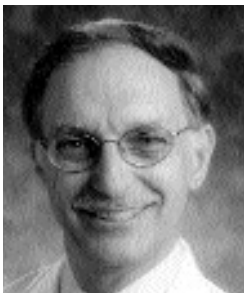
Editorial

Pharmacogenetics for the Individualization of Treatment of Rheumatic Disorders Using Azathioprine



Therapeutic drug monitoring has evolved to become a commonly employed clinical adjunct for decision making in a wide variety of conditions. Nevertheless, over 3 decades after its introduction into clinical practice, issues remain concerning the justification of the benefits of drug monitoring in the face of increasing competition for limited budgetary resources[1,2]. It has been suggested that the greatest benefit of therapeutic drug concentration monitoring accrues from targeted populations, such as those receiving immunosuppressive drugs[2]. In such situations, the benefits gained from drug monitoring are considered primary, while cost issues are secondary and economic justification is not generally required. When considering drug monitoring, however, both benefit and toxicity must be considered, as patients differ in their susceptibility to adverse drug reactions. Recent advances in molecular biology have led to the founding of pharmacogenetics, a novel scientific field aimed at understanding the genetic contribution to the variability in drug efficacy and toxicity[4]. The goal of these emerging technologies is to provide clinicians with the tools needed to predict how genetic polymorphisms determine the metabolism and transport of cytotoxic drugs, potentially identifying individuals at risk for drug induced toxicity[5].

The thiopurine drugs azathioprine (AZA) and 6-mercaptopurine (6-MP) are cytotoxic and immunosuppressant agents commonly employed in a number of rheumatological and autoimmune disorders[6]. 6-MP, including that derived from AZA, is cleared from the circulation via competing anabolic and catabolic pathways (Figure 1). Following their absorption, extensive first-pass metabolism occurs in the intestinal mucosa and liver, where xanthine oxidase and thiopurine methyltransferase (TPMT) convert 6-MP to the inactive metabolites 6-thiouric acid and 6-methyl-mercaptopurine (6-MMP), respectively[7]. The subsequent metabolic steps occur intracellularly, where hypoxanthine guanine phosphoribosyl transferase converts 6-MP to 6-thioinosine-5′-monophosphate (TIMP). The latter molecule is converted to the active, but potentially myelotoxic metabolites, the 6-thioguanine nucleotides (6-TGN)[8]. The therapeutic and immunosuppressive effects of 6-MP and AZA are thought to be primarily due to the intracellular formation of the 6-TGN after their incorporation into the DNA and RNA of cells (Figure 1). In a competing pathway, TPMT catalyzes the conversion of TIMP to the 6-MMP, whose overproduction has been associated with therapeutic failure and an increased risk of hepatotoxicity[9].

The enzymatic activity of TPMT is genetically determined[10]. Two alleles, $TPMT^{H}$ and $TPMT^{L}$, confer high and low TPMT activity, respectively, and are inherited in an autosomal codominant fashion. About 85-90% of individuals have the wild-type $TPMT^{H}$ allele ($TPMT^{H}/TPMT^{H}$) and have normal to high enzymatic activity. One out of 300 (0.3%) individuals is homozygous for the $TPMT^{L}$ allele ($TPMT^{L}/TPMT^{L}$) and has absent or negligible activity. The remaining 10–15% of the population are heterozygous ($TPMT^{H}/TPMT^{L}$) and have intermediate activity. As illustrated in Table 1, individuals with low or intermediate TPMT activities generate higher 6-TGN concentrations and are consequently more likely to respond to therapy but are at much higher risk for myelosuppression[11]. On the other hand, those with high levels of TPMT activity may have low levels of 6-TGN metabolites and are more likely refractory to treatment[9-11].

If treated with standard doses of thiopurines, TPMT deficient patients accumulate excessive 6-TGN levels in hematopoietic tissues, potentially leading to severe hematological toxicity that may be fatal[11]. In this issue, Marra and coworkers[12] have attempted to evaluate the clinical utility and cost effectiveness of implementing TPMT genotyping in patients with rheumatological disorders who are treated with thiopurine drugs. The authors applied a decision analytic model to determine whether utilizing molecular determination of TPMT polymorphisms would be cost effective compared to standard dosing without knowledge of TPMT genotype. The decision node in the genotyped patients would indicate a

See Practical pharmacogenetics: the cost effectiveness of screening for thiopurine s-methyltransferase polymorphisms in patients with rheumatological conditions treated with azathioprine, *page 2507*

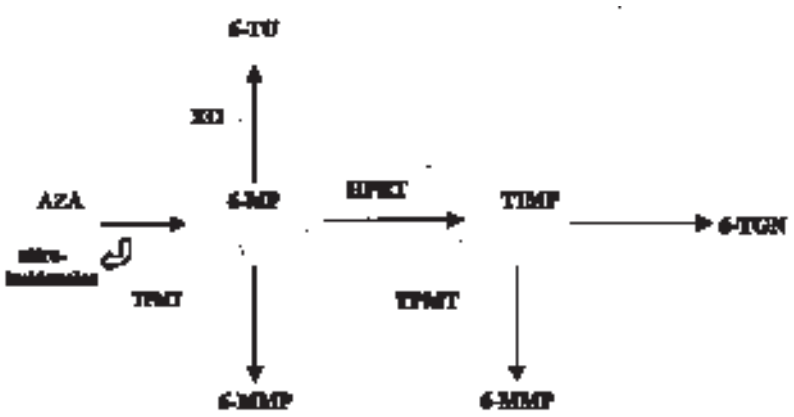


Figure 1. Thiopurine metabolism. Azathioprine (AZA) and 6-mercaptopurine (6-MP) are metabolized along 3 competing routes: an oxidative pathway via xanthine oxidase (XO), yielding thiouric acid (6-TU); S-methylation via thiopurine methyltransferase (TPMT), yielding 6-methylmercaptopurine ribonucleotides; and active nucleotide metabolite formation via hypoxanthine-guanine phosphoribosyltransferase (TPMT). 6-TGN: 6-thioguanine nucleotides.

Table 1. Thiopurine methyltransferase (TPMT) correlation with dose of azathioprine (AZA) and metabolic outcomes.

TPMT Genotype	TPMT Phenotype	AZA Recommended Dose, mg/kg/day	6-TGN Levels	6-MMP Levels	Myelotoxicity Risk	Hepatotoxicity Risk
TPMT*1 (wild-type)	Normal activity	2–3	Normal	Normal	Low	Low
TPMT*1 (wild-type)	High activity	2–3	Low	High	Low	High
TPMT*2, *3A, *3B, or *3C (heterozygote)	Low activity	0.75–1.25	High	Low	High (if full dose AZA)	Low
TPMT*2, *3A, *3B, or *3C (homozygote)	Negligible to absent	Drug best avoided	Very high	Very low	Very high, potentially fatal	Low

6-TGN: 6-thioguanine nucleotides. 6-MMP: 6-methyl-mercaptopurine.

reduction in dose for heterozygotes (from 2.5 to 1 mg/kg AZA) and a more drastic reduction for those identified as homozygotes (to 0.25 mg/kg). Their analysis revealed that TPMT genotyping with reflex dose reduction in TPMT deficient patients would yield a modest cost saving based on fewer hospitalizations and treatments for severe leukopenic events. The moderate cost savings would, however, be eclipsed by the reduction in morbidity generated by the genotype-based strategy. The estimated number needed to test in order to avoid one serious adverse event over 6 months was estimated to be 20.

This innovative report has important conclusions for practitioners, as it opens the horizon towards the use of pharmacogenetics to individualize and optimize therapy using thiopurine drugs in rheumatological disorders. Admittedly, the decision analytic model employed is based on numerous assumptions that could affect the results, including the estimated percentage of cases that would require hospitalization, as well as the needs for costs of medical services and treatments (e.g., antibiotics, granulocyte monocyte-colony stimulating factor), as well as for genotyping. Irrespective of these limitations, we believe that the benefits in terms of avoiding toxicity are such self-evident and compelling arguments that safe and humane practice would support genotyping as worthwhile without respect to cost. In a study in patients with rheumatoid arthritis, TPMT heterozygotes had an increased risk of severe side effects due to AZA (relative risk 3.3)[13], in support of the genotyping strategy.

A few other points raised in the report by Marra, *et al* warrant clarification. The first is that not all leukopenic events in patients treated with thiopurine drugs are due to TPMT deficiency. In a recent retrospective study by Colombel, *et al*[14], only 30% of leukopenic events in patients taking thiopurine drugs for inflammatory bowel disease were accounted for by TPMT polymorphisms. Further, cases with severe leukopenic events due to homozygous TPMT deficiency occurred early, within 6 weeks of initiating therapy, and necessitated cessation of the drug in all cases. On the other hand, heterozygotes presented later, at times more than 6 months later, often in the presence of another triggering event such as a viral infection or the use of a sulfa drug. The message to retain is that knowledge of normal TPMT genotype does not preclude monitoring white blood cell counts on a regular basis. The latter study also illustrates that although genetic polymorphism is the primary determinant of TPMT activity, other factors can also play a role. TPMT activity has been shown to be induced by AZA or 6-MP, whereas its activity is inhibited by sulfasalazine and 5-acetylsalicylic acid preparations, as well as by furosemide, thiazide diuretics, and nonsteroidal antiinflammatory drugs[15]. Thus, although the concomitant use of the drugs that inhibit TPMT could potentially increase 6-TGN levels by inhibiting 6-MMP, leukopenia may ensue[15]. It is also worth noting that xanthine oxidase inhibitors such as allopurinol are contraindicated in patients taking thiopurines, as they would dramatically increase the bioavailability of these drugs by inhibiting the first-pass metabolism by this enzyme (Figure 1).

In contrast to the statement by Marra and co-workers[12], assays to measure TPMT enzyme activity are commercially available and are, in fact, less expensive than genotyping. Phenotyping patients' enzyme activity has the theoretical advantage of detecting deficient patients from all ethnic backgrounds, as opposed to the 98% sensitivity of genotyping for the common polymorphisms. The relatively brief turnaround time for both genotyping and phenotyping TPMT allows for the practical implementation of these tests in the clinical setting.

The report by Marra, *et al*[12] supports the role of TPMT genotyping in order to individualize therapy with thiopurine drugs. It is also possible to optimize therapy using these drugs by measuring the intracellular levels of the major metabolites, 6-TGN and 6-MMP. As an example, optimal 6-TGN levels in the treatment of Crohn's disease are above a 235 pmol/8 x 10^8 erythrocyte threshold[7]. Levels exceeding 450 pmol/8 x 10^8 erythrocytes are associated with a higher risk of myelotoxicity. The therapeutic window of 6-TGN levels for optimal treatment of rheumatic disorders remains to be determined. As pointed out by Marra, *et al*[12], TPMT genotyping permits the selection of a safer starting dose of AZA or 6-MP, based on susceptibility to myelotoxicity. Most TPMT heterozygotes achieve therapeutic 6-TGN levels for the treatment of Crohn's disease or ulcerative colitis using a reduction in the standard dose of AZA (0.75–1.25 mg/kg/day AZA), without myelotoxicity[7,16]. These drugs are best avoided in homozygous mutants (Table 1). Further studies are needed to determine the therapeutic window of 6-TGN levels in patients with various rheumatological disorders, as has been achieved in patients with leukemia, inflammatory bowel disease, autoimmune hepatitis, and transplantation.

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