Journal of Bone Oncology 13 (2018) 143–147

Contents lists available at ScienceDirect

Journal of Bone Oncology

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

Research Paper

MTHFR variant is associated with high-dose methotrexate-induced toxicity in the Chinese osteosarcoma patients

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ARTICLE INFO

Keywords:
Osteosarcoma
MTX
Variants
MTHFR
Toxicity

ABSTRACT

Background: The role of Methylene tetrahydrofolate reductase (MTHFR) C677T and A1298C polymorphisms in the efficacy and toxicity of MTX-based therapy remains uncertain. Our purpose was to clarify whether these two polymorphisms are associated with the outcome of chemotherapy in a cohort of Chinese osteosarcoma (OS) patients treated by high-dose MTX.

Methods: 109 OS patients who had sequentially received high-dose MTX therapy were included in this study. Plasma MTX level was measured routinely at 0, 24, 48 and 72 h after the administration of MTX. Two variants of MTHFR were genotyped using TaqMan SNP Genotyping Assay, including rs1801133 (C667T) and rs1801131 (A1298C). The extent of toxicity induced by MTX, including hematological toxicity, hepatic toxicity, renal toxicity and mucositis, was scored from grade 1 to 4. Severe toxicity was defined as a grade score of ≥ 3. Patients were dichotomized as follows: grade < 3 or ≥ 3 for toxicity, and ≤ 0.2 μmol/L or > 0.2 μmol/L for plasma MTX level at 72 h. The frequencies of genotypes and allele were compared between the dichotomized groups with the Chi-square test.

Results: 24.8% (27/109) of the patients were found to have significantly high plasma MTX level at the 72 h. Patients with high MTX level at 72 h were found to have significantly higher frequency of genotype TT of rs1801133 (p = 0.002). As for rs1801131, no significant association was found with plasma MTX level. Patients with severe hepatic toxicity or mucositis were found to have remarkably higher incidence of genotype TT of rs1801133 than those with mild toxicity (33.3% vs. 14.8%, p = 0.04 for hepatic toxicity; 34.8% vs. 19.8%, p = 0.05 for mucositis).

Conclusions: Variant rs1801133 was confirmed to have remarkable influence on the MTX-induced toxicity. We recommend identification of the genotype of MTHFR variant prior to the application of high-dose MTX to OS patients, which could be an important predictor to screen severe toxicities and thus improve treatment outcomes.

1. Introduction

Osteosarcoma (OS) is the most common malignant bone tumor that primarily develops in the long bone of extremities [1,2]. With the application of neoadjuvant chemotherapy drugs, the overall 5-year survival rate of OS patients has dramatically improved to 60%–70% [3–5]. Currently, the most standard protocol of chemotherapy before surgery was as a triple-drug regimen composed of Methotrexate (MTX), doxorubicin, and cisplatin [6]. As an antifolate drug that inhibits several key enzymes involved in the metabolism of folate during DNA synthesis, high-dose MTX has been proven effective in controlling the proliferation of cancer cells such as OS and acute lymphoblastic leukemia [7]. To be noted, however, patients treated with MTX may have a variety of outcomes due to varied pharmacogenomic profiling [8,9]. Clarifying OS patients with good response to high-dose MTX therapy has become an important focus of research in pharmacogenomics. It was speculated that variations of the genes encoding the enzymes involved in folate metabolism might influence both efficacy and toxicity of MTX [10,11].

Methylene tetrahydrofolate reductase (MTHFR) is a key regulatory enzyme of the folic acid pathway and plays an important role in the regeneration of reduced folate [12,13]. Several studies have been published concerning the association of C677T and A1298C polymorphisms of MTHFR with the efficacy and toxicity of MTX in pediatric

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https://doi.org/10.1016/j.jbo.2018.10.002
Received 14 August 2018; Received in revised form 1 October 2018; Accepted 12 October 2018
Available online 01 November 2018

OS patients [14–17]. However, confined by relatively small sample size and heterogeneity of subjects, the results of these studies were not consistent. PATIÑO-GARCÍA et al. [17] observed that the polymorphisms MTHFR C677T was associated with increased MTX-induced toxicity in pediatric OS. Comparably, Windsor et al. [18] reported that MTHFR A1298C and C677T were associated with MTX-related nephrotoxicity and anemia. By contrast, Lambrecht et al. [15] reported that the MTHFR C667T polymorphism was not predictive for toxicity or overall survival. Park and Shin [16] concluded that C667T and A1298C polymorphisms were not associated with event-free survival or overall survival rates of the patients. Herein, the pharmacogenetic role of C677T and A1298C polymorphisms in the efficacy and toxicity of MTX-based therapy remains uncertain. To have a conclusive result about this association, we performed a study that included a cohort of OS patients treated by high-dose MTX in our center. Our purpose was to clarify whether the MTHFR polymorphisms C677T and A1298C are associated with the outcome of high-dose MTX treatment in Chinese OS patients.

2. Methods

2.1. Patients

This retrospective study was approved by our institutional ethical committees. Informed consents were obtained from the patients or from their guardians in case of <18 years old. 109 OS patients who had sequentially completed a total of 6 cycles of MAP chemotherapy were included in this study. Generally, the MAP protocol was composed of Adriamycin (30 mg/m^2/d for 3 days), MTX (10 g/m^2/d for 1 day), and cisplatin (100 mg/m^2/d for 1 day). Leucovorin rescue (15 mg/m^2 per 6 h) was used in twenty-four hours after the initiation of MTX infusion and maintained until the plasma MTX-level was less than 0.2 μmol/L as described by Holmboe et al. [19]. Plasma MTX level was measured routinely at 0, 24, 48 and 72 h after the administration of MTX, which was classified as normal level if ≤0.2 μmol/L at 72 h after administration and as high if >0.2 μ mol/L [16].

Clinical data was extracted from medical records including age at diagnosis, sex, tumor site, histology, histological response, time of relapse, and length of follow-up. Time to relapse was calculated as an interval between date of initial diagnosis and date of relapse. Good response to chemotherapy was histologically defined as >90% necrosis of tumor cells in the surgical resection specimen [20].

2.2. DNA extraction and genotyping

Blood samples were collected for each participant, from which a commercial kit (QIAGEN Inc., Tokyo, Japan) was used to extract DNA. 2 SNPs of MTHFR, including rs1801133 (C667T) and rs1801131 (A1298C), were genotyped using TaqMan SNP Genotyping Assay that was performed with ABI 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA). Twenty percent of the samples were randomly selected to validate the genotyping results. The reproducible rate was 100%.

2.3. Assessment of high-dose MTX-related toxicity

Blood samples acquired at 48 h after each administration of MTX were analyzed for creatinine level, hemoglobin, the enzymes aspartate transaminase (AST) and alanine transaminase (ALT). Anemia was assessed to determine hematological toxicity. Liver toxicity was defined on the basis of AST and ALT levels. Renal toxicity was evaluated by the elevated creatinine level. According to the Common Terminology Criteria for Adverse Events version 4.0 (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm), the extent of toxicity induced by MTX, including hematological toxicity, hepatic toxicity, renal toxicity and mucositis, was scored from grade 1 to 4. Severe toxicity was defined as a grade of ≥3 [18].

Table 1
Baseline characteristics of the patients.

Features	Number
Gender	
Male	58
Female	51
Age (years)	
>20	46
≤20	63
Histologic type	
Osteoblastic	75
Chondroblastic	34
Tumor location	
Femur	52
Tibia	23
Humerus	18
Others	16
Tumor recurrence	
Presence	51
Absence	58
5-year survival (%)	
Death	25
Survival	57

2.4. Statistical analysis

To evaluate the association of SNPs on efficacy and toxicity of MTX, patients were dichotomized as follows: event or no event for relapse, good or poor response to chemotherapy, grade <3 or ≥3 for toxicity, and ≤0.2 μmol/L or >0.2 μmol/L for plasma MTX level at 72 h. The frequencies of genotypes and allele were compared between the dichotomized groups with the Chi-square test. The odds ratios (OR) and 95% confidence interval (95% CI) were calculated with the minor allele used as reference. ANOVA was applied to compare continuous variables among different genotypes. Intergroup comparison was performed with the Student t test. All statistical analyses were performed using SPSS version 19.0 (SPSS Inc. Chicago, IL). $P < 0.05$ was considered statistically significant.

3. Results

3.1. Patients' characteristics

Clinical and pathological characteristics of the patients were shown in Table 1. The mean age of the patients was 33.8 ± 18.2 years, ranging from 10 to 65 years. There were 58 males and 51 females. Median time of follow-up was 3.1 ± 2.9 years (range: 1–7.4 years). 56.9% of the patients (62/109) were found to have good response to chemotherapy. At the time of analysis, 58 patients were alive with no event of relapse or disease progression. 26 patients were further treated for above-mentioned events. The overall 5-year survival rate was 69.5% (57/82).

3.2. Association of SNPs with serum MTX level

The average MTX concentrations after administration were 684.3 ± 193.6 μmol/L (range, 209.3–1220.1 μmol/L) at 0 h, 4.8 ± 12.1 μmol/L (range, 0.29–91.4 μmol/L) at 24 h, 0.76 ± 3.07 μmol/L (range, 0.05–24.5 μmol/L) at 48 h, and 0.19 ± 0.23 μmol/L (range, 0.02–1.55 μmol/L) at 72 h. 24.8% (27/109) of the patients were found to have significantly high plasma MTX level at the 72 h. As shown in Fig. 1, patients with genotype TT of rs1801133 were found to have significantly higher MTX level at 72 h. Table 2 summarized the distribution of genotype and allele of rs1801133 in different groups of patients. Patients with high MTX level at 72 h were found to have significantly higher frequency of genotype TT ($p = 0.002$) and allele T ($p = 0.007$). As for rs1801131, no

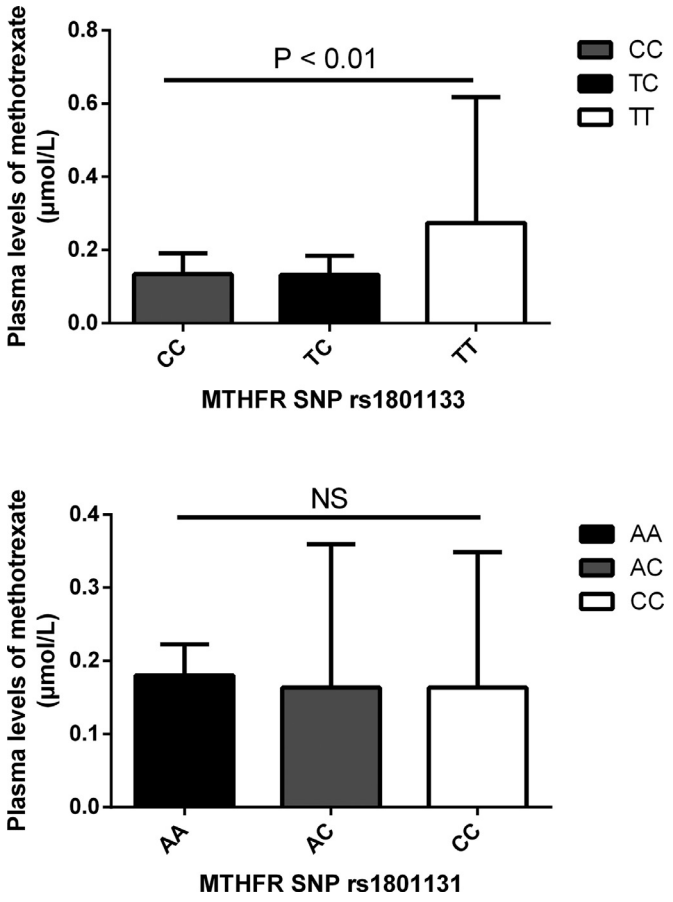

Fig. 1. Relationship between genotypes of MTHFR variants and plasma level of MTX at 72 h Genotype TT of rs1801133 was indicative of remarkably higher plasma level of MTX as compared with genotype CC (0.27 ± 0.31 μmol/L vs. 0.13 ± 0.07 μmol/L, $p < 0.01$). The plasma level of MTX was comparable among the three genotypes of rs1801131 ($p > 0.05$).

significant association was found with plasma MTX level after high-dose MTX therapy (Table 2).

3.3. Association of rs1801133 with MTX-induced toxicity and clinical outcome

The number of patients who developed severe hematological toxicity, hepatic toxicity, renal toxicity, or mucositis was shown in Table 3. Patients with severe hepatic toxicity or mucositis were found to have remarkably higher incidence of genotype TT of rs1801133 than those with mild toxicity (33.3% vs. 14.8%, $p = 0.04$ for hepatic toxicity; 34.8% vs. 19.8%, $p = 0.05$ for mucositis). Allele T was found to add to the risk of sever hepatic toxicity and mucositis by 1.98 fold (95% CI = 1.15–3.41, $p = 0.01$ for hepatic toxicity) and 2.06 fold (95% CI = 1.06–4.0, $p = 0.04$ for mucositis), respectively. We found no association of rs1801133 with hematological toxicity and renal toxicity. In terms of clinical outcomes, rs1801133 was found to have no association with histological response to chemotherapy ($p > 0.05$) or the event-free survival rate ($p > 0.05$).

3.4. Relationship between serum MTX level and toxicity

As shown in Table 4, MTX concentration at 72 h was significantly related to the severity of hepatic toxicity or mucositis. Patients with severe hepatic toxicity and mucositis were found to have remarkably higher MTX concentrations (0.20 ± 0.26 vs. 0.13 ± 0.05, $p = 0.03$ for hepatic toxicity; 0.29 ± 0.35 vs. 0.12 ± 0.06, $p = 0.01$ for mucositis). No significant relationship between MTX concentration and hematological toxicity and renal toxicity was found.

4. Discussion

The overall survival of OS has been dramatically improved by neoadjuvant chemotherapy, which however may also result in significant early and long-term side effects [3,5,19]. Herein, early prediction of patients at risk of severe chemotherapy-related toxicity may facilitate early intervention to minimize subsequent morbidity. To date, understanding the genetic variants involved in drug response and toxicity remains a great challenge in cancer chemotherapy [9,21,22]. For the first time, we investigated the impact of two common variants of MTHFR on the pharmacokinetics and toxicities of high-dose MTX treatment in Chinese OS patients. In our study, rs1801133 was found significantly associated with both serum level of MTX and the severity of MTX-induced toxicities. Patients with genotype TT had obviously more severe hepatic toxicity and mucositis than patients with genotype CC. Meanwhile, the serum level of MTX at 72 h was remarkably higher in patients harboring allele T. Overall, our findings were in line with previous studies which reported the influence of C677T variant (rs1801133) on the plasma level of MTX and related toxicity [17,18].

For the C677T variant, the substitution of the C nucleotide by the T nucleotide can lead to an amino acid change from alanine to valine. It has been reported to be associated with decreased activity of MTHFR, elevated plasma homocysteine levels and altered distribution of folate [23]. It was speculated that patients with TT genotype were more vulnerable to potential MTX-induced toxicity since the diminished MTHFR enzyme activity may lead to slower folate metabolism and slower cell repair [15]. With regard to A1298C variant (rs1801131), we found no association between the genotypes and the plasma level of MTX. It was reported that rs1801131 may be associated with decreased MTHFR activity but at a lower extent as compared with rs1801133 [24]. It is possible that there existed a mixed influence of MTHFR variants on the enzyme activity. The role of rs1801131 in the metabolism of MTX needs to be further studied in future studies.

It has been widely reported that germ-line variants in the MTX metabolic pathway are associated with histological response and overall survival of cancer patients treated with MTX [18]. Jabeen et al. [14] reported that OS patients harboring genotype AA of rs1051266 in RFC1 had significantly worse survival as compared with patients

Table 2
Association of the MTHFR variants with serum MTX level.

SNPs	MA	Genotype[a]		P	MAF		P	Odds ratio (95% CI) [b]
		High level	Low level		High level	Low level		
rs1801133	T	13/8/6	12/44/26	0.002	0.629	0.415	0.007	2.41 (1.27–4.52)
rs1801131	C	3/10/14	8/26/48	0.54	0.296	0.256	0.59	1.22 (0.62–2.41)

MA, minor allele; MAF, minor allele frequency; CI, confidential interval.

[a] The three values in the 'genotype' column indicate the numbers of homozygotes with respect to the minor allele, heterozygotes and homozygotes with respect to the major allele, respectively.

[b] Calculated with the minor allele as reference.

Table 3
Allele and genotype distribution of rs1801133 in patients stratified by severity of toxicity and clinical outcome.

	Genotype			p	Allele		p	Odds ratio (95% CI [a])
	TT	TC	CC		T	C		
Hepatic toxicity				0.04			0.01	1.98 (1.15–3.41)
Grade 1–2 (n = 61)	9 (14.8%)	30 (49.1%)	22 (36.1%)		48 (39.3%)	74 (60.7%)		
Grade 3–4 (n = 48)	16 (33.3%)	22 (45.8%)	10 (20.9%)		54 (56.3%)	42 (43.7%)		
Mucositis				0.05			0.04	2.06 (1.06–4.0)
Grade 1–2 (n = 86)	17 (19.8%)	40 (46.5%)	29 (33.7%)		74 (43.0%)	98 (57.0%)		
Grade 3–4 (n = 23)	8 (34.8%)	12 (52.2%)	3 (13.0%)		28 (60.9%)	18 (39.1%)		
Anemia				0.48			0.46	1.34 (0.64–2.79)
Grade 1–2 (n = 92)	21 (22.8%)	42 (45.7%)	29 (31.5%)		84 (45.7%)	100 (54.3%)		
Grade 3–4 (n = 17)	4 (23.5%)	10 (58.9%)	3 (17.6%)		18 (52.9%)	16 (47.1%)		
Renal toxicity				0.71			0.76	1.12(0.61–2.01)
Grade 1–2 (n = 81)	20 (24.7%)	37 (45.7%)	24 (29.6%)		77 (47.5%)	85 (52.5%)		
Grade 3–4 (n = 28)	5 (17.9%)	15 (53.6%)	8 (28.5%)		25 (44.6%)	31 (55.4%)		
Event-free survival				0.57			0.42	1.28 (0.75–2.17)
Yes (n = 58)	11 (19.0%)	29 (50.0%)	18 (31.0%)		51 (44.0%)	65 (56.0%)		
No (n = 51)	14 (27.5%)	23 (45.0%)	14 (27.5%)		51 (50.0%)	51 (50.0%)		
Response to chemotherapy				0.67			0.59	1.16 (0.68–1.99)
Good (n = 62)	13 (21.0%)	30 (48.4%)	19 (30.6%)		56 (45.2%)	68 (54.8%)		
Poor (n = 47)	12 (25.5%)	22 (46.8%)	13 (27.7%)		46 (48.9%)	48 (51.1%)		

[a]indicates aIndicates confidential interval.

Table 4
Relationship between the severity of toxicity and the Serum MTX level at 72 h.

	Serum MTX level at 72 h (μmol/L)	p
Hepatic toxicity		0.03
Grade 1–2 (n = 61)	0.13 ± 0.05	
Grade 3–4 (n = 48)	0.20 ± 0.26	
Mucositis		0.01
Grade 1–2 (n = 86)	0.12 ± 0.06	
Grade 3–4 (n = 23)	0.29 ± 0.35	
Anemia		0.72
Grade 1–2 (n = 92)	0.17 ± 0.19	
Grade 3–4 (n = 17)	0.15 ± 0.03	
Renal toxicity		0.65
Grade 1–2 (n = 81)	0.15 ± 0.23	
Grade 3–4 (n = 28)	0.17 ± 0.11	

harboring genotype GG. Krajinovic et al. reported that acute lymphoblastic leukemia patients with 677T/1298A haplotype of MTHFR had significantly worse prognosis of event-free and disease-free survival [25]. Overall, our analysis revealed no association between the MTHFR polymorphisms and overall outcome including histological response and event-free survival rates in OS patients. In line with our findings, Lambrecht et al. [15] did not observe any association of MTHFR 677TT genotypes with the overall survival rates in pediatric OS patients either. It is possible that variants other than those of folate metabolic pathways may influence the overall outcome of osteosarcoma patients.

The primary limitation of our study lies in that the sample size was relatively small for genetic association study, considering the fact that OS is a rare cancer with low incidence. Also, the inclusion criterion of treatment with high-dose MTX has confined the number of patients eligible for the study. The second limitation is that the all the patients were retrospectively reviewed and included in the study. MTX therapy was discontinued in 4 patients due to severe complications, who were therefore excluded from the study. It is probable that the exclusion of these patients may lead to the bias of the distribution of genotyping in this cohort. Despite these limitations, it is noteworthy that all the patients were from ethnically homogenous population. Our results should be considered preliminary and validations in larger sample size are warranted.

To summarize, we report for the first time on the role of MTHFR variants in the disposition and treatment outcome of high-dose MTX in Chinese OS patients. Variant C677T was confirmed to have remarkable influence on the MTX-induced toxicity. Therefore we recommend identification of the genotype of MTHFR variant prior to the application of high-dose MTX to OS patients, which could be an important predictor to screen severe toxicities and thus improve treatment outcomes.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (Grant Nos. 81501849 and 81201385) and by the Nanjing Key Program of Medical Science and Technology Development (Grant No. ZKX14021).

Conflict of interest statement

No benefits in any form have been or will be received from a commercial party related directly or indirectly to the subject of this manuscript.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jbo.2018.10.002.

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