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Original Research Article

Methylenetetrahydrofolate reductase polymorphism and capecitabine-induced toxicity in patients with gastric cancer

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Abstract

Purpose: *To evaluate the effect of methylenetetrahydrofolate reductase (MTHFR) polymorphism on toxicity in gastric cancer (GC) patients treated with capecitabine.*
Methods: *One hundred and twenty-six GC patients were treated with capecitabine in the study. DNA from GC patients was genotyped for MTHFR A1298C using direct sequencing. Toxicity evaluations were graded. Clinical response was assessed.*
Results: *In 87.3 % of the patients, capecitabine toxicity was observed. As for MTHFR A1298C polymorphism, 55.6 % patients who exhibited it were associated with reduced MTHFR activity. MTHFR A1298C was associated with capecitabine-related toxicity (p = 0.008); in addition, MTHFR A1298C was significantly associated with gastrointestinal toxicity (p = 0.026), but not with other types of toxicity.*
Conclusion: *The findings suggest that MTHFR A1298C may be useful for predicting toxicity in GC patients receiving capecitabine treatment, especially gastrointestinal toxicity.*

Keywords: *MTHFR, Polymorphism, Gastric cancer, Toxicity*

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INTRODUCTION

Gastric cancer (GC) is one of the main causes of cancer death all over the world [1,2]. GC accounts for about 35 – 40 % of new cases and deaths each year in China [1,2]. Although early-stage and locally-advanced GC can be treated by surgery, many patients have local recurrence or distant metastasis after gastrectomy [3,4]. However, about a quarter of patients have lost the best time to perform surgery at the time of diagnosis [5]. It is generally believed that palliative chemotherapy for advanced diseases and perioperative chemotherapy can improve the survival rate and quality of life of GC patients [6]. Capecitabine, a prodrug of 5-fluorouracil (5-FU), is widely used to treat gastrointestinal cancer, including GC. However, chemotherapy may sometimes bring serious, unpredictable toxicity to patients without any tumor response. Genetic variation may play an important role in toxic and side effects induced by chemotherapeutic drugs. Increasing evidence suggests that individual

variations in drug metabolizing enzymes may affect the efficacy of anticancer drugs [7].

Methylenetetrahydrofolate reductase (MTHFR) is a key enzyme in folate metabolism, and reduced activity of *MTHFR* could augment the cytotoxicity of fluoropyrimidines [8]. Therefore, mutations in *MTHFR* may cause enzyme deficiency resulting in toxicity with capecitabine therapy. Furthermore, a number of patients have been described as having reduced activity of the enzyme and severe toxicity of fluoropyrimidines including capecitabine [9,10]. A common SNP in *MTHFR* causes reduced enzyme activity in homozygous individuals: *MTHFR* 1298A>C (rs1801131) polymorphism results in a 30 – 40% reduction in its activity [8].

However, the relationship between the rs1801131 mutation in *MTHFR* and the efficacy and toxicity of capecitabine in GC patients has not been reported in central China.

The aim of the present study was to evaluate the relationship between rs1801131 in *MTHFR* and the efficacy of capecitabine chemotherapy in GC patients to assist in therapeutic selection.

EXPERIMENTAL

Samples

In total 126 GC patients treated with capecitabine were enrolled in the Hubei hospital from 2015 to 2018 who met the following criteria [2,11]: age > 18; histologically-confirmed GC; Eastern Cooperative Oncology Group was 0 – 2; no malignant coexistence; adequate organ function; patients received capecitabine treatment; patients had completed at least two cycles of chemotherapy. Ethics approval was obtained from the ethics committee of Hubei Hospital (approval no. 20150501) and followed the guidelines of the Helsinki Declaration [12], and all the patients gave written informed consent.

Capecitabine (Roche, NJ, USA) was orally administered at a dose of 1,000 mg/m^2 twice daily from day1 to day14 for 3 weeks. Continue treatment until disease progression, unacceptable toxicity, patient refusal, or medical determination to stop treatment.

Assessment of efficacy and toxicity

Follow-up procedures including intermediate medical history, physical examination, hematology, and diagnostic imaging. Toxicity evaluations was graded as previously described [13]. Computed tomography was used to evaluate clinical response based on Response Evaluation Criteria in Solid Tumors [11]. The toxicities include hematopoietic, gastrointestinal, hand-foot syndrome and hair-skin toxicities. All reactions and toxicities were recorded.

MTHFR genotyping

Genomic DNA was extracted using the Kit (TIANGEN, Beijing, China). Primers for *MTHFR* 1298A>C were designed using Primer 5.0 (CA, USA). PCR reactions were performed in a volume of 25 μL. The sequence of the primers was shown in Table 1. The PCR products were determined using DNA sequencing (ABI 3100, Shanghai, China).

Statistical analysis

Analyses were done using SPSS 19.0. Allele frequency and number of adverse reactions were determined by counting. The relationships between polymorphism of *MTHFR* and the existence of toxicity was analyzed using Chi square test. A $p < 0.05$ was considered statistically significant.

RESULTS

Patient characteristics

A total of the 126 GC patients treated with capecitabine are shown in **Table 2**, with 74 males (58.7 %) and 52 females (41.3 %), and the median age of 59 (range 27 - 79 years). The histopathological types were classified as adenocarcinoma (83.3 %), adenosquamous cell carcinoma (7.9 %), and squamous cell carcinoma (8.8 %), respectively. The stages of the tumors were classified as stage Ⅲ (41.3 %), and stage IV (58.7 %), respectively. The proportion of patients with complete remission, partial remission, stable disease and progressive disease were 1.6, 40.5, 38.1 and 19.8 %, respectively.

Table 1: Sequence of the primers

Primer	Sequence (5'→3')	T_m (℃)
rs1801131	Forward: TTC TAC CTG AAG AGC AAG TCC Reverse: CAC TCC AGC ATC ACT CAC TT	56.0

Table 2: Patients' clinical characteristics

Characteristic		Patients (%)
Gender n (%)	Male	58.7
	Female	41.3
Age at diagnosis	≤60	43.7
	>60	56.3
Histopathological type	Adenocarcinoma	83.3
	Others	16.7
Stage	Ⅲ	41.3
	Ⅳ	58.7
Comorbidities	< 2	46.0
	≧2	54.0
Clinical response	Complete remission	1.6
	Partial remission	40.5
	Stable disease	38.1
	Progressive disease	19.8

Adverse reactions related to treatment

All patients were followed up after treatment with capecitabine. 87.3 % of the patients suffered from toxicities. Treatment was prematurely stopped in 10 % of the patients due to severe gastrointestinal or haematological adverse events, and no toxic death was reported. The most frequently observed toxicities were hematological and gastrointestinal, but hair and skin, and hand-foot syndrome were observed only rarely. Details of toxicity classification are shown in Table 3.

Genotype frequencies

Genotypes and allele frequencies are listed in Table 4. For the *MTHFR* 1298A>C locus, 56 (44.4 %) patients were wild type, 12 (9.5 %) patients were homozygote type and 58 (46.0 %) patients were heterozygote, respectively, and the allele frequencies of A and C were 67.5 and 32.5 % in this study.

Correlation between *MTHFR* genotypes and toxicity

Patients with *MTHFR* A/C + C/C genotypes had a significantly higher incidence of toxicities than patients with *MTHFR* A/A genotype (P = 0.008) (**Table 5**). The *MTHFR* A1298C genotype was significantly associated with the incidence of gastrointestinal toxicity (p = 0.026) (Table 6), however, *MTHFR* A1298C genotype has no significant correlation with other types of toxicity.

Table 3: Adverse reactions related to treatment

Toxicity type	Toxicity grade			
	1	2	3	4
Gastrointestinal	25 (22.7%)	12 (10.0%)	5 (4.5%)	5 (4.5%)
Hematopoietic	11 (10.0%)	13 (11.8%)	10 (9.1%)	10 (9.1%)
Hair and skin	4 (3.6%)	1 (0.9%)	2 (1.8%)	2 (1.8%)
Hand-foot syndrome	4 (3.6%)	5 (4.5%)	2 (1.8%)	1 (0.9%)

Table 4: Genotype and allelic frequencies among all patients

Polymorphism	Amino acid change	Genotype frequency			Allelic frequency	
		AA	AC	CC	A	C
MTHFR 1298A>C	E429A- Exon7	56 (44.4%)	58 (46.0%)	12 (9.5%)	170 (67.5%)	82 (32.5%)

Table 5: *MTHFR* A1298C genotype and all toxicities caused by capecitabine

MTHFR A1298C	All toxicities		Risk ratio	*P*
	-	+		
AA	8 (7.3%)	32 (29.1%)	7.096	0.008
AC+CC	2 (1.8%)	68 (61.8%)		

Table 6: *MTHFR* A1298C genotype and hematopoietic toxicity caused by capecitabine

***MTHFR* A1298C**	**Gastrointestinal toxicity (n)**		**Risk ratio**	***P***
	-	+		
AA	6 (12.8%)	13 (27.7%)	4.970	0.026
AC+CC	1 (2.1%)	27 (57.4%)		

DISCUSSION

It is generally believed that there are many factors influencing the outcome of drug treatment including mixed environmental factors, physiological factors, genetic variation of drug-metabolizing enzymes, drug transporters, and drug targets, therefore, genetic polymorphisms play a key role in determining toxicity. The MTHFR polymorphisms result in decreased enzyme activity and then low levels of plasma folate and high homocysteine. Folate takes part in various biochemical reactions to provide or accept one-carbon units [14]. Folate deficiency is likely to develop into cancer, possibly due to DNA structural defects [15].

The study is based on the MTHFR pharmacogenetics of capecitabine in the treatment of GC patient. MTHFR 1298A>C genotype was significantly correlated with the incidence of adverse events in patients with GC treated with capecitabine, especially in gastrointestinal toxicity, however, *MTHFR* A1298C genotype has no significant correlation with the other types of toxicity.

In this study, 87.3 % of the patients suffered from toxicities when treated with capecitabine, and the MTHFR A1298C frequency (46.0 % heterozygote mutants, 9.5 % homozygote mutants) was significantly higher than previous reports [13], but similar to another study [8], and the reasons are as follows, sample size, differences in race and region.

Several studies examined genetic variants related to capecitabine therapy, such as MTHFR and thymidylate synthase, but without conclusive results, and the role of pharmacological genetic markers in predicting the toxicity and efficacy of capecitabine remains to be explored. Liu *et al* found that MTHFR A1298C polymorphism had no significant association with GC patients' survival [16]. Zhao *et al* suggested that MTHFR 1298CC genotype showed protective effect for all GC patients, and MTHFR 1298A > C polymorphisms may be useful prognostic biomarkers for GC patients [17]. In addition, van Huis-Tanja e*t al* concluded that MTHFR 677C>T and 1298A>C polymorphisms were not related to the occurrence of severe toxicity (and efficacy) of capecitabine-based chemotherapy in metastatic colorectal cancer [18]. Thus, the association of MTHFR A1298C remained unclear.

However, capecitabine is increasingly being included in the first-line treatment of solid tumors, and its efficacy is comparable to that of 5-FU single or combined [18]. The toxicity spectrum is different, capecitabine causes higher incidence of hand-foot syndrome than 5-FU injection [19], therefore, reducing drug adverse reactions requires better drug management.

Limitations of the study

This is the first study on the role of pharmacogenetics in capecitabine toxicity in GC patients in central China. There are some limitations of the present study that should be considered. (i) The present results need to be validated in a larger cohort. (ii) capecitabine was given as monotherapy (iii) folic acid is not added in the 5-FU protocol; therefore, the methylenetetrahydrofolate pool is directly controlled by MTHFR; (iv) the patients received chemotherapy, ruling out the possibility of acquired chemotherapy resistance. Therefore, it cannot be ruled out that the observed difference in MTHFR effect may be caused by other factors.

CONCLUSION

The results of this study suggest that MTHFR A1298C can be used for the prediction of capecitabine toxicities in patients with GC; in particular, it can predict gastrointestinal toxicity. These findings, as well as other supporting or opposing conclusions need to be validated in a larger cohort.

DECLARATIONS

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities

pertaining to claims relating to the content of this article will be borne by the authors.

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