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Clinical relevance of thiopurine S-methyltransferase gene polymorphisms

Minireview

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The therapeutic response to thiopurines may result in either severe toxic or inadequate effect based on the interindividual genetic variability. Same drug doses of various anticancer drugs cause considerable interindividual differences in the therapeutic response. Genetic factors have a major impact on effectiveness of several anticancer drugs such as mercaptopurine, 5-fluorouracil, platinum agents, and cyclophosphamide. Heredity related differences in interindividual response to thiopurine therapy represent perhaps the most compelling evidence of pharmacogenomics‘ usefulness in identification of patients in risk for adverse drug reactions. A number of variations in the gene for thiopurine methyltransferase (TPMT) have been associated with the low activity of this enzyme. Patients with intermediate and low activity of TPMT have a greater incidence of thiopurine toxicity. This minireview summarizes results of studies assessing the role of genetic polymorphisms in the gene encoding TPMT and their relationship to the toxicity of thiopurines.

Key words: toxicity, thiopurines, thiopurine methyltransferase (TPMT), polymorphism.

High interindividual variability in the response to a great number of drugs is well-known fact in clinical community. Same doses of numerous drugs cause considerable differences in patients' therapeutic responses that may result in fatal complications of the therapy. The effects of anticancer and immunosuppressive agents are influenced by numerous modifying factors, for example age, renal and liver function, drug interactions, and compliance. Genetic factors significantly contribute to the toxicity of several anticancer drugs and account for as much as 95% of the variability in drug disposition and effects [1].

This variability is usually caused by common genetic polymorphisms in genes encoding drug-metabolizing enzymes, drug transporters or drug targets [2,3]. Polymorphisms represent common variations in a DNA sequence leading to reduced or increased activity of the encoded protein. Unlike somatic mutations, polymorphisms are stable and heritable.

The metabolism of anticancer drugs is a complex process - metabolic cascades consist of a significant number of enzymes, transport and regulating proteins. Most anticancer drug effects are determined by the interplay of multiple gene products throughout the entire drug pathways [4-8].

Toxicity of thiopurine drugs - azathioprine (AZA), 6-mercaptopurine (6-MP) and thioguanine (TG) may be predicted by detection of single gene polymorphism for thiopurine S-methyltransferase (TPMT) [4,5].

Polymorphisms of thiopurine S-methyltransferase. TPMT (EC 2.1.1.67) is a cytosolic enzyme encoded by a 27-kb gene on human chromosome 6p22.3. TPMT exhibits autosomal codominant polymorphism [6].

To date, at least 29 variants have been reported in the TPMT gene. More than 20 types of these variants are associated with reduced enzyme activity. The most common polymorphisms associated with low activity are TPMT*2, 3A, 3B, 3C. These alleles are responsible for over 95% of cases of low enzyme activity (9).Other alleles are rare [4, 5, 10].

TPMT in metabolism of thiopurines. The inactivation of active thiopurine compounds is provided in human tissues by two enzymes – TPMT and xantinoxidase (XO) (Fig. 1). Due to the lack of XO in the bone marrow, inactivation of

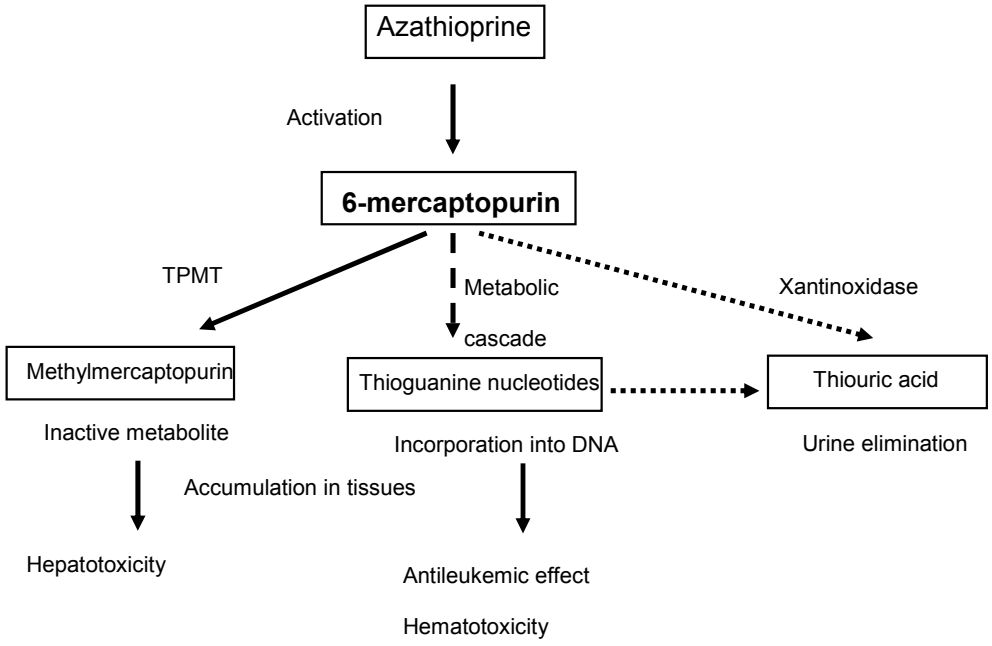

Fig. 1 Metabolism of thiopurine analogues (according to 10).

active thiopurine compounds depends only on the quality of S-methylation by TPMT.

In wild type alleles, protein degradation halftime is about 18 hours, but in mutated forms only 15 minutes [4, 10, 11]. Decreased degradation halftime leads to longer exposure of haematopoietic tissue to active thiopurine metabolites and severe myelotoxicity.

In vivo tests on TPMT knock-out murine model indicate that 6-MP is more affected by the TPMT polymorphism than 6-TG [12].

In the population low, intermediate and high activity of TPMT is encountered. Patients with decreased activity of TPMT are at risk of severe adverse haematopoietic toxicity after administration of standard doses of thiopurines [12, 13].

Approximately 10% of population is heterozygous for mutant alleles and tolerates approximately 65% of standard dosage.

Homozygous form of mutant alleles is found in 0.3-0.5% of population [4, 5, 10]. Patients with this form tolerate only 1/10 – 1/15 of standard dosage (12). Patients with TPMT deficiency, who are treated with thiopurines, are at increased risk of serious cytopenia, infections and therapeutic failure.

The physiological role of TPMT is unknown. The endogenous substrate of TPMT has not been identified yet. The synthesis of purines and pyrimidines in murine knock-out model for TPMT remains intact. Krynetski et al [14] suggest that TPMT is involved in the processes of methylation in detoxification cascades. Carriers of deficient phenotype are not distinguishable from non-deficient ones, except in the presence of thiopurine drugs [15]. Other enzymes such as XO, inosine triphosphate pyrophosphohydrolase also affect metabolic cascade of thiopurine drugs [14].

Age may impact the activity of TPMT. Ganiére – Monteil et al. (2004) suggest that TPMT activity is already mature at birth, however TPMT activity in children is slightly lower than in adults [16]. On the other hand, McLeod et al (2005) suggest that erythrocytes of newborns have higher amount of TPMT protein which correlates with the higher activity of TPMT in them when compared to TPMT activity in adults. Conflicting results from these studies highlight the need for accurate measurement of TPMT activity in children [17].

Geographical distribution of TPMT polymorphisms as described in studies of European, American, African and Asian populations shows significant ethnic differences [18-27]. In Chinese, Japanese and in African -Americans TPMT*3C allele is mostly present. In Sub-Saharan region TPMT *8 is responsible for low activity of TPMT. However, in the population of Caucasians TPMT *2 and TPMT*3A alleles are mostly present. In 2007, TPMT*3A/*23 genotype association with nearly undetectable activity of TPMT was discovered [18]. Most prevalent TPMT variations in selected populations in Europe are listed in Tab. 1. Data for Slovak population will become available soon.

Drug interactions. The activity of TPMT can be altered by concomitant drugs influencing the pharmacokinetics of AZA or 6-MP.

Allopurinol, a substrate for XO, may lead to increased plasma concentrations of active thioguanine metabolites and to higher toxicity.

Tab. 1 TPMT Variations in selected populations in Europe

Europe		
Population	1st most prevalent deficient allele	2nd most prevalent deficient allele
British	TPMT * 3A	TPMT * 2
Bulgarian	TPMT * 3A	TPMT * 2
Czech	TPMT * 3A	TPMT * 3C
French	TPMT * 3A	TPMT * 2
German	TPMT * 3A	TPMT * 3C
Portuguese	TPMT * 3A	TPMT * 2
Italian	TPMT * 3A	TPMT * 3C
Sardinian	TPMT * 2	TPMT * 3C
Swedish	TPMT * 3A	TPMT * 3C
Norwegian		
Saami	TPMT * 3C	
Caucasian	TPMT * 3A	TPMT * 3C
Polish	TPMT * 3A	TPMT * 2
Russian		
Caucasians	TPMT * 3A	
Ugur	TPMT * 3C	
Asian	TPMT * 3C	
Serbian	TPMT * 3A	TPMT * 3B
Slovenian Caucasian	TPMT * 3A	TPMT * 3C TPMT * 3B
Slovak	Not published yet	

Methotrexate (MTX) inhibits the XO activity in tissues. Although, the inhibition of XO activity of clinical significance is questionable, it has to be also of concern in patients treated with MTX and thioguanines, especially in patients with inflammatory bowel disease (IBD) [6].

Reports about the inducing or inhibiting role of 5-aminosalicylate drugs are conflicting [38, 41]. Drug interactions of 5-aminosalicylates and thiopurines in IBD patients are studied in vitro and in vivo. Recent reports indicate that sulfasalazine or 5-aminosalicylates inhibit TPMT activity (38- 40). The addition of aminosalicylates to ongoing AZA or 6-MP therapy results in increased blood levels of 6-MP and leucopenia. Since mesalazine, sulfasalazine and olsalazine are inhibitors of TPMT, careful monitoring of patients with concomitant therapy with AZA or 6-MP is recommended. Measurement of TPMT activity prior to treatment could help to minimize the myelotoxicity in patients with IBD [42, 43].

6-MP decreases anticoagulant effects of warfarin. The mechanism by which 6-MP interferes with effects of warfarin is not fully understood. However, discontinuation of 6-MP leads to bleeding in patients previously treated with warfarin [6].

Weyer et al. (2001) reported that uraemia in renal transplant recipients is a significant inducer of TPMT activity [44]. In these patients the administration of angiotensine-converting enzyme inhibitors (ACEI) along with thioguanines resulted in severe anaemia. However, pharmacokinetic interactions between AZA and ACEI were not observed.

Diuretics inhibit TPMT activity in vitro. Clinical significance of inhibition of TPMT by diuretics has not been evaluated.

NSAIDs (nonsteroidal anti-inflammatory drugs) were also investigated as potential inhibitors of TPMT in recent in vitro studies. Because of the wide usage of NSAIDs the in vivo studies are essential [4, 6].

Defect TPMT polymorphisms and secondary malignancies. Cancer survivors had almost seven times higher risk of a second malignant neoplasm than the general population after an average follow-up of 6.5 years [46]. Thiopurines are widely used in oncological patients, especially in those with acute lymphoblastic and myeloid leukemia and nonHodgkin lymphoma.

Low activity genetic polymorphisms might be a risk factor for the occurrence of therapy-dependent secondary leukaemia [47]. Low activity of TPMT in patients seems to be a good predictor of an earlier onset of secondary acute myeloid leukaemia (AML) when compared to patients with normal TPMT activity after the treatment of primary malignancy [48, 49].

Several studies have identified relationship between cranial radiotherapy, incidence of brain tumours and levels of 6-MP. Reduced TPMT activity may be associated with increased risk of secondary malignancies, such as leukaemia and brain tumours in children suffering from acute lymphoblastic leukaemia (ALL) who are treated with 6-MP combined with cranial irradiation, or etoposid application. The year cumulative incidence of brain tumours among children with defective TPMT that received prophylactic cranial radiotherapy was 42.9% [50].

Several authors confirmed that carriers of mutant allele either in homozygous or heterozygous form had lower risk of relapse of ALL in comparison to homozygotes for wild type allele [51].

Leukemia patients with reduced TPMT activity had higher response rate to 6-MP and better prognosis for being cured in comparison to ALL patients with wild type alleles (52). Reduced TPMT activity is significant predictor of event-free survival in ALL [9, 51, 52].

TPMT deficient patients were at higher risk of hepatotoxicity, nodular regenerative hyperplasia, veno-occlusive disease and development of oesophageal varices after the therapy with thioguanines and busulphan[(6].

Importance of thiopurine S-methyltransferase gene investigation in clinical practice Thiopurine drugs are used mainly in oncology, gastroenterology, dermatology, rheumatology and transplantology (Tab.2).

The primary purpose for TPMT screening is an identification of patients with deficient activity of TPMT thus increasing the efficiency of thiopurine therapy and preventing severe adverse reactions. The therapeutic window of thiopurines is very narrow. The dosage of anticancer and immunosuppressive therapy based either on body weight or body surface - ´one size fits all´ modality – may lead to an inadequate treatment or to overdosing with adverse drug reactions especially myelo-

Table 2. Representative indications for thiopurines

	Diagnosis	Reference
Oncology	Acute lymphoblastic leukaemia	10, 14, 28
	Acute myeloblastic leukaemia	10
	Non-Hodgkin´s lymphoma	10
Gastroenterology	Crohn Disease	29
	Ulcerative Colitis	30
	Autoimmune hepatitis	31
Rheumatology	Rheumatoid arthritis	32
	Systemic lupus erythematosus	33
Dermatology	Psoriasis	34
	Severe atopic eczema	35
	Pomphylox (dyshidrotic eczema)	36
Transplantology	Solid organ transplantations	37

toxicity. [4, 51]. Patients with high TPMT activity are at high risk of hepatotoxicity caused by an accumulation of methylated metabolites in liver tissue. Several strategies have been suggested to individualize thiopurine dosage – most used are genotyping and phenotyping [53-57].

In phenotype assays, the accurate activity of TPMT enzyme is evaluated. TPMT from red blood cells lysate is incubated with 6-MP or 6-TG with the methyl donor S-adenosyl-L-methionine. The production of methylated products is detected by, for example, radiochemical enzymatic assay method using liquid chromatography [38, 56, 57], high-performance liquid chromatography (HPLC) with absorbance or fluorescence detection [58, 59, 60], or capillary electrophoresis (61). Identification of low enzyme activity may uncover patients with not-usually evaluated but deficient polymorphisms.

TPMT activity cannot be accurately measured in patients receiving transfusions of red blood cells due to interference from donor cells. Misinterpretation of results in transfusion recipients may lead to serious toxicity [62].

Genotyping is essential for patients who received red cells transfusions 2-3 months prior to the testing. Routine genetic testing prior to thiopurine therapy and adjustment of doses of these drugs was approved by the Food and Drug Administration (FDA) and is performed on routine basis in some oncologic centres not only in the USA, but also in the United Kingdom and Spain [63, 64].

Conclusion. Over the past decade genetic polymorphisms have been widely investigated, especially in association with drugs with narrow therapeutic window [4, 6, 64].

"One size fits all" modality does not guarantee the best outcome of anticancer therapy. The use of pharmacogenetics may help prevent adverse drug reactions. TPMT and thiopurines provide a successful example of the application of pharmacogenetics into individualized human drug therapy.

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