Relevance of thiopurine methyltransferase status in rheumatology patients receiving azathioprine

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Azathioprine (AZA) is widely used in the management of rheumatological diseases. Despite its efficacy, AZA can often cause bone marrow suppression, notably leucopenia, which has been recorded in up to 17% of patients taking AZA for rheumatoid arthritis, though this can be considered clinically significant in about 3% overall. Severe myelosuppression, associated with abnormal AZA metabolism, is linked to the thiopurine methyltransferase (TPMT) genetic polymorphism. TPMT status can be assessed prior to AZA treatment by measuring enzyme activity or genotyping techniques. Analysis of recent data suggests that by optimizing the AZA dose on the basis of TPMT status testing (with a substantial reduction in dose for patients homozygous for mutant TPMT alleles), a reduction in drug-induced morbidity and cost savings can be made by avoiding hospitalization and rescue therapy for leucopenic events. In this article we review the pharmacogenetic and clinical implications of the TPMT polymorphism, emphasizing its relevance to rheumatologists managing diseases with AZA.

KEY WORDS: Myelosuppression, Thiopurine methyltransferase (TPMT), Azathioprine, Mercaptopurine.

Drug metabolism

In human tissue AZA is broken down to 6MP non-enzymatically. The breakdown is potentiated by biogenic thiols in blood (e.g. glutathione) and results in the release of 6MP from the imidazole ring of the AZA molecule [9]. 6MP is a purine antimetabolite designed to disrupt the synthesis of purine de novo and purine salvage pathways and to interfere with the supply of normal purine nucleotides for DNA synthesis by the provision of abnormal alternatives. AZA-derived 6MP is metabolized intracellularly to eventually form the therapeutically active 6-thioguanine nucleotides (TGNs), initially by hypoxanthine phosphoribosyltransferase (HPRT) and then by a series of metabolic conversions [10] (Fig. 1). It is the 6MP-derived TGNs that are eventually incorporated into DNA as a false base [11, 12]. 6MP can also be methylated [13] by TPMT [14–15] and oxidized by xanthine oxidase (XO) [16] to inactive metabolites. TPMT can also methylate 6MP nucleotide, a precursor of the TGNs, to produce the methylmercaptopurine nucleotides. Variations in the extensive metabolism of 6MP undoubtedly play a role in the toxicity and efficacy of AZA-derived 6MP. Of the enzymes involved, only TPMT has been extensively studied with respect to its variation in specific patient groups, namely in leukaemic patients taking 6MP [17, 18] and in the AZA immunosuppression of IBD [19, 20]. However, variability in HPRT-catalysed thionucleotide formation, 5-nucleotidase-catalysed intracellular nucleotide turnover [21–23] and the pharmacogenetics of XO [24, 25] could all influence thiopurine metabolism and thus toxicity from AZA-derived 6MP. Although population studies have indicated 4-fold inter-individual variation in liver XO activity [25], none of these latter enzymes have been studied extensively in large relevant populations taking thiopurine drugs. XO exists primarily in the xanthine dehydrogenase (EC 1.1.1.204) form, which is converted to XO (EC 1.1.3.22) by proteolysis. However, variations in in vivo XO activities are difficult to quantify due to the lack of functional XO activity in circulating blood cells and easily accessible tissues, and inter-individual XO variation can only be implied from differences in caffeine metabolism [26]. It has been reported that lung XO activity
can be regulated by iron, with greater activity in individuals with elevated iron body stores [27]. In vitro studies have linked AZA-induced hepatotoxicity to XO-induced oxidative stress [28]. Despite the lack of biochemical or pharmacogenetic studies directly assessing XO, the potential importance of XO to AZA toxicity is illustrated ultimately by the well-documented myelosuppression resulting from the co-administration of the XO inhibitor allopurinol alongside 6MP [29]. Allopurinol inhibition of 6MP oxidative metabolism increases the amount of drug available for cytotoxic metabolite formation.

**Pharmacogenetics of TPMT**

TPMT activities in human tissue are controlled by a genetic polymorphism inherited as an autosomal codominant trait [15, 30]. TPMT deficiency results in AZA- and 6MP-induced profound bone marrow toxicity, a consequence of the intracellular accumulation of grossly elevated cytotoxic TGN concentrations [7]. Through the 1990s, and across different disciplines, case reports have confirmed this association [22, 31–37]. In many cases toxicity occurred soon after starting the relevant thiopurine drug. Furthermore, during AZA therapy drug-induced bone marrow toxicity may be potentiated by drug–drug interactions. This may be the case for sulphasalazine [38], olsalazine [39], salicylate and other salicylic acid derivatives that can potently inhibit TPMT [40–41] and are co-prescribed along with AZA [42–43].

About 1 in 300 Caucasians are homozygous for TPMT deficiency (homozygosity for TPMT<sup>−</sup>; two variant alleles are present) and at highest risk of life-threatening bone marrow suppression when treated with thiopurine drugs. Intermediate TPMT activity (heterozygosity for TPMT<sup>1</sup>/TPMT<sup>−</sup>; one variant allele present) occurs in 11% of individuals and about 89% are homozygous for wild-type alleles, which appears to be well conserved through different ethnic and racial groups [45], and a series of variant alleles, almost all involving single-nucleotide polymorphisms (SNPs) that result in mis-sense or nonsense codons [30] (Table 1). The wild-type allele is denoted TPMT<sup>∗1</sup>. The commonest variant allele for low enzyme activity in a Caucasian population is a double mutant containing two SNPs [44], which leads to an increased rate of degradation of the altered enzyme [46]. This allele is denoted TPMT<sup>∗3A</sup>, but each mutation can occur independently (alleles TPMT<sup>∗3B</sup> and TPMT<sup>∗3C</sup>; Table 1).

Importantly, there is dissimilarity in the type and frequency of variant alleles between ethnic groups, and this may be important in the clinical use of AZA [45, 47–55]. In African-American and African (Kenyan and Ghanaian) populations the wild-type:mutant allele ratio is the same as recorded in Caucasians but TPMT<sup>∗3C</sup> is the most prevalent mutation [48–50]. In Korean, Japanese, South-West and South-East Asian populations TPMT<sup>∗3A</sup> is absent and TPMT<sup>∗3C</sup> again is the most frequent allele [45, 51–54]. In addition, the frequency of variant alleles varies amongst ethnic groups, being recorded in up to 5% in Asian/Oriental populations [47] (TPMT<sup>∗3C</sup>) and up to 10% in Caucasian and African-American populations (TPMT<sup>∗3A</sup>, *3B and *3C). TPMT<sup>∗3C</sup> is thought to be the oldest mutation and TPMT<sup>∗2</sup> the most recent, and TPMT<sup>∗4</sup> to TPMT<sup>∗8</sup> are thought to be isolated mutations [55].

**TABLE 1. The TPMT<sup>∗3</sup> family, the most frequent SNPs of the TPMT gene**

<table>
<thead>
<tr>
<th>Nucleotide position</th>
<th>Allele</th>
</tr>
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<tbody>
<tr>
<td>460</td>
<td>G</td>
</tr>
<tr>
<td>719</td>
<td>A</td>
</tr>
</tbody>
</table>

TPMT<sup>∗2</sup> and TPMT<sup>∗4</sup> to *8 (which are isolated mutations) are not shown.

**FIG. 1. Simplified scheme of the metabolism of azathioprine and 6MP to 6-thioguanine nucleotides (TGNs).** TPMT, thiopurine methyltransferase; XO, xanthine oxidase; HPRT, hypoxanthine phosphoribosyltransferase.
TPMT phenotype, genotype and AZA/6MP metabolite monitoring

In theory, the TPMT status of patients can be measured by phenotype (TPMT activity) or genotype. Because of the uncertainty in interpreting the consequences of novel polymorphism detection in different racial groups and the chance of missing clinically relevant allelic variation, genotype testing may be viewed as potentially unreliable. The TPMT genotype can, in theory, predict the 1 in 300 with TPMT deficiency who are at risk of profound myelosuppression when treated at standard AZA doses and the individuals with intermediate TPMT activity (11% of the population) who may experience a greater frequency of drug-induced side-effects. Standard genotyping techniques cannot, as yet, predict those individuals with very high TPMT activities who may not respond to standard AZA doses. Phenotype (activity) testing will quantify the biologically active enzyme and may give a more reliable reflection of in vivo events. A number of different methods for assessing TPMT status are available.

TPMT phenotype is measured in red blood cells (RBCs) using radiochemical [14, 56] or high-performance liquid chromatography (HPLC) assays [57, 58]. Measurement of TPMT activity has become a standard clinical test in some centres [59] but the technique is limited to specialized laboratories. The phenotype assay requires 100 μl RBC lysate and genotyping requires 5-10 μl of DNA, usually obtained from leucocytes. There is concordance between genotype and phenotype in Caucasian populations [18, 45]. The TPMT genotype can be defined using standard techniques, and a number of variant alleles for low populations [18, 45]. The TPMT genotype can be defined using standard techniques, and a number of variant alleles for low TPMT activity have been documented [44, 45, 60]. In addition to the regulation of TPMT activity by SNPs in the open reading frame, a variable-number tandem repeat within the TPMT promoter modulates levels of RBC TPMT enzyme activity [61].

Therapeutic drug monitoring of AZA active metabolites, the active cytotoxic TGNS and the methylmercaptopurine nucleotides (MeMPs; Fig. 1), have been used to identify potential reasons for no response to AZA or drug-related toxicities. Myelosuppression is associated with elevated TGNS and suboptimal therapy with low TGNS. Monitoring both TGNS and MeMPs can identify compliance problems. Those who have low TGNS because of high TPMT activity will have high intracellular MeMPs and those who develop toxicity have high TGNS and low MeMPs. The TPMT-deficient individual lacks MeMPs [32] and the non-compliant patient lacks both sets of metabolites [62].

The available assays for the quantitation of parent drug or intracellular thionucleotide metabolites are based on HPLC technology, and the biological tissues sampled include plasma [63], urine [64], RBCs [65], neutrophil granulocytes and lymphocytes [66]. The major intracellular metabolites of AZA and 6MP, the TGNS and MeMPs, can be monitored by reverse-phase HPLC with UV detection [65, 66], and they are measured in the same assay. A common feature of many published methods is the hydrolysis of the thionucleotide back to the parent thiopurine prior to chromatographic separation. If required, the specific thionucleotide metabolites can be measured with gradient elution from an anion-exchange column [67]. Simple HPLC methods can be used for the measurement of plasma 6MP concentrations and these assays have been used in routine analysis [68, 69]. The major catabolite thiouric acid can also be measured in plasma [70], but the lack of a commercially available thiouric acid standard has perhaps hindered the development of routine assays. The clinical use of thiouric acid monitoring remains unproven, but in theory urine concentrations could be used as a compliance indicator.

Pharmacogenetics and drug efficacy

Genetic variation in TPMT activity may not only influence toxicity but may influence the efficacy of AZA and 6MP. Decreased therapeutic efficacy linked to high TPMT activity was observed originally in children with leukaemia who responded variably to standard-dosage 6MP chemotherapy [17]. Similarly, TPMT activity has been inversely related to renal allograft function in patients receiving AZA immunosuppression after transplantation. Those patients with a better clinical outcome had significantly lower TPMT activities than those with reduced allograft function. However, lower TPMT activity was associated with myelosuppression, severe leucopenia and AZA intolerance being observed in two patients, one of whom lacked and the other had very low TPMT activity [71]. Likewise, in dermatological patients high TPMT activity was associated with a poor clinical response, and a higher incidence of bone marrow suppression was observed in patients with intermediate TPMT activity [72]. In a retrospective evaluation of AZA in severe atopic eczema, TPMT activity was used to identify patients with 'normal' (i.e. high, wild-type) TPMT activity in order to establish therapy at 2.5–3.5 mg/kg and to reduce the frequency of bone marrow and liver function tests [73]. Many recent publications have investigated the efficacy of AZA in IBD patients, and in this patient group pretreatment TPMT evaluation has been used to determine the initial dosage regime [74]. In IBD patients with intermediate TPMT activity, gene mutations correlated with TPMT activity, and this subgroup had an increased risk of AZA toxicity, whilst very high TPMT activities were associated with treatment failure [75]. In IBD patients intolerant of AZA, those who had neutropenia had significantly lower TPMT activities than those who had other toxicities, such as pancreatic, hepatic and dermatological problems [20]. In a study of TPMT status in a group of patients with autoimmune hepatitis, TPMT activities were significantly lower in patients who were intolerant of azathioprine; however, there was discordance between TPMT status and the response to AZA [76]. Some patients with intermediate TPMT activity and variant alleles appeared to tolerate the drug well whilst other patients with wild-type (high activity) enzyme were intolerant of azathioprine.

Clinical implications for rheumatology

Only a few studies of TPMT status [22, 37, 77–80] and none of 6MP metabolite monitoring have been reported in patients with rheumatological disease receiving AZA.

TPMT status testing prior to prescribing AZA

The 1 in 300 of the population deficient in TPMT (homozygotes for the mutant allele) will be at risk of the development of life-threatening bone marrow suppression within days of initiating AZA therapy. Even mutant allele heterozygotes are at risk of leucopenia, possibly necessitating AZA withdrawal within the first month of treatment [77], and prior detection of the deficient TPMT phenotype or mutant allele homozygotes/heterozygotes will identify those at risk and enable clinicians to prescribe a reduced AZA dosage [31]. In one study, dose-related toxicities resulted in AZA discontinuation rates of 10–20% and it was estimated that the number of patients who would need TPMT analysis in order to avoid one serious adverse event over 6 months would be 20 [81]. TPMT status testing prior to taking AZA has been shown to be cost-effective when modelled in a variety of theoretical situations [79, 82, 83]. Analysis of some recent data suggests that by optimizing the maximum AZA dose between 0.75 and 3 mg/kg/day depending on TPMT status testing (with a drastic reduction in dosage for patients homozygous for mutant TPMT alleles), considerable cost savings can be made by avoiding hospitalization and rescue therapy for leucopenic events [81]. One potentially valuable role for TPMT status testing is prior to intravenous AZA loading for prompt control of disease. This strategy has been shown to be safe in Crohn’s disease [84] but is unreported in the treatment of rheumatological disease.
Routine haematological monitoring or not?

There is a logical argument that testing for TPMT status should not be viewed as a reason to change current haematological monitoring for patients on AZA. Even patients with wild-type TPMT are susceptible to myelosuppression on long-term AZA therapy [85-88]. The point is emphasized by the report that TPMT genotyping failed to predict the majority of drug-induced neutropenias in a cohort of patients on long-term AZA therapy for SLE [78]. Autoimmune neutropenia in rheumatological disease is not uncommon [89] and factors such as the disclosure of previously mild, disease-associated but clinically less relevant levels of leucopenia after introduction of AZA, concomitant drug use [90] and ethnic case mix may all be relevant factors in influencing bone marrow suppression in such patients.

TPMT status testing and 6MP metabolite monitoring to predict AZA efficacy

It should be remembered that, in treating rheumatological diseases, the commonest cause for withdrawal of AZA is lack of therapeutic effect [77, 80]. Recommending higher than usual (> 3 mg/kg/day) AZA doses for non-responding patients with RA or SLE who have either high TPMT activity or are homozygous for the wild-type TPMT allele has not been systematically tested. However, the practice may be viable, particularly if combined with the monitoring of 6MP metabolites (TGNs and MeMPS). This approach has proved clinically useful in monitoring AZA treatment of Crohn's disease [86] and in organ transplant patients [66]. The therapeutic window of TGN concentrations for optimal treatment of different rheumatological diseases is unknown [81] and perhaps should be established systematically in specific disease groups.

Concluding remarks

TPMT status testing prior to starting AZA has been investigated in patients with a variety of diseases and has provided a reliable way of predicting life-threatening bone marrow toxicity. A number of studies have shown pretreatment TPMT status testing to be cost-effective, and therefore consideration should be given to the incorporation of TPMT status testing into routine clinical practice. In rheumatological practice there is little evidence that TPMT genotyping failed to predict the majority of drug-induced neutropenias in a cohort of patients on long-term AZA therapy for SLE [78]. Autoimmune neutropenia in rheumatological disease is not uncommon [89] and factors such as the disclosure of previously mild, disease-associated but clinically less relevant levels of leucopenia after introduction of AZA, concomitant drug use [90] and ethnic case mix may all be relevant factors in influencing bone marrow suppression in such patients.

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