PHARMACOGENOMIC RELATED TOXICITY OF 6-MERCAPTOPURINE IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA

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ABSTRACT:
Polymorphisms in drug metabolizing enzymes, transporters and/or pharmacological targets of drugs may profoundly influence the dose-response relationship between individuals sometimes exacerbating the drug toxicity. Mercaptopurine (6-MP) is a pro drug that has been used for past 35 to 40 years in the treatment of various types of cancers especially acute lymphoblastic leukemia (ALL) of childhood. Thiopurine S-methyltransferase (TPMT) is a cytosolic enzyme that catalyzes its inactivation through methylation. It has been found that the activity of TPMT possesses genetic polymorphism, as an autosomal recessive trait. Continuous efforts of researchers and scientists to find out the molecular basis for altered activity of this enzyme now has been defined with various rapid and inexpensive assays especially allele specific polymerase chain reaction (AS-PCR) and restriction fragment length polymorphism (RFLP). These assays are now internationally available for the four commonest signature TPMT variant alleles 2*, 3A*, 3B* and 3C*. Various studies on TPMT genotyping and phenotyping in patients using 6 MP demonstrated its close association with the risk of myelotoxicity. Alarmingly altered TPMT genotype may influence the risk of secondary malignancies also, including brain tumors and acute myelogenous leukemia. This review highlights the current approaches to improve the clinical impact of 6-MP in childhood ALL in context with polymorphic TPMT gene. Some of these investigations are entering routine clinical practice internationally but a lot of work is required in determining their optimal use in patients with ALL at our part of the world.

Keywords: Mercaptopurine, thiopurine methyltransferase, polymorphism, mutant allele, ALL.

1. Metabolism of 6-mercaptopurine (6MP):
Mercaptopurine (6-MP) is one of the widely used medications for childhood acute lymphoblastic leukemia (ALL) (Relling et al., 1999a) in a daily oral dose of 50-90mg/m²/day for about 2-3 years during maintenance phase of the treatment. It is a pro-drug, metabolized to thioguanine nucleotides (TGNs) (Krynetski et al., 1999) which get incorporated into DNA and RNA to exert its cytotoxic effects on leukemic blast cells (Figure 1). Normally TGNs are formed by a multi-step pathway which is initiated by hypoxanthine-guanine phosphoribosyl transferase (HGPRT) (Krynetski et al., 1996). Alternatively 6-MP can undergo two other conversions namely (a) S-methylation to methylmercaptopurine (MeMP) catalyzed by thiopurine methyltransferase (TPMT) and (b) oxidation to thiouric acid (TA) via xanthine oxidase (XO). Either conversion reduces the formation of the active TGNs (Lennard et al., 1992).

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2. Fatal myelotoxicity by 6MP:
When ALL patients are treated with conventional doses of thiopurines (6MP), almost half of the patients with heterozygous deficiency of TPMT and all homozygous deficient patients develop hematopoietic toxic effects, which are almost fatal in the homozygous group (Evan et al., 2004). During the last two decades the focus of ALL treatment has not only been shifted from standard dosage regimes to dose titration but also directed towards expansion of pharmacogenetic studies related to 6-MP. (Christine et al., 2007)

3. Pharmacogenetics in Acute Lymphoblastic Leukemia role of TPMT:
It has evolved now that the efficacy and toxicity of all the anti-neoplastic drugs depends upon the genetic polymorphisms of targets such as drug transporters, receptors, and drug-metabolizing enzymes (Evan et al., 2004; Kishi et al., 2007). The traditional approach of pharmacogenomic studies in the field of oncology including ALL, has been the candidate gene approach, in which a gene or pathway is identified and then explored. Candidate gene overtures focus on one or a small number of genes known to be important in the pharmacokinetics or pharmacodynamics of a particular drug. This approach has yielded important and clinically relevant information within the field of oncology, mainly with the study of genes involved in drug metabolism, transport and DNA repair (Bosch et al., 2006; Efferth et al., 2005). Petros WP has explained in his study that the best known example of successful application of the candidate gene approach is thiopurine methyltransferase (TPMT) (Petros et al., 2004). Zamm and Lsafoli in 1980s told that oral dose of 6-MP has a half life of 1-2 hour with large inter- and intra-individual variations (Zimm 1983 and Lsafoli 1989) In early 21st century, Weinshilboum, Schmiegelow and others have found out that the major determinant of these large inter-individual variations in 6-MP pharmacokinetics reflects polymorphisms in the TPMT activity (Reuther et al., 2003) and (Weinshilboum 2001).

Later on Relling MV and Stanulla M explained the utility of identification of TPMT polymorphisms to improve the treatment outcome of ALL (Relling et al., 2006; Stanulla et al., 2005). TPMT polymorphism is inherited as an autosomal co-dominant genetic trait, representing itself by a number of allelic variants ranging from TPMT*2 to TPMT*24, all showing single nucleotide polymorphism

![Figure 1. Schematic representation of the metabolism of 6-mercaptopurine (McLeod, 2000).](image-url)
(SNP) with decreased enzymatic activity (Ujiee et al., 2008). These variations ultimately leads to decreased methylation (inactivation) of 6MP at the cellular level. If this routine process of inactivation of the drug after its therapeutic use has been interrupted; it leads to the diversion of the metabolic pathway more towards the formation of its active metabolites such as TGNs. All this ultimately increases the risk of toxicity (McLeod et al., 2000).

4. Significance of 6-mercaptopurine in acute lymphoblastic leukemia despite myelotoxicity

Research during last two decades in the field of anticancer drugs has approved globally that all the ALL patients showed excellent improvement with minimal risk of relapse after a course of continuation/maintenance therapy for approx. 2.0-2.5 years which include daily 6-MP and weekly methotrexate (MTX) (Pui et al., 2008). After several randomized trials (Harms et al., 2003, Stork et al., 2001 and Vora et al., 2002), it has been concluded that 6MP is proved to be the drug of choice for maintenance phase of ALL. Still many investigators advocate that its dosage be adjusted to maintain leucocyte counts below 3x10^9/L and neutrophil counts between 0.5 and 1.5x10^9/L to ensure adequate dose vividness during the continuation/maintenance phase (Pui et al., 2006).

5. Relationship between thiopurine methyl transferase polymorphism and 6-mercaptopurine induced myelotoxicity and relapse:

Pharmacogenetic studies during last few years have stressed that polymorphism at the TPMT gene locus plays a significant role in the incidence of life-threatening myelosuppression, a serious dose-related toxicity of thiopurine drugs (Colombel et al., 2000). In addition to toxicity, clinical implications of the TPMT polymorphism may also include variations in drug efficacy and drug interactions (Corominas et al., 2004). As already mentioned half of the patients with heterozygous deficiency of TPMT and all homozygous deficient patients develop hematopoietic toxic effects, which can be almost fatal in the homozygous group (Evan et al., 2004). Conversely patients with high levels of enzyme activity are at greater risk of relapse due to decreased exposure of leukemic cells to active drug metabolites like TGN (Stanulla et al., 2005).

6. Thiopurine methyl transferase deficiency leading to secondary malignancies:

In a study of secondary brain tumors in ALL children treated at St Jude Children's Research Hospital, Memphis, USA, Relling et al reported that those patients who are exposed to fatal side effect of myelotoxicity due to TPMT polymorphism are also found to be at a higher risk of development of secondary malignancies like radiation-induced brain tumors (Relling et al., 1999b). In another study from the same group, there was also a trend for patients treated for ALL who developed secondary AML to have a lower TPMT activity than controls and an association between an earlier onset of secondary AML and lower TPMT activity (Relling et al., 1998).

These conclusions are supported by a study from the Nordic Group who reported a significant association between low TPMT activity and the risk of secondary myelodysplasia or AML (Thomsen et al., 1999).

7. Significance of thiopurine methyl transferase genotyping:

Until 2004, TPMT genotyping of the three commonest variant alleles accounting for more than 90% of cases with low or intermediate TPMT enzyme activity (Petros et al., 2004) was done prior to therapy. This was helpful in identifying the risk group of ALL patients and tailoring of the chemotherapeutic regimen. Various methods and latest technologies are now available to detect approximately 24 different types of TPMT alleles (Ujieii et al., 2008). As various studies have realized and explained well that mutations present within TPMT enzyme are most probably responsible
for the fatal side effect of 6-MP induced myelotoxicity (McLeod et al., 2000). Christine M H and M Eileen D in their review recommended that genotyping of the three commonest variant alleles of TPMT i.e. 2*, 3A* and 3C* is beneficial if done prior to 6MP therapy. Genotyping, helps in the identification of most of the patients at greatest risk for severe thiopurine induced myelotoxicity and hence the tailoring of chemotherapeutic regime (Christine et al., 2007).

8. Detection of thiopurine methyl transferase deficiency by polymerase chain reaction:

The promptest and a very reliable method for detection of TPMT genotyping is allele specific polymerase chain reaction (AS-PCR) and restriction fragment length polymorphism (RFLP) (Christine et al., 2003). The Food and Drug Administration (FDA) has recommended that patients with clinical evidence of severe toxicity, particularly myelosuppression, should be considered for TPMT genotyping (Wei et al., 2006). Although this methodology of identifying the high risk group of patients is performed at a limited number of academic centers internationally (Christine et al., 2007), this important aspect of managing a serious outcome of 6MP therapy is not even considered at our end of the world.

9. Effect of 6-mercaptopurine dosage according to thiopurine methyl transferase genotyping on outcome of disease

There is a dire need for dose reductions in ALL patients with homozygosity or heterozygosity for a variant TPMT allele as compared to those who were homozygous wild-type (Relling et al., 1999a). This becomes entirely an easy effort once the TPMT alleles are duly categorized. Many studies have concluded that 6- MP doses are adjustable based on the TPMT genotyping.

Colombel et al. and Regueiro & Mardini have observed that azathioprine and 6-mercaptopurine metabolized by polymorphic TPMT is inversely proportional to the risk of developing acute leucopenia. They have further shown that the risk of azathioprine-induced acute leucopenia can be greatly reduced by selecting the initial azathioprine dose based on TPMT genotype or phenotype (Colombel et al., 2000). A Korean genotype-based dosing strategy through PCR-based thiopurine methyltransferase (TPMT) polymorphism screening proved it to be very cost-effective and more reliable than the conventional weight-based dosing strategy (Oh, 2004) as it was associated with a marked reduction in the number of serious adverse events. However, controversial reports that average 78% of adverse drug reactions (ADRs) were not associated with TPMT polymorphism in six clinical studies correlating the adverse effects of these drugs with TPMT genotype are also available in literature review. Pharmacogenetic testing will thus not eliminate the need for careful clinical monitoring of ADRs (Schwab et al., 2002).

CONCLUSION

Mercaptopurine therapy is the back-bone for maintenance phase of ALL treatment and is used in almost all of the ALL treatment protocols. TPMT responsible for its inactivation (S-methylation) exhibits genetic polymorphism resulting in one of the hazardous side effect of this drug namely myelotoxicity which is even lethal in some patients. Many studies have discovered and concluded that 6- MP doses are adjustable based on the TPMT genotyping especially in the homozygous TPMT -mutant group. Despite disputable reports, clinical application of this discovery remains practicable and is being adapted in many parts of the world. So now studies into the role of TPMT in governing response to treatment with the thiopurine drugs has moved from the research laboratory into clinical practice and assessment of RBC TPMT activity and these have become a routine in the investigation of thiopurine associated myelosupression at these centres. In a few instances pretreatment assessment (i.e., TPMT genotyping) has become mandatory and this has been argued to
be cost-effective, physicians still preferred to rely on the monitoring of the white cell count after initiation of therapy. However, the onset of neutropenia in patients with biallelic mutations can be very swift and life threatening. Unfortunately, we are still treating our ALL patients without pre treatment assessment with TPMT genotyping. It is to be hoped that the increased availability of simple genetic tests and non-isotope based TPMT activity assays will enable us to get the valuable information about TPMT mutations in governing drug response to be put into everyday clinical practice at our part of the world also.

REFERENCES


