was identified to be of 52% & 74% for heterozygous and homozygous, respectively [20], contrary to our results with RFC, the presence of the polymorphism MTHFR 667C>T wasn't associated with decreased overall survival and relapse-free-survival. Similar results have also been identified in Mexican [22] and Brazilian [23] patients.

The evidence available of MTX gene-drug interactions have not yet been well characterized to make a recommendation regarding pharmacogenetic testing. Schmiegelow concludes that even if the toxicities where feasible to prevent the test and its prevention would only be justified if it did not hamper the cure rates of the non-at-risk population [18]. Howard et al. did not recommend any prevention and pharmacogenomics assessment but did prioritize the clinical evolution and use of the folinic acid [19]. Rudin et al. recommend that each case should be individualized. In patients experiencing gastrointestinal toxicity, the SLCO1B1 should be genotyped for variants [2].

5. Conclusion

The development of MTX toxicity can be suggested with the identification of certain mutations in genes that encode for proteins that regulate the entrance, metabolism, and exit of the drug from the cells. The use of universal genotype analysis has no scientific basis yet and is economically prohibitive. The best of our knowledge isn't routinely used by any group for screening all patients that will be exposed to MTX. It would seem that for the moment, continuing with folinic acid prevention, use of hydration and testing in patients that develop certain toxicities associated with MTX such as; gastric (SLCO1B1), hepatic (RFC G80A), mucosal (ABCC2, ABCC4, ABCB1) a genotype should be obtained.

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