Optimizing therapy with 6-mercaptopurine and azathioprine: to measure or not to measure?

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Ther Adv Gastroenterol
(2010) 3(5) 275–279
DOI: 10.1177/1756283X10376121

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Inflammatory bowel disease (IBD) is a broad term that encompasses a heterogeneous group of inflammatory conditions of the small and large bowel but generally refers to ulcerative colitis (UC) and Crohn's disease (CD). These conditions are believed to occur when, with the appropriate genetic predisposition, gut flora activate an exaggerated immune response. The resultant inflammation leads to myriad symptoms, a possible need for surgery, an increased risk of cancer, and a decreased quality of life, to name just a few.

Given the role that the immune system plays in these diseases, the hallmark of therapy is immune modulation (except for mild-to-moderate ulcerative colitis, in which topical anti-inflammatory therapy is usually adequate). There are many pharmacologic agents that have been proven to be effective in IBD, including corticosteroids (short term), methotrexate, anti-tumor necrosis factor (anti-TNF) α agents, cyclosporine, and emerging agents that block novel targets in the inflammatory cascade (e.g. anti-integrin and anti-interleukin-12/23 therapy). One of the oldest agents shown to be an effective steroid-sparing immunomodulator in both CD and UC was azathioprine (and its metabolite, 6-mercaptopurine, henceforth collectively referred to as AZA/6-MP). Based on the Nobel Prize-winning work of Elion and Hitchings, who demonstrated differences in nucleic acid metabolism in normal versus cancer cells [Hitchings *et al.* 1950], purine analogs were originally developed to intercalate during DNA synthesis and halt cell division. Used as chemotherapy for leukemia in the early 1950s [Burchenal *et al.* 1953], AZA/6-MP inhibits DNA synthesis due to its structural similarity to purines, thereby disrupting leukocyte proliferation. Owing to its broad T-cell effects, AZA/6-MP is effective in blunting autoimmune responses in a variety of diseases, as well as preventing allograft rejection in the setting of transplantation [Elion and Hutchings, 1975]. More recently, studies have demonstrated that 6-thioguanine triphosphate (6-TGTP) inhibits Rac1 required for T-cell stimulation, resulting in T-cell apoptosis [Tiede *et al.* 2003]. Kirsner and colleagues at the University of Chicago were amongst the first to show its efficacy in UC nearly 45 years ago [Bowen *et al.* 1966], and it was subsequently shown to be beneficial in CD as well [Present *et al.* 1980; Willoughby *et al.* 1971].

Unfortunately, the metabolites of AZA/6-MP also predispose to adverse events. Bone marrow toxicity has been described in the earliest reports of AZA/6-MP use in leukemia and IBD; in fact, Kirsner and colleagues' original case series in UC urged caution with its use due to leukopenia [Bowen *et al.* 1966]. Over the years, we have come to appreciate two types of side effects from AZA/6-MP: idiosyncratic side effects such as pancreatitis and fever, and dose-dependent effects such as hepatotoxicity and bone marrow toxicity. If one could predict the likelihood of development of the dose-dependent adverse events, these could be significantly diminished. In addition, data suggest that metabolite levels can predict efficacy [Dubinsky *et al.* 2000]; to that end, knowing the metabolite levels can help optimize therapy by providing a surrogate marker prior to the development of therapeutic efficacy.

Recent advances now allow us to address both of these issues. We can measure thiopurine

methyltransferase (TPMT) enzyme activity or genetic polymorphisms to predict leukopenia and bone marrow toxicity, and we can check metabolite levels in patients on AZA/6-MP therapy to both assess response and clarify toxicity. But what should we measure, and when? To answer this question, it is first important to understand the metabolic pathway of azathioprine.

The oral form of azathioprine is quickly and nonenzymatically cleaved to 6-MP. At that point, there are three competing pathways that can be taken, based on enzyme activity levels. Most of the 6-MP (about 85%) is catabolically converted into the inactive 6-thiouric acid (6-TU) by xanthine oxidase (XO); the remaining 6-MP can be catabolically converted into an inactive 6-methyl-mercaptopurine (6-MMP) by TPMT or anabolically converted into 6-thioinosine 5'-monophosphate (6-TiMP) by hypoxanthine phosphoribosyltransferase (HPRT). Two more enzymatic reactions then cleave 6-TiMP to yield the active 6-thioguanine nucleotides (6-TGN); alternatively, 6-TiMP can be converted by TPMT to the active 6-MMP ribonucleotides (6-MMPR) [Lennard, 1992].

In 1980, pharmacogenetic studies showed the genetic variation in TPMT activity; 1 in 300 (0.3%) people are homozygous for low enzyme activity, 11% are heterozygous, and the remaining 89% are homozygous for high activity [Weinshilboum and Sladek, 1980]. It was later discovered that low TPMT levels correlate with higher 6-TGN levels [Lennard *et al.* 1987]. The same authors had previously shown that 6-TGN levels are inversely correlated with leukopenia [Lennard *et al.* 1983]. These findings have allowed us to apply this data clinically to help predict those patients at greatest risk for leukopenia (patients with low or absent TPMT enzyme activity). Conversely, since increased levels of 6-TGN are also associated with therapeutic efficacy [Dubinsky *et al.* 2000], those with intermediate TPMT enzyme activity are at the greatest likelihood to benefit from AZA/6-MP [Cuffari *et al.* 2004]. One could then also reasonably extrapolate that those with normal enzyme activity may need to use higher doses of AZA/6-MP to gain a clinical response; to that end, knowing the TPMT status allows physicians to start at higher doses of AZA/6-MP in those with normal enzyme activity, decreasing the time patients are on steroids and not in remission (by avoiding the 'start low–go slow' technique) [Dubinsky *et al.* 2005]. Therefore, it seems reasonable to check TPMT enzyme activity in all patients prior to initiating AZA/6-MP therapy. Two cost-effectiveness studies, albeit with different presumptions and endpoints, have shown the superiority of TPMT testing to standard dosing of AZA/6-MP [Dubinsky *et al.* 2005; Winter *et al.* 2004]. Lewis and colleagues showed that the rate of severe myelosuppression decreases after the first 8 weeks of therapy, thereby likely decreasing the need for intensive complete blood count (CBC) monitoring [Lewis *et al.* 2009]; since checking TPMT best predicts early leukopenia, the intensity with which blood counts are checked can be decreased with TPMT evaluation [Lennard *et al.* 1987]. In a study looking at the relationship between severe leukopenia and TPMT genotype, patients with TPMT mutations had early leukopenia [Colombel *et al.* 2000]. Unfortunately, neither genetic testing nor enzymatic activity predicts late leukopenia; intercurrent viral infections may explain some of these [van Asseldonk *et al.* 2009].

So which should be checked, the genotype looking for mutant TPMT alleles or the phenotype evaluating enzyme activity? Data from a UK study in 2007 suggest that measurement of enzyme activity is a better predictor of significant myelosuppression than genotype assessment [Winter *et al.* 2007]. Since TPMT phenotyping measures erythrocyte concentrations of enzyme activity, perhaps genotyping would be preferred in patients with a recent blood transfusion.

But what about when a patient is already on AZA/6-MP therapy? Can we take advantage of our understanding of AZA/6-MP metabolism to improve patient outcomes? As noted above, Dubinsky and colleagues showed that higher 6-TGN levels are associated with higher therapeutic efficacy; neither the dose of 6-MP (in mg/kg/day) nor the 6-MMPR level had such a correlation [Dubinksy *et al.* 2000]. A 6-TGN >235 pmol/8×10^8 red blood cells (RBC) corresponded to a statistically significantly higher likelihood of clinical remission. A subsequent meta-analysis of 43 studies demonstrated a strong correlation between 6-TGN >230–260 pmol/8×10^8 RBC and clinical remission (pooled odds ratio 3.27) [Osterman *et al.* 2006].

Metabolite monitoring can also be used to detect the risk of adverse events. In the same 2000 Dubinsky and colleagues study, hepatotoxicity increased threefold with a 6-MMPR >5700

Table 1. Proposed diagnostic algorithm for checking thiopurine methyltransferase (TPMT) and metabolite levels.

(1) Considering use of AZA → check TPMT enzyme activity level:
   a. if low → consider alternative strategy
   b. if intermediate → start at 1 mg/kg 6-MP or 2 mg/kg AZA, still monitor CBCs closely in first 2 months (e.g. weeks 3, 6, 9), then less often after that if OK
   c. if high → start 6-MP or AZA at higher dose, still monitor CBCs as above

(2) After starting treatment, check metabolites 3 weeks later (along with CBC and liver chemistries):
   a. 6-TG OK, 6-MMP OK → same dose of AZA/6-MP
   b. 6-TG low, 6-MMP OK or 6-MMP/6-TG ratio <10–15 → increase dose of AZA/6-MP
   c. 6-TG low, 6-MMP high or 6-MMP/6-TG ratio >15 → consider changing to methotrexate or anti-TNF α therapy
   d. 6-TG OK, 6-MMP high → same dose of AZA/6-MP unless elevated AST/ALT; if elevated AST/ALT, consider changing to anti-TNF α therapy
   e. 6-TG low, 6-MMP low → nonadherence, re-educate patient and continue same dose of AZA/6-MP

TPMT, thiopurine methyltransferase; AST/ALT, aspartate aminotransferase/alanine aminotransferase; AZA, azathioprine; CBC, complete blood count; 6-MP, 6-mercaptopurine; 6-MMP, 6-methyl-mercaptopurine; 6-TG, 6-thioguanine; anti-TNF, anti-tumor necrosis factor.

pmol/8×10^8 RBC, and there was no relationship between hepatotoxicity and the 6-MP dose or the 6-TGN level; leukopenia, which is known to be associated with higher 6-TGN levels, was not associated with higher levels of 6-MMPR or 6-MP dose. Nonetheless, hepatotoxicity is not exclusively dependent on 6-MMPR levels. Nearly 90% of patients with a 6-MMPR >5300 pmol/8×10^8 RBC had no hepatotoxicity, so dose reduction should be reserved for those who have elevated aminotransferases [Shaye *et al.* 2007].

Another way to interpret metabolite data is to use the ratio of metabolites to suggest the predominant metabolic pathway in a particular patient. For example, if the 6-MMPR is far greater than the 6-TGN, it suggests that in that individual, TPMT is the predominant enzymatic pathway; meanwhile, if the ratio of 6-MMPR to 6-TGN is smaller, that individual has less TPMT activity. In a study of patients not responding to AZA/6-MP therapy, the group who responded to dose escalation had a 6-MMPR/6-TGN ratio of 2.5 before dose escalation and a ratio of 9.1 afterwards; in the nonresponders, the ratio went from 18 to 66, pointing to the exaggerated production of 6-MMPR in patients with high TPMT activity [Dubinsky *et al.* 2002]. So perhaps those with very high 6-MMPR/6-TGN ratios (correlating with high TPMT enzyme activity) are more likely to reach toxic 6-MMPR levels before achieving therapeutic levels of 6-TGN; these are the patients in whom alternative therapies (e.g. anti-TNF agents or methotrexate) should be considered when not responding to AZA/6-MP therapy. On the other hand, for those with lower ratios (<10–20), dose escalation should allow for augmentation of 6-TGN levels and therefore a greater chance at clinical remission without increased risk of hepatotoxicity.

Finally, metabolite monitoring has allowed some to devise novel therapeutic strategies. Investigators at the University of Chicago found that in those who preferentially metabolize to 6-MMPR, the addition of a XO inhibitor could optimize 6-TGN and reduce 6-MMPR; the addition of allopurinol in these patients (with concurrent dose reduction of the AZA/6-MP for safety purposes) led to a statistically significant increase in 6-TGN levels and a steroid-tapering effect [Sparrow *et al.* 2007, 2005]. This has given us yet another way to optimize the utility of the thiopurines before condemning them to failure.

In conclusion, advances in our understanding of the metabolism of azathioprine and the ability to measure enzyme and metabolite levels has allowed us to risk-stratify patients and determine the likelihood of response to therapy and need for more aggressive dosing strategies even before therapy (see Table 1). At our institution, we routinely check TPMT enzyme activity levels on all patients in whom thiopurine therapy is to be initiated; this helps us to not only optimize dosing for safety and efficacy, but it also helps us to predict the response, a tool we use in our risk/benefit discussion with patients. While on therapy, we can assess appropriate dosing for clinical efficacy, the potential for adverse events, and also the probability of success with dose escalation.

The metabolites can either be checked routinely after a few weeks of therapy to modify the dose (as some at our institution do) or in those who are failing treatment after 3 months, when full therapeutic potential is expected (as others in our center do). Nonetheless, many significant questions remain unanswered. The cost-effectiveness data has been heterogeneous, with differing assumptions of risk and calculations of complications. And perhaps more importantly, we still do not know if measuring TPMT or metabolites improves long-term clinical outcomes, both of efficacy and safety. Until these issues are more definitively addressed, heterogeneity in the utility of these tests will remain.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Conflict of interest statement

Dr Abreu is a consultant for Prometheus, a company that does metabolite measurements. Dr Deshpande has no relevant conflicts of interest.

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