RESEARCH COMMUNICATION

Relationship Between Antimetabolite Toxicity and Pharmacogenetics in Turkish Cancer Patients

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Abstract

Introduction: Antimetabolites may cause severe toxicity and even toxic death in cancer patients. Our aim was to evaluate the relationship between antimetabolite toxicity and pharmacogenetics in patients with severe clinical toxicity or alanine transaminase (ALT) elevation after fluorouracil (5FU), capecitabine or methotrexate administration. Patients and Methods: Cancer patients with severe antimetabolite toxicity were evaluated for methylenetetrahydrofolate reductase (MTHFR) gene C667T, thymidilate synthase (TS) gene 5´UTR variable number of tandem repeats (VNTR), dihydroprymidine dehydrogenase (DPYD) gene IVS14+1G/A, Xeroderma pigmentosum (XPD) gene Lys751Gln and X-ray repair cross-complementing group 1 (XRCC1) gene Arg399Gln polymorphisms. Results: Eighteen patients were enrolled, with a male/female ratio of 0.8. They had osteosarcoma in methotrexate group (n=7), gastrointestinal malignancies in 5FU group (n=9) and breast cancer in the capecitabine group (n=2). Mucositis and dermatitis occurred in all groups, together with ALT elevation in the methotrexate group and 2 toxic deaths were encountered. DPYD, TS, MTHFR, XPD and XRCC1 gene polymorphism rare allele frequencies were observed to be higher than in the general population. Conclusion: Pharmacogenetics might contribute to tailored therapy.

Keywords: Pharmacogenetics - antimetabolite toxicity - methotrexate - fluorouracil

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Introduction

Antimetabolites may cause severe toxicities, even lethal toxicities, in some of the cancer patients receiving chemotherapy (CT) including antimetabolites, like methotrexate and 5-fluorouracil (5FU). Rate of severe toxicity with 5FU-based chemotherapy was reported as 30% in a meta-analysis (Meta-analysis group in cancer, 1998).

5FU inhibits thymidilate synthase (TS), an enzyme which converts deoxyuridylate to thymidylate. Methotrexate inhibits tetrahydrofolate generation by dihydrofolate reductase. Tetrahydrofolate has role in carbon unit transfer which is essential for thymidylate synthesis. Therefore, both 5FU and methotrexate disturb DNA synthesis via TS inhibition. TS activity can also be analyzed by immunhistochemistry (IHC), but DNA analysis by genotyping has been reported to be more predictive (Van Kuilenburg, 2004)). Dihydroprymidine dehydrogenase (DPYD) is a rate limiting enzyme of pyrimidine metabolism. More than 80% of 5-FU is catabolized by DPYD (Van Kuilenburg et al, 2000). DPYD deficiency or low activity is closely related to fluoropyrimidine and 5FU toxicity. Enzyme activity can be measured in peripheral mononuclear cells, and DPYD mutations leading to decreased enzyme activity can be analyzed by genotyping.

Methotrexate is an antifolate agent. It is commonly used in the treatment of leukemia, lymphoma and almost in all cases of osteosarcoma. 5,10-methylenetetrahydrofolate reductase (MTHFR) is involved in maintaining folic acid homeostasis and converts 5,10-metylenetetrahydrofolate to 5-metylenetetrahydrofolate (Kantar et al, 2009). MTHFR gene is located on 1p36.13 (Goyette et al, 1998). C677T and A1298C polymorphisms are well-known MTHFR gene polymorphisms both of which cause to decrease enzyme activity and lower folate levels (Toffoli et al, 2008).

DNA repair genes, such as xeroderma pigmentosum group D (XPD) and X-ray repair cross-complementing group 1 (XRCC1), have roles in genomic stability via DNA repair pathways, like nucleotide excision repair, base excision repair and double-strand break repair (Sreeja et al 2008; López-Cima et al 2007). Gene polymorphisms may occur at codon 194, 280 and 399 for XRCC1 whereas they may occur at codon 312 and 751 for XPD. Arg399Gln (substitution of Arg with Gln) and Lys751Gln (substitution of Lys wiyh Gln) are well-known gene polymorphisms

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for XRCC1 and XPD, respectively. However, the effect of DNA repair gene polymorphisms in cancer promotion or progression is controversial (López-Cima et al 2007; Sliwinski et al 2009; Wang et al 2009).

MTFHR gene T allele increases both methotrexate toxicity risk and 5FU response rate. DPYD A allele also increases risk of 5FU toxicity while TS 3R, XPD Gln and XRCC1 Gln alleles decrease 5FU response or increase 5FU resistance. Therefore, all these gene polymorphisms have relevant importance in pharmacogenetic analysis of patients to be treated with these drugs. Tailored therapy based on pharmacogenetic data may provide the administration of the most appropriate and cost-effective treatment with less toxicity.

The aim of this study was to define these polymorphisms which might have been related to antimetabolite toxicity and response to the treatment in a group of patients who had suffered from grade 3-4 clinical or biochemical toxicity.

Materials and Methods

The patients who had suffered from grade 3-4 clinical or biochemical toxicity were analyzed for gene polymorphisms which might have been related to toxicity. DNA was isolated from peripheral blood samples by standard salting-out extraction.

MTHFR gene C667T and TS gene 5´UTR VNTR polymorphisms were investigated for methotrexate toxicity. TS gene 5´UTR VNTR, DPYD gene IVS14+1G/A, MTFHR gene C667T, XPD gene Lys751Gln and XRCC1 gene Arg399Gln polymorphisms were analyzed for 5FU efficacy and toxicity. TS polymorphism was evaluated by polymerase chain reaction (PCR) while DPYD, MTHFR, XPD and XRCC1 polymorphisms were evaluated by PCR-RFLP.

Table 1. Patient Characteristics

	Methotrexate	5 Fuorouracil based chemotherapy	
		5 Fluorouracil	Capecitabine
Male/female	1.3 (4/3)	1.57 (4/5)	0 (0/2)
Age (median, range)	20 (16-36)	60 (45-83)	
Osteosarcoma (n)	7	-	-
Gastric cancer (n)	-	5	-
Colon cancer (n)	-	3	-
Cholangiocellular carcinoma (n)	-	1	-
Breast cancer (n)	-	-	2

Results

Eighteen patients were enrolled between January 2008 and November 2008. Patient characteristics are summarized in table 1. Most of the patients were female (10/18), but methotrexate and 5FU groups had male predominance. Gene polymorphisms and treatment toxicities in our patient group are shown in Table 2, and frequencies of common and rare alleles of these polymorphisms in our patient group and general population are given in Table 3.

In methotrexate group, the most common toxicity was liver toxicity (i.e. ALT elevation). In this group, five patients had ALT elevation, one patient had high ALT level with grade 4 mucositis and another one had grade 3 mucositis. Median methotrexate cycle (12g/m2, max 20g/d, on days 21 and 28, every 5 weeks) was 2 (range=1-3) with a median interval between methotrexate administration and toxicity onset as 2 days (1-6). Median methotrexate level at 24h was 9.05 μmol/L (1.45-32, N<10). Median ALT level was 854 U/L (248-2885, N<40) with median increment as 22.4-fold (6.2-72,1). MTHFR gene T allele frequency was 47.2% in our patients who had methotrexate toxicity. Population frequency of this rare allele is 10-40%.

In 5FU / capecitabine group, nine patients had grade 4 mucositis, one patient had grade 4 mucositis with grade

Table 2. Characteristics with Gene Polymorphisms of the Patients.

No	DPD	TS	MTFHR	XPD	XRCC1	Treatment ALT level	Toxicity (X UL)	Max	ALT level	24h Mtx	İnterval between ALT peak and Mtx
1	GG	3R/3R	CT	Gln/Lys	Arg/Gln	capecitabine	mucositis/derrmatitis	-	-	-	-
2	GG	2R/3R	CT	?	?	5FU	mucositis	-	-	-	-
3	GA	2R/2R	CT	Lys/Gln	Gln/Gln	5FU	mucositis	-	-	-	-
4	GG	2R/3R	CT	Lys/Lys	Arg/Gln	capecitabine	mucositis	-	-	-	-
5	GG	3R/3R	CT	Lys/Gln	Arg/Arg	5FU	mucositis	-	-	-	-
6	GG	2R/2R	CT	Lys/Gln	Arg/Gln	5FU	mucositis	-	-	-	-
7	GG	2R/2R	CT	Lys/Gln	Arg/Gln	5FU	mucositis	-	-	-	-
8	GG	2R/2R	CT	Lys/Gln	Arg/Gln	5FU	mucositis	-	-	-	-
9#	GG	2R/3R	CC	Lys/Lys	Arg/Gln	5FU	mucositis	-	-	-	-
10#	GG	3R73R	TT	Lys/Gln	Arg/Gln	5FU	mucositis-FEN**	-	-	-	-
11	GG	2R/3R	CT	Gln/Gln	Arg/Arg	5FU	mucositis	-	-	-	-
12	-	3R/3R	TT	-	-	mtx*	High ALT	279	6.97	18.94	2
13	-	3R/3R	CT	-	-	mtx	High ALT	962	24	9.05	6
14	-	2R/3R	CT	-	-	mtx	mucositis	n	n	14.4	2
15	-	3R/3R	CC	-	-	mtx	High ALT	945	23.6	4.93	6
16	-	2R/2R	CT	-	-	mtx	High ALT	854	21.3	2.9	1
17	-	3R/3R	CC	-	-	mtx	High ALT	248	6.2	1.45	1
18	-	2R/3R	CT	-	-	mtx	High ALT	2885	72.1	32	1

*methotrexate, ** febrile neutropenia #toxic deaths

Table 3. Common and Rare Allele Frequencies of Genetic Polymorphisms in Patients Compared with the Population.

	common			rare		
	Allele (%)	Population (%)	Our study	Allele (%)	Population (%)	Our study
DPYD						
IVS14+1G	99		95.5	IVS14+1A	1	4.5
TS						
3R	60-80		55.5	2R	20-40	44.5
MTHFR						
C667	60-90		52.8	667T	Oct-40	47.2
XPD						
Lys751	70-72		55	751Gln	28-30	45

4 dermatitis (hand-foot syndrome) and another one had grade 4 mucositis with febrile neutropenia (grade 4). DPYD IVS14+1A allele frequency was 4.5 fold higher than population. TS common allele (3R) frequency was found to be slightly lower in our patient group. On the other hand, it showed differences between 5FU and methotrexate groups; it was lower in 5FU group but in normal population range in methotrexate group

For XPD and XRCC1 gene polymorphisms associated with decreased 5FU response, the frequency of Gln alleles for both genes was found to be 45% which is higher than the population frequency.

There were two toxic deaths. The first patient had 5FU, cisplatin and docetaxel for advanced stage gastric cancer. He had disseminated grade 4 mucositis after first cycle of CT. Co-administration of docetaxel with 5FU might have contributed to grade 4 mucositis. He had been applied supportive care including total parenteral nutrition. He died because of sepsis. The second patient was given 5FU, folinic asid and oxaliplatin (FOLFOX-4 regimen) for colorectal cancer. He suffered from grade 4 neutropenia and grade 4 mucositis. He died because of febrile neutropenia in spite of supportive care including parenteral antibiotics.

Discussion

Thirty-nine different mutations and polymorphisms leading to decreased enzyme activity and therefore to incerased risk of toxicity for 5-FU have been identified in DPYD gene with an overall heterozygote frequency of 3 % (Van Kuilenburg 2004; Dervieux 2005). The most frequent inactivating mutation is IVS14+1G>A leading to skipping exon 14 and therefore missing 165 nucleotide in mRNA and the corresponding 55 aminoacids in the protein product. The frequency of IVS14+1G>A mutation in patients with severe toxicity after 5-FU treatment is controversial. Van Kuilenburg et al. reported that the frequency of IVS14+1G>A mutation was 50 % in patients with grade IV neutropenia either in homozygote or heterozygote state and was 56 % in patients with low DPYD level (Van Kuilenburg, 2002). However, Magnè et al. reported the frequency of the same mutation as low as 2.2 % in a group of patient with 5FU-related toxicity and concluded that screening for IVS14+1G>A mutation have limited effectiveness in identifying patients at risk, at least for French population (Magnè et al, 2007). Recently, Gross et al. reported another mutation, c.496A>G with a high frequency of 43 % in patients with severe drug-adverse effects while the frequency of IVS14+1G>A mutation was found to be as low as 5.4 % (Gross et al, 2008). Furthermore, association of C496A>G mutation with toxicity was observed in patients with gastroesophageal and breast cancer but not in patients with colorectal malignancies. In our study, IVS14+1G>A mutation was found in only one case in heterozygote state with an overall frequency of 4.5 % (n=11) which is still 4.5 fold higher than the population frequency. The discrepancy between the results of individual studies has been attributed to ethnic differences of patients, different therapy regimens and tumor type (Magnè et al 2007; Gross et al 2008). Moreover, it was suggested that not only a few mutations but all functionally important mutations and polymorphisms must be screened in order to identify patients at risk for 5-FU related toxicity. Magnè et al. also suggested the need for the development of a large scale screening test for DPYD with good sensitivity and specificity by using genetic and/or biochemical techniques (Magnè et al., 2007). However, also the efficacy of 5-FU is effected by the functional polymorphisms of genes encoding metabolizing enzymes. The main target of 5-FU is the enzyme thymidilate synthase and higher levels of this enzyme is associated with reduced response to 5-FU treatment. Since TS gene 3R allele is related to the elevated expression of TS, individuals with 3R allele are less likely to respond to 5-FU. On the other hand, higher levels of MTHF increase the inhibitory effect of 5-FU. MTHF is the substrate of MTHFR and since MTHFR 677T allele reduces the activity of the enzyme and therefore cause an increase in the MTHF levels, individuals with T allele have e better response to 5-FU. In our study, TS 3R allele frequency was found to be lower than the population frequency in 5FU group while MTHFR T allele frequencies were found to be higher. Taken together, 5FU toxicity may be due to the increased efficacy of 5FU as a result of increased TS 2R and MTHFR T allele frequencies. However, a second polymorphism, a G>C single nucleotide polymorphism, in the second repeat of the 3R allele dramatically effects the expression level of this allele in a way that 3R alleles harboring C nucleotide (named as 3RC allele) have the same expression level of 2R allele while 3R alleles with G nucleotide (named as 3RG allele) have a higher level of expression. As a result, 3R/3R genotype does not necessarily mean higher expression levels. It may be significant to investigate G>C polymorphism in 3R/3R genotypes. Furthermore, a third polymorphism, a 6 bp deletion in 3' untranslated region, was found to be associated with 5-FU response in such a way that 3R/-6bp alleles had a better response. In MTHFR gene, a second polymorphism, 1298A>C namely, also reduces the activity of the enzyme and has a better response to 5-FU.

Analysis of all relevant polymorphisms associated with the efficacy and toxicity of a given drug is recommended as a pharmacogenetic approach to clarify the efficacy and toxicity of theurapeutic agents (Toffoli

and de Mattia, 2008). In this study, TS gene 2R/3R polymorphism were also investigated in methotrexate group since this polymorphism seem to affect not only 5FU based therapies but also methotrexate based treatments, in terms of response or toxicity. Campalani et al reported that 2R/3R polymorphism might have affected response to methotrexate (Campalani et al, 2007). They also reported that TS 3R/3R genotype and MTHFR 677T alleles have contributed to methotrexate toxicity. Kantar et al reported a relationship between A1298C polymorphism of MTHFR gene and methotrexate toxicity in Turkish pediatric patients with leukemia and lymphoma who were applied CT, including methotrexate, but they failed to show an association between C677T polymorphism and methotrexate toxicity (Kantar et al., 2009). In this study, the frequency of 2R allele was found to be 28.6% which was in normal population range. MTHFR 677T allele frequency was also very close to the normal population range though it seemed to be slightly higher. Our results suggest a combinatory affect of 2R and T alleles on higher ALT levels and the interval between methotrexate administration and ALT peak.

In conclusion, antimetabolites are widely used in cancer patients. Some patients may have higher rates of toxicity, even lethal toxicity. We consider that pharmacogenetic analysis might contribute to individualized therapy ('tailored therapy'). It will not only increase response rates but also decrease toxicity. Individualized therapy will be also cost-effective by avoiding administration of unnecessary chemotherapy to the patients who will have no benefit. It should be emphazised that we only analyzed gene polymorphisms of the patients who had severe toxicity. We evaluated gene polymorphisms after toxicity. However, it will be better if suitable patients are selected before CT. We believe that we need further pharmacogenetic trials with more patients to have better clinical outcome.

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