Toxic Encephalopathy and Delayed MTX Clearance after High-dose Methotrexate Therapy in a Child Homozygous for the *MTHFR* C677T Polymorphism

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Abstract. *Background: High-dose methotrexate (HD-MTX) is one of the most important agents in the therapy of osteosarcoma (OSC). Acute and delayed toxicities still constitute clinical problems. Methylenetetrahydrofolate reductase (MTHFR) has a key role in the folate cycle. In case of homozygosity of the 677C→T polymorphism, treatment with antimetabolites, such as MTX, can cause additional toxicity. Case Report: In the present work, we describe the case of a 10-year-old boy with OSC. After the first HD-MTX infusion (12 g/m^2/6 h) acute neurological disturbances were detected followed by severe hepatotoxicity. Plasma concentrations of MTX and 7-OH-MTX showed delayed clearance. Calcium folinate was administered to the patient until +186 hours. Tha patient was homozygous for the 677 polymorphism and wild-type for the 1298 polymorphism of the MTHFR gene. Conclusion: We hypothesize that MTX toxicity can be explained by the association between homozygosity of the MTHFR C677T polymorphism causing disturbances in the folate status and thus an enhanced vulnerability of the central nervous system to antimetabolites and to the prolonged MTX exposure due to delayed MTX clearance.*

Methotrexate (MTX) is a folate antagonist which is widely used in the treatment of malignancies and in non-neoplastic diseases as an anti-inflammatory or immunosuppressive drug. High-dose methotrexate (HD-MTX) is one of the most important agents in the neoadjuvant and adjuvant therapy of osteosarcoma (OSC) (1). The incidence of drug-related deaths can be reduced with leucovorin rescue together with urinary alkalization and hydration (2). However, acute and delayed toxicities such as vomiting, gastrointestinal mucositis, hepatic toxicity, myelosuppression, renal dysfunction and neurological disturbances still constitute clinical problems. MTX-induced neurotoxicity includes nausea, emesis, headache, dizziness, poor memory, lethargy, aphasia, blurred vision, seizures, hallucinations and leukoencephalopathy (3). Acute toxicity is usually transient without permanent damage. The exact pathophysiological mechanisms of neurotoxicity are still not understood, although this issue has been widely discussed in the past decade. It has been postulated that the neurotoxicity associated with MTX is a consequence of its direct damaging effect on the central nervous system (CNS). In addition, MTX induces metabolic alterations, which could at least partly be responsible for the observed neurotoxicity (4).

Methylenetetrahydrofolate reductase (MTHFR) has a key role in the folate cycle and catalyses the reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. This generates active folate for methylation of DNA, homocysteine and DNA synthesis. The most common polymorphism of *MTHFR* is a C→T substitution at nucleotide position 677 that causes a substitution of valine for alanine in the functional enzyme, which decreases its activity by 35% in persons who are heterozygous for the mutation and by 70% in those who are homozygous (5). The *MTHFR* C677T TT genotype results in imbalance in the intracellular folate pools and treatment with antimetabolites, such as MTX, can increase homocysteinaemia, causing additional toxicity. It has been suggested that homocysteine is at least partly responsible for ischaemic white matter changes, mineralising microangiopathy and focal neurological deficits observed after MTX treatment (6). There are some reports of *MTHFR* C677T polymorphism and MTX toxicity in patients receiving MTX (7-13). The 1298 polymorphism of this gene is still not as well defined.

In the present work, we describe a patient with osteosarcoma treated with HD-MTX. After the MTX

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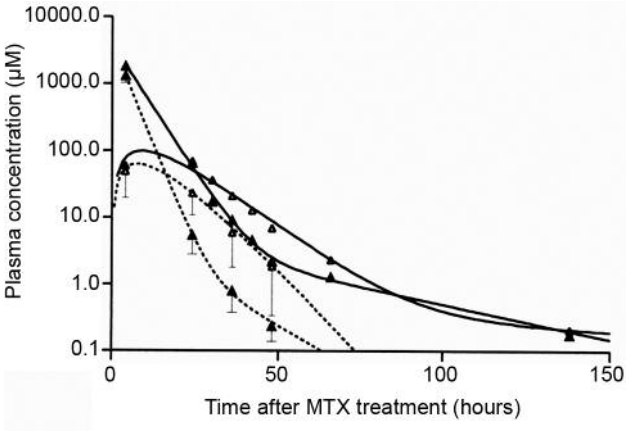

Figure 1. *Change of serum methotrexate (MTX) and 7-OH-MTX concentration after high-dose MTX administration (12 g/m^2/6 h). Black triangles show MTX and white triangles 7-OH-MTX levels. Solid lines indicate plasma concentration values of the referred patient; dotted lines show mean plasma concentration values ± standard deviation of 56 osteosarcoma patients, treated with high-dose MTX. Plasma concentration values of MTX and 7-OH-MTX were fitted by MEDUSA™ Version 1.5 (CheMicro LTD, Budapest, Hungary) computer program.*

infusion, acute neurological disturbances were detected followed by severe hepatotoxicity and delayed MTX clearance. The patient was found to be homozygous for the *MTHFR* C677T polymorphism, which can be causative in these MTX-induced toxic effects. To our knowledge, this kind of correlation has not been described before.

Case Report

A 10-year-old boy presented in December 2000 with pain and a palpable mass on the left fibula. The bone biopsy was diagnostic of osteosarcoma. He was treated according to the Cooperative Osteosarcoma Study 1996 protocol (14). Twenty minutes after the end of the first HD-MTX infusion at the recommended dose of 12 g/m^2/6 h, the patient started to become somnolent, had urinary incontinency, decreased reflexes, mild nystagmus and his pupils were narrow, but reactive to light. His vital parameters were within the normal range. Parenteral dexamethasone was administrated immediately due to suspicion of brain oedema. Forced diuresis was begun with 4,000 ml/m^2 infusion with administration of furosemide every 6 hours. The neurological symptoms disappeared in 24 hours. Serum alanine aminotransferase (ALT) (33 U/l) and γ-glutamyl transpeptidase (GGT) (18 U/l) were in the physiological range, prior to starting HD-MTX therapy. ALT was significally elevated at the time of the acute neurological disturbances (1,349 U/l). The highest level of ALT was detected 24 hours after HD-MTX infusion at 2,231 U/l and it was followed until normalization (55 U/l) on day 35. The serum GGT level was 263 U/l on day 11 and normalized in two months. There were no disturbances in the renal function of this patient. Both the level of serum creatinine and creatinine clearance were in the normal range. The plasma concentration of MTX and 7-OH-MTX was monitored by high-pressure liquid chromatography and showed delayed elimination (Figure 1). The fitted MTX and 7-OH-MTX plasma concentration *vs*. time curves of the patient with toxic treatment in comparison with the plasma concentration values of 56 osteosarcoma patients with normal MTX elimination (obtained HD-MTX treatment as well) are shown in Figure 1. The elimination half-life values ($t_{½}$) are summarized in Table I.

Table I. *Comparison of the elimination half-life values.*

Measured parameter	Elimination half-life (hours)	
	$t_{1/2}\alpha$	$t_{1/2}\beta$
MTX (toxic case)	3.96	29.45
7-OH-MTX (toxic case)	8.55	99.75
MTX (N:56)	2.44	10.87
7-OH-MTX (N:56)	5.28	-

Calcium folinate was administrered to the patient until 186 hours. During subsequent anticancer treatment, no MTX was applied. The patient had a limb salvage operation and his active treatment was finished after 8 months. After a 6-year follow-up period, the patient is in complete oncological remission.

The *MTHFR* genotype of our patient was analysed in a DNA sample isolated from peripheral blood lymphocytes, using standard procedures. Genetic polymorphism of the two *MTHFR* variants, C677T and A1298C, were studied by restriction fragment length polymorphism analysis. The C677T polymorphism was analyzed by polymerase chain reaction (PCR) followed by digestion with HinfI, using the primers described by Frosst *et al.* (5). A1298C polymorphism was analyzed by PCR followed by digestion with Mbo II, using the primers described by Yi *et al.* (15). After restriction enzyme digestion, PCR products were evaluated by capillary electrophoresis and laser-induced fluorescence detection (ABI PRISM TM 310).

Discussion

In patients homozygous for the *MTHFR* C677T polymorphism, the activity of the enzyme is reduced by 70% (5). The consequence is impaired synthesis of 5-methyltetrahydrofolate, the main circulating form of folate, which leads to a decrease in plasma folate concentration (16). Folates are transported into cells by two active systems: the membrane folate receptors (FRs) and the reduced folate carrier (RFC). At high

concentration, MTX uses passive diffusion as well as low-affinity transporter (17). The FRs have a higher affinity for folic acid as compared with the reduced folates (18). Increased *FR* expression has been observed in extracellular folate deficiency in normal cells (19). The RFC has a relatively high affinity for reduced folates and folate analogues, such as MTX as compared with folic acid (18). A low concentration of folate induces overexpression of the *RFC* gene, which results in a marked increase in influx and free intracellular MTX levels without any measurable changes in efflux kinetics at all (20). Methotrexate, like physiological folates, is converted to polyglutamate forms by folylpolyglutamate synthase (21). The intracellular formation of MTX polyglutamate plays a major role in the cytotoxicity of MTX (22).

The roles of the polymorphisms of the *MTHFR* gene in the risk of toxicity after MTX have been studied, but the results are inconsistent. Several studies have reported a higher incidence of toxicity after MTX therapy among patients homozygous for the C677T polymorphism (7, 13, 23). Others could not find any correlation between these polymorphisms and MTX toxicity (24, 25), while some authors confirmed the association of MTX-induced toxicity and the A1298C but not C677T polymorphism (26, 27). Imanishi *et al*. reported association of the TT genotype at *MTHFR* C677T with the residual concentration of MTX at 48 h (28).

As a conclusion, we hypothesize that the association between homozygosity of the *MTHFR* C677T polymorphism and MTX toxicity can be explained by disturbances in the folate status and by prolonged MTX exposure due to delayed MTX clearance. Homozygosity for the *MTHFR* C677T polymorphism leads to low serum folate levels with consequent overexpression of the *FR* and *RFC* genes. In cases of antifolate treatment, such as MTX, the overexpression of these transporters leads to a high intracellular concentration of MTX. After polyglutamation, this higher level of intracellular MTX becomes more cytotoxic and causes delayed MTX clearance.

We suggest that the *MTHFR* polymorphism should be determined prior to HD-MTX in patients with malignant disorders to avoid severe, life-threatening complications.

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