LEADING ARTICLE

TPMT in the treatment of Crohn's disease with azathioprine

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Azathioprine induced profound myelosuppression linked to TPMT deficiency has now been documented in many patient groups, including those with Crohn's disease. At the start of azathioprine or mercaptopurine therapy, measurement of TPMT activity has a role in identifying the 1 in 300 patients who are at risk of severe myelosuppression when treated with standard thiopurine dosages. During the initial months of azathioprine therapy a knowledge of TPMT status warns of early bone marrow toxicity. In patients established on azathioprine these is no clear evidence to suggest that TPMT is predictive of clinical response or drug toxicity, indicating a role for TPMT in the prediction of early events rather than long term control. In patients with Crohn's disease on long term azathioprine therapy, it is clear that myelosuppression, particularly leucopenia, is caused by other factors in addition to variable TPMT activity and therefore monitoring of blood cell counts throughout treatment is essential.

THE IMPORTANCE OF TPMT

The first indication that thiopurine methyltransferase (TPMT) deficiency was associated with profound myelosuppression came from observations in adults taking azathioprine as an immunosuppressive agent. Accumulation of grossly elevated concentrations of mercaptopurine derived thioguanine nucleotide (TGN) cytotoxic metabolites[1 2] was linked to a lack of red blood cell TPMT activity.[3] These observations of azathioprine induced profound myelosuppression linked to TPMT deficiency have now been documented in many patient groups and confirmed by numerous reports.[4–7]

HOW AZATHIOPRINE WORKS

Azathioprine has no indigenous immunosuppressive activity; it is a prodrug. The first step in biotransformation is non-enzymic cleavage to form mercaptopurine which in turn undergoes extensive metabolism.[8] Mercaptopurine can be oxidised, methylated, or formed into a variety of active thionucleotide metabolites. It is drug derived thioguanine nucleotide which is eventually incorporated into DNA as a false base.[9]

The thionucleotide metabolites of mercaptopurine compete with their endogenous counterparts in many biochemical pathways. Nucleotides play a variety of important roles in all cells: they are precursors of DNA and RNA, they are essential carriers of energy (for example, ATP and GTP), and they also function as cellular second messengers.[10] It is the importance of these nucleotide dependent processes to functioning and dividing cells that has endowed the thiopurine antimetabolite azathioprine with both immunosuppressive and cytotoxic properties.

THE ROLE OF TPMT IN THE CLINICAL PHARMACOLOGY OF THIOPURINE DRUGS

TPMT methylates azathioprine metabolites at the expense of TGN formation. Both mercaptopurine and mercaptopurine nucleotide (thioinosine monophosphate) are good substrates for TPMT, but TGNs are poor substrates.[11] In the mercaptopurine treatment of childhood leukaemia, TPMT activities show a significant negative correlation to red blood cell TGN concentrations[12] and TPMT deficiency is associated with grossly elevated TGN concentrations and profound myelosuppression.[13] Multivariate analysis has confirmed that higher TGN concentrations[3] and lower TPMT activity[14] tend to be associated with better outcomes. Therefore, in children with lymphoblastic leukaemia, TPMT activity has been shown to reflect mercaptopurine efficacy and toxicity.[12–15]

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During chemotherapy, one reason for mercaptopurine "resistance" is very high TPMT activities; extensive methylation results in suboptimal cytotoxic TGN formation.[12] A second cause of low TGN concentrations is simply not taking the tablets. The two cases of low TGNs can be distinguished by measurement of methylmercaptopurine nucleotide metabolites (products of the TPMT reaction) alongside TGNs.[16] Reports based on therapeutic drug monitoring suggest that 10% of patients fail to take their mercaptopurine or azathioprine tablets.[16 17]

Thiopurine drugs are potential inducers of liver damage and this is reflected in the abnormal liver function tests which are frequently reported in

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Abbreviations: TPMT, thiopurine methyltransferase; TGN, thioguanine nucleotide; IBD, inflammatory bowel disease; MeMPs, methylmercaptopurine nucleotide metabolites; HBI, Harvey Bradshaw index.

children undergoing mercaptopurine or thioguanine long term maintenance chemotherapy for lymphoblastic leukaemia.[18] The exact mechanisms of induced hepatotoxicity are unknown but TPMT produced methylated mercaptopurine metabolites have been implicated in the resultant liver damage[19]; children with higher TPMT activities develop more hepatotoxicity.[15]

TPMT: THE BASICS

TPMT activity in the red blood cell and other human tissues is under the control of a common genetic polymorphism.[20] The frequency distribution of TPMT activity in Caucasian populations is trimodal: approximately 89% of the population have high enzyme activity and are homozygous for the wild-type allele ($TPMT^{H}$), 11% inherit intermediate levels of enzyme activity with one wild-type and one variant allele (heterozygous $TPMT^{H}/TPMT^{L}$), while 1 in 300 subjects have no functional activity (two variant alleles, homozygous $TPMT^{L}$).

A number of variant TPMT alleles have been described,[21] and ethnic differences in the incidence of these variant alleles may be important in the clinical use of thiopurines. *TPMT*3A*, a double mutant, is the most frequently occurring variant allele ($TPMT^{L}$) in white Caucasians but each mutation can occur independently (*TPMT*3B* and *TPMT*3C*). In African-Americans the mutant allele frequency was the same as recorded in Caucasians, but *TPMT*3C* was the most prevalent mutant allele.[22] In a Korean population *TPMT*3C* was the most frequent variant allele, and the *TPMT*3A* allele was absent.[21]

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Variant alleles were only detected in 2.0% of South West Asians (*TPMT*3C*) and 4.7% of Chinese (*TPMT*3C*) compared with 10% of Caucasians (*TPMT*2*, **3A,* and **3C*), indicating that *TPMT*3C* is the oldest mutation and *TPMT*2* the most recent, while variant alleles *TPMT*4* to *TPMT8* are thought to be isolated mutations.[23]

TPMT AND CROHN'S DISEASE

The thiopurine drugs azathioprine and mercaptopurine are well established in the treatment of Crohn's disease.[24] Non-allergic clinical toxicities appear to be dose dependent and may correlate with aspects of azathioprine metabolism.[24] Leucopenia is a frequently reported side effect in patients with inflammatory bowel disease (IBD), and the observations made with respect to the TPMT genetic polymorphism and bone marrow toxicity in childhood leukaemia were initially translated to the treatment of Crohn's disease in 1996 by Cuffari and colleagues.[25] This study investigated mercaptopurine efficacy and toxicity in 25 adolescent patients with Crohn's disease. TPMT activity was investigated indirectly by measurement of the products of the TPMT reaction the methylmercaptopurine nucleotide metabolites (MeMPs). Remission (assessed by a modified Harvey Bradshaw index (HBI)) correlated with TGN concentrations ($p<0.5$) but not MeMPs. Mercaptopurine complications however were "generally associated" with increased MeMPs. However, Cuffari observed that although a lack of clinical response (high HBI) was associated with low TGNs, a satisfactory clinical response (low HBI) was associated with a wide range of red blood cell TGNs. In a larger patient group (n=82), disease remission was shown to correlate with TGNs over 250 pmol/8×10^{8} red blood cells.[26 27] However, MeMPs, potentially an indirect measure of TPMT activity, did not correlate with TGN concentrations.[27] The question now was, can direct measurement of TPMT phenotypic activity or genotype assist clinicians in optimising the therapeutic response to mercaptopurine?

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A dramatic amplification of interest in the TPMT genetic polymorphism arose with the commercial availability of the thiopurine enzyme and metabolite assays.[28 29] This, coupled with a number of institutions capable of "in house" pharmacogenetic analysis, has produced a plethora of abstracts in recent years, but many of these have yet to be translated into peer reviewed articles. Dubinsky and colleagues[28] in a study of 92 paediatric IBD patients, 79 of whom had Crohn's disease, reported that patients heterozygous for TPMT (8/92) had higher TGN concentrations ($p<0.001$) and all responded to therapy. This study confirmed an association between high red blood cell TGNs and clinical response to mercaptopurine. The best probability of patient response was not significantly increased until red blood cell TGN concentrations were >235 pmol. The odds ratio of a therapeutic response for red blood cell TGNs greater than 235 pmol was 5.0 (95% confidence interval 2.6–9.7; $p<001$). The products of the TPMT reaction (the MeMPs) did not correlate with disease activity but patients generating high MeMP concentrations did however experience more hepatotoxicity. Although hepatotoxicity was experienced at low MeMP concentrations, the risk increased threefold at MeMPs above the third quartile (>5700 pmol).

In this study,[28] analysis of TPMT genotypes showed that only one of 13 patients who experienced leucopenia was heterozygous for TPMT. The vast majority (97%) of patients with drug related toxicity had a wild-type TPMT genotype. In addition, the range of mercaptopurine dosages were similar in the heterozygotes and wild-type TPMT genotypes.[28] Subsequently, this group analysed TPMT activities in 51 IBD patients, of whom 35 had Crohn's disease.[29] Again, there was no significant relationship between TPMT activity and thiopurine dosage. In a subgroup of 42 patients, seven had intermediate TPMT activity (that is, presumed heterozygotes) and all were non-responders. In the group as a whole, red blood cell TGNs were positively correlated with therapeutic efficacy and MeMPs with drug related toxicity which, in the majority of cases, was hepatotoxicity.

Colombel and colleagues[30] reported TPMT genotype analysis in 41 Crohn's disease patients. All of this patient group had experienced leucopenia (white cell count $<3.0\times10^{9}$/l) or thrombocytopenia (platelets $<100\times10^{9}$/l) leading to a reduction in thiopurine dose (17% of patients) or withdrawal (83% of patients). Four patients (10%) were TPMT deficient (homozygous variant allele), seven (17%) were heterozygotes, and the reminder had wild-type activity. From the start of thiopurine therapy, the time to bone marrow toxicity was less than 1.5 months in the four TPMT deficient patients, ranged from 1 to 18 months in the heterozygotes, and from 0.5 to 87 months in patients with wild-type TPMT. Variant alleles associated with lack of or lower TPMT activity are over represented in this small study group compared with the general population but it was clear that myelosuppression was caused by other factors in these patients with Crohn's disease.[30]

By far the largest and perhaps the most extensive study on TPMT activities in IBD patients was reported by Lowry and colleagues.[31] Of 170 patients studied, 130 had Crohn's disease and the duration of constant dose azathioprine (n=115) or mercaptopurine (n=55) therapy ranged from 3.5 to 102 months. All patients had responded to, and were tolerant of, thiopurines. Patients with intermediate TPMT activity (n=23, 13.5%) had significantly higher red blood cell TGN concentrations, but for the group as a whole there was no difference in red blood cell TGNs between those in clinical remission

(n=114, 67%) and those with active disease. In Lowry's report,[31] active disease was recorded in 33% of those with wild-type homozygous "normal" TPMT activity and 33% of patients with a heterozygous intermediate TPMT activity. Neither pretreatment or on-therapy TPMT activities were associated with leucopenia but the self selected nature of this cohort would have excluded those individuals with early leucopenia.

A recent report by Campbell and colleagues[32] of TPMT activities in 113 IBD patients (61 with Crohn's disease) investigated those who were currently taking azathioprine (n=63), those who had discontinued azathioprine (n=24), and those who had never taken azathioprine (n=26). TPMT activities were similar in all three groups (range 9–41 units; median 25). TPMT activity correlated (r=0.41, p<0.001) with the lowest neutrophil count in the first four months of azathioprine therapy. In the intolerant group, those experiencing neutropenia had significantly lower TPMT activities than those experiencing other toxicities (for example, pancreatitis, hepatitis, dermatological problems). Survival analysis, based on time to first relapse, was performed on a subgroup of patients taking low dose (<2.0 mg/kg) azathioprine. Those individuals with lower TPMT activities (<20 units) had a statistically significant relapse free advantage (log rank χ^2=4.0; p<0.05).[32] In this low dose azathioprine cohort, mean TPMT activities in patients with stable disease (n=20) were significantly lower (19.8 *v* 27.6 units) than those measured in patients who had experienced active disease (n=14).

Similar apparently contradictory results have emerged when assessing the clinical value of TPMT in the context of azathioprine immunosuppression for other patient groups. In the long term treatment of systemic lupus erythematosus with azathioprine, TPMT genotyping failed to predict the majority of thiopurine induced neutropenias.[33] In contrast, in rheumatic disease, TPMT genotype predicted therapy limiting toxicity induced by azathioprine, five of the six patients with variant alleles experienced leucopenia within one month of starting thiopurines. Within two months of starting azathioprine, seven patients had abnormal liver function tests. Six of the seven had wild-type TPMT alleles.[34] Taken together, the results of all of the studies detailed above indicate a potential role for TPMT in the prediction of early events rather than long term control.

DRUG-DRUG INTERACTIONS

The clinician should be aware that a number of compounds, which could be coadministered with azathioprine or mercaptopurine, may potentially influence TPMT activity. After a therapeutic dose of aspirin, plasma concentrations of salicylic acid are within the range for TPMT inhibition[35] and the loop diuretic frusemide inhibits TPMT at concentrations within the therapeutic range for frusemide.[36]

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Balsalazide, olsalazine, sulphasalazine, and 5-aminosalicylic acid are potent inhibitors of TPMT in vitro.[37 38] In a large long term clinical study of IBD, mercaptopurine was withdrawn in 10% of patients because of the occurrence of adverse reactions. Over half of the patients in that study were treated simultaneously with mercaptopurine and sulphasalazine.[39] A possible drug-drug interaction, linked to TPMT inhibition, was reported in a patient with refractory Crohn's disease who developed bone marrow suppression while receiving daily mercaptopurine and olsalazine.[40]

In the report of Dubinsky and colleagues,[28] 48 patients (52%) received concomitant mesalamine therapy but coadministration of mesalamine was reported not to influence TGN or MeMP concentrations. This was later confirmed in a subsequent study reporting TPMT activities in 51 IBD patients (35 with Crohn's disease).[29] Concurrent mesalamine did not influence TPMT activity or mercaptopurine derived metabolite concentrations. This report is in contrast with the findings of Lowry and colleagues[41] who compared red blood cell TGNs in the same patient prior to and during coadministration of 5' aminosalicylic acids. Mesalamine, balsalazide, and sulphasalazine all produced increased red blood cell TGNs in the 29 Crohn's disease patients studied. This was taken to indicate the possibility of in vivo TPMT inhibition.[41] Inhibition of an enzyme in vivo will not necessarily be reflected by lower TPMT activities because TPMT is measured in vitro in the absence of the inhibitor.

Patients receiving concurrent mesalamine, sulphasalazine, or olsalazine had significantly lower median white cell counts compared with patients not taking aminosalicylates.[31] In addition to the possible myelosuppressive influence of increased TGN concentrations, there are other factors that can influence leucocyte count in these patients. An additional variable in the occurrence of leucopenia is the ability of the individual to metabolise aminosalicylates by acetylation. Variations in the ability to form the major metabolite N-acetyl 5'aminosalicycylic acid (that is, "slow acetylator" status) have been linked to leucopenia. This may also contribute to the myelosuppression observed with sulphasalazine.[41 42]

CONCLUSIONS

When reflecting on TPMT activity in the treatment of Crohn's disease with azathioprine, it is clear that one is dealing with a mixed cohort of patients receiving a wide variety of thiopurine dosages supplemented to various degrees by other immunosuppressive agents.[24] At the start of azathioprine or mercaptopurine therapy, measurement of TPMT activity has a role in identifying the 1 in 300 patients who are at risk of severe myelosuppression when treated with standard thiopurine dosages. In addition, identification of the heterozygote intermediate TPMT individual identifies those prone to early leucopenic episodes. During the initial four months of thiopurine therapy, lower TPMT activities correlate with low neutrophil counts.[32] Thus a knowledge of TPMT status warns of early bone marrow toxicity. Indications are that identification of the heterozygote would indicate those patients who could be safely managed on lower (<2.0 mg/kg) azathioprine dosages.[32] We await a formal analysis of TPMT activities with respect to the frequency of active disease recurrence in those patients on long term thiopurine immunosuppression.

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In patients established on azathioprine, TPMT was not predictive of clinical response or drug toxicity.[31] These observations are perhaps due in part to the self selected nature of this group of patients in whom early events had already occurred. Thus during long term thiopurine therapy, it appears that by careful titration of dosages to clinical response by frequent monitoring of blood cell counts and liver function tests the TPMT genetic polymorphism could have been circumvented. As yet, there is no evidence to suggest a specific role for TPMT in the management of patients already established on azathioprine/mercaptopurine immunosuppression. However, taken together the results of the studies detailed in this article indicate a role for TPMT in the prediction of early events rather than long term control.

In the patient with Crohn's disease on long term azathioprine therapy it is clear that myelosuppression, particularly leucopenia, is caused by other factors in addition to variable TPMT activity and therefore monitoring of blood cell counts throughout treatment remains essential.[15]

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