Thiopurine toxicity

Introduction

Thiopurines are a class of immunosuppressant and antineoplastic drugs developed by Gertrude Elion and George Hitchings and patented in 1953 for use to treat leukaemia, then used to prevent transplant rejection (Marx, 2005). Thiopurines are used in dermatology, rheumatology and to treat inflammatory bowel disease. Their use in transplantation has been succeeded by mycophenolate mofetil. The thiopurine family comprises azathioprine, mercaptopurine and thioguanine.

Thiopurines have limitations as therapeutic agents. Individual variance in efficacy impairs outcomes and side effects contribute to non-adherence. Tailored dosing and monitoring have helped to some degree in reducing toxicity while maintaining efficacy.

Mechanism of action

Azathioprine is a prodrug for mercaptopurine and there is little difference between their toxicity profiles (Gisbert et al, 2007; Gisbert and Gomollón, 2008). Azathioprine is used in autoimmune and chronic inflammatory diseases; mercaptopurine in lymphoid malignancies. There is some evidence that mercaptopurine is better tolerated than azathioprine.

Thioguanine bypasses much of the metabolism of azathioprine or mercaptopurine and patients can therefore tolerate higher concentrations of thioguanine nucleotide (the main immunosuppressive metabolite of thiopurines – *Figure 1*) (Relling et al, 2011). However, concerns over its safety profile, particularly hepatoxicity, have so far limited its use to treatment of childhood leukaemia (Gisbert et al, 2007).

Levels of thioguanine nucleotide are partly determined by the activity of the enzyme thiopurine methyltransferase (TPMT). Low levels of TPMT activity mean mercaptopurine is preferentially converted to thioguanine nucleotide, enhancing immunosuppression and increasing the risk of toxicity. TPMT activity is a monogenic co-dominant trait, with three single-nucleotide polymorphs accounting for over 90% of variant alleles. Between 3 and 14% of the population are heterozygous, and up to 0.56% are homozygote. In homozygous or compound heterozygote deficiency, TPMT activity is very low, effectively zero (Relling et al, 2011).

Types of thiopurine toxicity

Allergic reactions are very rare. Idiosyncratic hypersensitivity reactions are rare but important to consider, as they can mimic systemic sepsis or disease recurrence, especially in patients with inflammatory bowel disease. Patients typically present with rash, fever, myalgia, arthralgia and nausea, or occasionally with hepatitis or interstitial nephritis (Gisbert and Gomollón, 2008). Very rarely these idiosyncratic reactions can lead to shock, pneumonitis and pancreatitis (Meggitt et al, 2011).

Adverse reactions may be dose related, although underlying mechanisms are not fully understood and are likely multifactorial. These include nausea, hepatotoxicity and myelosuppression. TPMT phenotyping and/or genotyping and therapeutic drug monitoring can limit toxicity.

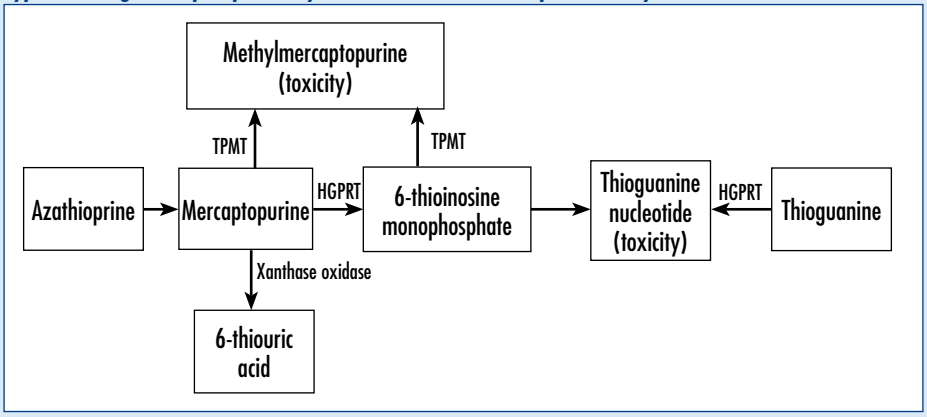
Nausea

Nausea is the most common reason to stop thiopurines. It is usually isolated and dose-dependent. The imidazole moiety of azathioprine is implicated in nausea. Switching to mercaptopurine, which lacks this moiety, can often circumvent this side effect. TPMT phenotype also plays a role; one study showed 17% with intermediate TPMT activity developed nausea compared to 8% of those with normal activity (Gisbert et al, 2006b). Nausea can be managed with dose reduction. Lower drop-out rates occur if the starting dose is low and increased, compared with fixed dosing. Co-prescribing an antiemetic and taking at night, with food, are recommended. Although usually isolated, clinicians should be alert to the possibility that nausea can form part of a hypersensitivity reaction (Meggitt et al, 2011).

Myelotoxicity

Myelosuppression affects 3% of patients per year of treatment, with an overall risk

Figure 1. Mechanism of action of thiopurines. Azathioprine converts rapidly to mercaptopurine, and then mercaptopurine is metabolized to thioguanine nucleotide via the salvage pathway for purines. Mercaptopurine is also metabolized by competing enzymes. It is oxidised by xanthase oxidase in the liver and intestinal mucosa to produce 6-thiouric acid. Mercaptopurine is also a substrate for thiopurine methyltransferase to produce the metabolite methylmercaptopurine. Similarly, thioguanine is metabolized to thioguanine nucleotide and to other inactive metabolites (van Asseldonk et al, 2011). HGPRT = hypoxanthineguanine phosphoribosyltransferase; TPMT = thiopurine methyltransferase.



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of death 0.94%. While mild leucopenia may spontaneously resolve, if leucocytes fall below 3–4x10^9/litre, or neutrophils below 1.5x10^9/litre, a dose reduction is indicated (Gisbert and Gomollón, 2008).

Those with negligible TPMT activity (homozygote variant TPMT) are at increased risk of myelosuppression. A meta-analysis of 67 studies showed 86% of these patients developed myelosuppression (Higgs et al, 2010), while other studies have put this figure at 100% (Relling et al, 2011).

In those with intermediate TPMT activity, a prospective study of 394 inflammatory bowel disease patients found the risk of myelosuppression was 14.3% compared to 3.5% of patients with normal activity (Gisbert et al, 2006b). Other studies of intermediates have found no evidence of increased risk (Newman et al, 2011).

Toxicity cannot entirely be predicted by TPMT phenotype. Myelosuppression can also occur at normal TPMT activity (Gisbert et al, 2006a); up to half of patients with normal TPMT developing leukopenia. Other factors implicated include polymorphisms in ITPase and other enzymes, parvovirus and other intercurrent illness, imidazole sensitivity, and concurrent use of aminosalicylates, allopurinol, co-trimoxazole and diuretics (Duley and Florin, 2005).

Although the desired therapeutic effect of thiopurines, immunosuppression also confers indirect toxicity through increasing the risk of opportunistic infections. These are mainly viral infections, including herpes zoster and simplex, Epstein–Barr virus and cytomegalovirus, although bacterial infection can occur (Rahier et al, 2009).

Hepatotoxicity

Hepatotoxicity is common, with a mean annual incidence of 1.4% in patients with inflammatory bowel disease. Researchers have found difficulty in attributing hepatotoxicity specifically to thiopurine therapy, but it can generally be split into four groups (Gisbert et al, 2007; Meggitt et al, 2011):

1. Idiosyncratic liver injury. Abnormal liver function tests are seen in 10% after the first few months to 1 year, but usually resolve spontaneously. This can be cholestatic (occasionally progressive despite withdrawal) or hepatocellular
2. Nodular regenerative hyperplasia. This is seen after 10 years of use, mainly in patients with inflammatory bowel disease
3. Hepatomegaly
4. Portal hypertension.

TPMT phenotype is a factor influencing the risk of developing hepatotoxicity:

- High TPMT levels correlate with high methylmercaptopurine concentrations, which are believed to contribute to idiosyncratic liver injury
- Co-prescribing allopurinol can decrease the risk of hepatotoxicity of thiopurines caused by high methylmercaptopurine levels. As a xanthine oxidase inhibitor, allopurinol allows mercaptopurine to be preferentially converted to thioguanine nucleotide, rather than methylmercaptopurine. By reducing the dose of thiopurines to 25–50% and adding allopurinol 100 mg once a day, it is possible to maintain thioguanine nucleotide levels and therefore drug efficacy while decreasing methylmercaptopurine levels (Gisbert et al, 2007).
- Conversely, high thioguanine nucleotide levels (low TPMT activity) may correlate with nodular regenerative hyperplasia (Dubinsky et al, 2003).

Risk of malignancy

Malignancy poses additional risk to patients taking thiopurines. Immunosuppression is associated with an increased risk of malignancy. The risks seen with azathioprine are documented from transplant patient and inflammatory bowel disease cohorts: non-melanoma skin cancer and lymphoma being most common, and possibly cervical cancer. These malignancies appear more aggressive in nature in these cohorts.

Non-melanoma skin cancer

Non-melanoma skin cancer occurs predominantly in solid organ transplant patients taking thiopurines (Maddox and Soltani, 2008). Most of the non-melanoma skin cancer risk associated with sun exposure in transplant patients is ultraviolet B-related. However, thiopurines confer a specific sensitivity to ultraviolet A radiation, through the action of reactive oxygen species. Mutagenesis also involves incorporation of rogue thiopurine nucleotides into DNA. Some studies have linked cumulative dose of thiopurines with cancer risk, which appears to return to baseline after stopping (Smith et al, 2010).

Limiting the duration of medicating, protecting against excess sun exposure (ultraviolet A and B), and vaccinating against infection with human papillomavirus can reduce these risks.

Lymphoproliferative disorders

Lymphoma tends to occur in patients with inflammatory bowel disease. Data from the CESAME cohort show that patients receiving thiopurines were 5.28 times more likely to develop lymphoproliferative disorders than those who had never received the drug (Beaugerie et al, 2009). However, several studies convincingly show the benefits in this cohort outweigh the risk (Kwon and Farrell, 2005), and the absolute incidence is low.

Predicting toxicity and tailored dosing

Individualizing the risk profile enables tailored dosing to minimize toxicity. Patient selection is the first important step: elderly patients, those with a history of malignancy or concurrent immunosuppressant use may prohibit thiopurines. The areas of tailored dosing are discussed below.

Pre-administration testing

Pre-testing of TPMT activity is fundamental in identifying patients at risk of myelosuppression. A survey in 2006 found that only 60% of gastroenterologists and 47% of rheumatologists routinely test for either, despite apparently being cost-neutral (Meggitt et al, 2011).

Pre-testing of TPMT can be done by assessing genotype to identify homozygous variant type and heterozygotes, or by directly measuring TPMT phenotype (enzyme activity). It is now recommended that both methods be used (Relling et al, 2013). Those with negligible TPMT activity or homozygous variant type should not receive thiopurines. Heterozygotes should receive reduced doses (*Figure 2*).

Other recommendations for pre-administration testing include baseline full blood count, renal profile and liver function tests. The European Crohn's and Colitis Organisation recommends thorough work-up to prevent opportunistic infections before starting any immunomodulator therapy (*Table 1*) (Rahier et al, 2009).

Blood monitoring

Although there is no clear evidence base for the schedule of blood monitoring, *Table 2* suggests a strategy (Lichtenstein, 2004).

Metabolite monitoring

The level of thioguanine nucleotide in red blood cells can be used to determine the adequacy of thiopurine dose.

- Levels of thioguanine nucleotide from 250–400 pmol/8x10^8 red blood cells tend to correlate with a good clinical response (Dubinksy et al, 2000; Cuffari et al, 2001)
- Levels below 250 pmol/8x10^8 red blood cells tend to correlate with an inadequate clinical response. This can reflect one of three scenarios: non-compliance; subtherapeutic dose, in which case dose can be increased by 25%, providing white cell count is normal; or preferential metabolism to methylmercaptopurine (Goldenberg et al, 2004)
- Above thioguanine nucleotide levels of 400 pmol/8x10^8 red blood cells, myelosuppression may occur.

Methylmercaptopurine is also measured in patients receiving thiopurines.

- Patients with high methylmercaptopurine (above 5000 pmol/8x10^8 red blood cells) are more likely to develop hepatotoxicity (van Asseldonk et al, 2011)
- Measuring thioguanine nucleotide and methylmercaptopurine in combination can help determine therapeutic strategies (*Figure 2*).

There are several limitations of metabolites as monitoring tools, such as variability in levels of thioguanine nucleotide within the same patient (Duley and Florin, 2005). There are also concerns that red blood cell thioguanine nucleotide levels are not a reliable surrogate for leucocyte thioguanine nucleotide levels (Duley and Florin, 2005). Mean corpuscular volume has been put forward as an independent correlator of red blood cell thioguanine nucleotide levels, although currently sensitivity and specificity remain limited (van Asseldonk et al, 2011).

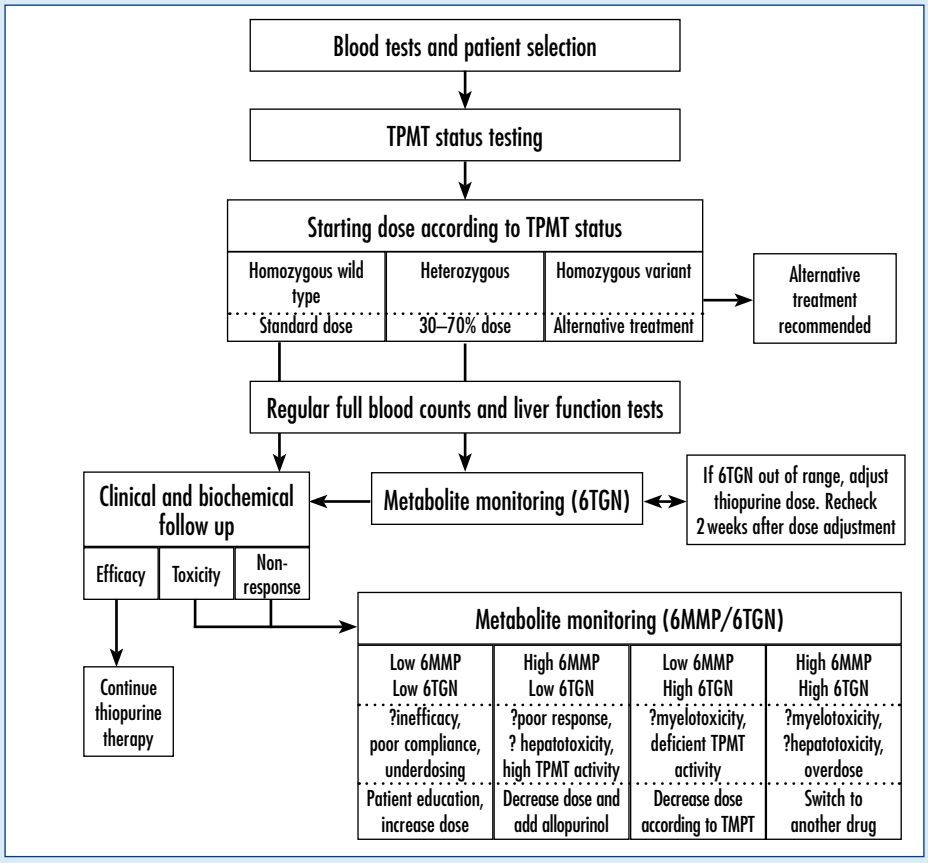

Figure 2. Therapeutic algorithm for thiopurine therapy management. From Chouchana et al (2012). MMP = methylmercaptopurine; 6TGN = thioguanine nucleotide; 6TPMT= thiopurine methyltransferase.

Conclusions

Toxicity with thiopurine use is usually manifested as nausea, myelosuppression and idiosyncratic drug reactions which can mimic a wide range of acute presentations or be limited to hepatotoxicity. Malignancy and opportunistic infections are a known association of azathioprine use.

Tailored dosing is recommended. TPMT levels are recommended in all patients and are cost-neutral. Thioguanine nucleotide monitoring is recommended. If levels are substantially above 200–400 pmol/8x10^8 red blood cells thiopurine dose should be reduced. Methylmercaptopurine can be measured to monitor for toxicity.

More research is needed into mechanisms of malignancy and the potential for use of mean corpuscular volumes or other biomarkers to monitor myelosuppression. **BJHM**

Conflict of interest: none.

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TOP TIPS

- Test for thiopurine methyltransferase status before deciding to start any patient on thiopurines.
- Work patients up thoroughly before commencing treatment. This should include immunisations, screening for infections and baseline blood tests.
- Regular monitoring is vital. Full blood count and liver function tests should be weekly for the first 6–8 weeks, and then 3-monthly. Metabolites should be monitored, aiming for thioguanine nucleotide levels between 200 and 400 pmol/8x10^8 red blood cells.
- Monitor patients for signs and symptoms of toxicity, e.g. nausea, bleeding, infections and signs of liver injury.
- Tailor thiopurine dose according to metabolite levels, biochemical and clinical signs of toxicity, and efficacy of treatment.

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KEY POINTS

- Thiopurines are a class of immunosuppressant and antineoplastic drugs, comprising azathioprine, mercaptopurine and thioguanine, which are used in dermatology, rheumatology and inflammatory bowel disease.
- Thiopurines have a variety of idiosyncratic and dose-related toxic effects, and are also associated with malignancy and opportunistic infections.
- The risk of some forms of toxicity can be decreased through tailored dosing. This involves checking thiopurine methyltransferase status, thorough patient work-up and regular monitoring.

Table 1. Suggested work-up before starting thiopurines

Medical history	Bacterial infections, for example urinary tract infections
	Fungal infections
	Tuberculosis risk (latent/active): contacts, travel history, treatment
	Date of BCG (bacillus Calmette–Guérin) vaccination
	History of herpes simplex viral infection or varicella-zoster (chicken pox or shingles)
	Immunization status for hepatitis B
	Future travel plans
Physical examination	Local or systemic signs of infection
	Cervical smear
Laboratory tests	Neutrophil and lymphocyte cell count, C-reactive protein
	Urinalysis in patients with prior history of urinary tract infections or urinary symptoms
	Varicella zoster virus serology if no previous immunization
	Hepatitis B or human immunodeficiency virus (HIV) serology
	Eosinophil cell count, stool examination and strongyloidiasis serology (for returning travellers)
Tuberculosis screening	Risk stratification (see medical history)
	Chest radiograph
	Tuberculin skin test or interferon gamma release assay
Vaccination (immunization status checked and vaccination given if necessary)	Tetanus, diphtheria, poliomyelitis
	Varicella zoster virus
	Human papilloma virus
	Influenza (trivalent inactivated vaccine)
	Pneumococcal polysaccharide vaccine
	Hepatitis B vaccine in all hepatitis B seronegative patients

Table 2. Recommended blood monitoring schedule

Test	Frequency	Comment
Full blood count	Weekly or fortnightly for the first 6–8 weeks, and after dose change, then 3-monthly. If hepatic or renal impairment is present, more frequent full blood count measurement is recommended	If white cell count is <4.0/mm^3, stop or reduce dose by 50%
Liver function tests	Weekly or fortnightly for the first 6–8 weeks, and after dose change, then 3-monthly	Any deviation from normal requires further monitoring or action