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Clinical Case Study

Poor Response to Thiopurine in Inflammatory Bowel Disease: How to Overcome Therapeutic Resistance?

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CASE DESCRIPTION

A 24-year-old woman (53 kg) with a 5-year history of steroid-dependent ulcerative colitis with mild and extensive ulcerations presented to the gastroenterology clinic for symptom recurrence. She was given 100 mg/day (1.9 $mg \cdot kg^{-1} \cdot day^{-1}$) azathioprine (AZA)[5] for 1 month, after which the dose was increased to 125 mg/day (2.3 $mg \cdot kg^{-1} \cdot day^{-1}$). Four months later, the patient was tapered off steroid therapy. Her symptoms persisted after 7 months of AZA therapy, however, and she experienced gastrointestinal side effects. The patient was switched to another thiopurine drug, 6-mercaptopurine (6-MP), at 75 mg/day (1.4 $mg \cdot kg^{-1} \cdot day^{-1}$), which was well tolerated but similarly ineffective (8 stools daily). A brief course of steroid therapy rapidly produced a substantial but short-lived clinical improvement.

To understand this patient's unresponsiveness to 2 thiopurine agents, we quantified thiopurine metabolites *(1)* 1 year after initiating AZA therapy. Intraerythrocyte concentrations of 6-thioguanine nucleotides (6-TGNs) were low (132 pmol/$8 \cdot 10^8$ erythrocytes; therapeutic interval, 230–400 pmol/$8 \cdot 10^8$ erythrocytes), and 6-methylmercaptopurine ribonucleotides (6-MMPRs) were very high (11 666 pmol/$8 \cdot 10^8$ erythrocytes; therapeutic interval, <5800 pmol/$8 \cdot 10^8$ erythrocytes). A second quantification of thiopurine metabolites 3 months later confirmed these results (6-TGNs, 127 pmol/$8 \cdot 10^8$ erythrocytes; 6-MMPR, 26 304 pmol/$8 \cdot 10^8$ erythrocytes). The patient had an unusual and extremely high thiopurine *S*-methyltransferase (TPMT) activity in erythrocytes [61.5 $nmol \cdot h^{-1} \cdot (mL\ erythrocytes)^{-1}$; reference interval, 8.5–15 $nmol \cdot h^{-1} \cdot (mL\ erythrocytes)^{-1}$]. The lack of clinical efficacy for 6-MP, together with the evidence of pharmacologic resistance, prompted discontinuation of 6-MP therapy. Thereafter, we administered the tumor necrosis factor-α (TNF-α) antagonist adalimumab, but we quickly replaced it with infliximab, which has a good clinical efficacy and safety profile.

QUESTIONS TO CONSIDER

1. What is the clinical utility of assessing the TPMT phenotype or genotype?
2. What is the rationale for therapeutic drug monitoring of thiopurines?
3. What causes of resistance should be considered before switching to another drug class in patients with apparent thiopurine resistance?
4. How can thiopurine treatment be optimized in patients with a very high TPMT activity?

DISCUSSION

The thiopurines AZA and 6-MP are cytotoxic and immunosuppressive drugs that constitute the cornerstone of maintenance therapy for inflammatory bowel disease (IBD). Both drugs cause severe hematologic toxicity (e.g., neutropenia) and hepatotoxicity in up to 10% and 13% of patients, respectively *(2)*. Resistance to thiopurines occurs in about 40% of patients *(3)*.

AZA and 6-MP are inactive molecules that require bioactivation via a complex enzymatic metabolism. After administration, AZA is rapidly transformed to 6-MP, which is then metabolized via 3 competitive enzymatic pathways, 2 of which are catabolic [xanthine oxidase (XO) and TPMT]. The anabolic pathway, via hypoxanthine-guanine phosphoribosyltransferase (HPRT), produces active 6-TGN metabolites (Fig. 1) *(2)*. This metabolism is largely regulated by TPMT, which catalyzes the conversion of 6-MP to 6-methylmercaptopurine (6-MMP) and related

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[5] Nonstandard abbreviations: AZA, azathioprine; 6-MP, 6-mercaptopurine; 6-TGN, 6-thioguanine nucleotide; 6-MMPR, 6-methylmercaptopurine ribonucleotide; TPMT, thiopurine *S*-methyltransferase; TNF-α, tumor necrosis factor-α; IBD, inflammatory bowel disease; XO, xanthine oxidase; HPRT, hypoxanthine-guanine phosphoribosyltransferase; 6-MMP, 6-methylmercaptopurine; 5-ASA, 5-aminosalicylic acid.

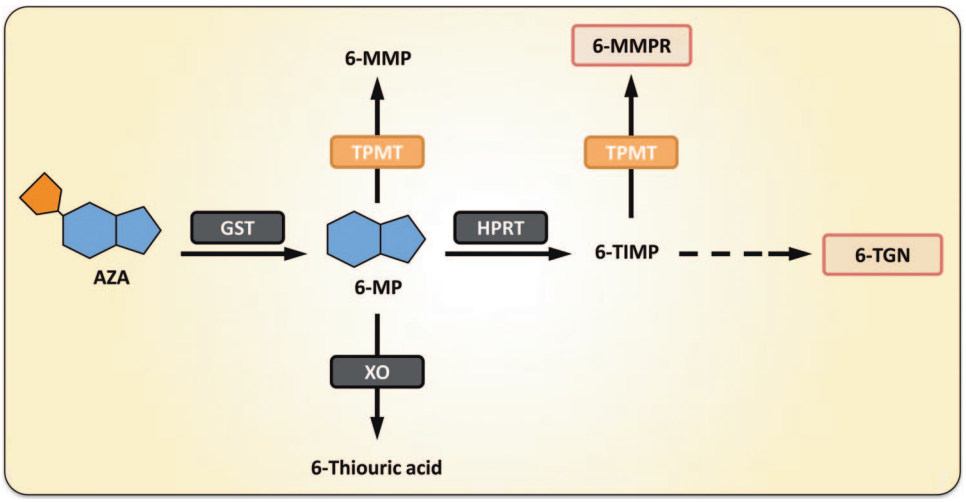

Fig. 1. Summary of thiopurine metabolism.
For a detailed version, see Chouchana et al. *(2)*. GST, glutathione-*S*-transferase; 6-TIMP, 6-thioinosine monophosphate.

6-MMPRs. TPMT activity varies widely across individuals, and this variation is due to genetic polymorphisms in the TPMT gene and in unrelated sequences *(2)*. About 0.03%–0.6% of patients have low TPMT activity (homozygous mutant), approximately 3%–14% have an intermediate activity level (heterozygote), and approximately 86%–97% present a normal or high activity (homozygous wild type), with large variation occurring among ethnic groups *(4)*. Moreover, in approximately 15% of the patients, TPMT activity is very high (over reference interval) compared to patients within the reference interval with normal/high TPMT activity *(2)*.

TPMT activity (or genotype) is negatively correlated with the blood 6-TGN concentration, and a cutoff of approximately 235 pmol/8 · 10^8 erythrocytes has been correlated with a therapeutic response *(2)*. Thus, patients with low or intermediate TPMT activity (homozygous mutant or heterozygotes) are more likely to have toxic 6-TGN concentrations and are therefore at greater risk for neutropenia or even lethal bone marrow suppression, especially in homozygous patients *(2, 3)*. These patients should therefore receive a lower thiopurine dosage. Conversely, patients with a very high TPMT activity have low 6-TGN concentrations, and their thiopurine metabolism preferentially produces methylated derivatives (6-MMPRs) associated with hepatotoxicity *(2)*. The abundance of data on TPMT deficiency and hematologic toxicity contrasts with the paucity of information on thiopurine resistance. Patients with a very high TPMT activity are more prone to have low 6-TGN concentrations. Thus, standard thiopurine dosages may be inadequate, leading to thiopurine resistance with a high 6-MMPR/6-TGN ratio *(3)*. In this situation, therapeutic drug monitoring with measurements of 6-TGN and 6-MMPR concentrations could highlight that a thiopurine dosage above the standard range is necessary to overcome the pharmacologic resistance. Patients like the one we describe, however, have a very high TPMT activity with predominately methylated derivatives. Dosage escalation in such cases can lead to preferential 6-MMPR production, risking hepatotoxicity *(3)*. Furthermore, dosage escalation is not consistently effective in patients with resistance to standard dosages, regardless of the TPMT activity *(3)*. Worth noting is that an unfavorable 6-MMPR/6-TGN ratio (i.e., high 6-MMPR and low 6-TGN) has also been reported in patients with a normal TPMT activity *(5, 6)*.

Therapeutic response can be improved in patients with increased blood 6-MMPR concentrations, which are usually related to a very high TPMT activity, by using the XO inhibitor allopurinol to potentiate 6-TGN production. The well-known interaction between thiopurines and allopurinol can cause serious adverse hematologic events, and this combination is contraindicated by the US Food and Drug Administration. Allopurinol was first used to shift the metabolism of thiopurine compounds from 6-MMP to 6-TGN in poor responders to AZA or 6-MP *(7)*. The authors of this retrospective study, which included 20 patients who had failed to respond to AZA or 6-MP and who had high 6-MMPR concentrations, gave the patients 100 mg allopurinol daily and decreased the thiopurine dosage to 25%–50% of the original dosage. This allopurinol–thiopurine combination increased 6-TGN concentrations 2-fold and decreased 6-MMPR concentrations 5-fold *(7)*. Clinically, this

combination seemed safe and effective, improving the clinical disease activity and diminishing the steroid requirements. In addition, adding allopurinol in patients with an aminotransferase increase during thiopurine therapy restored normal aminotransferase activities and alleviated hepatotoxicity *(7)*.

That allopurinol improves the 6-MMPR/6-TGN ratio in vivo has been abundantly documented, but the mechanism underlying this drug–drug interaction remains unclear. Allopurinol inhibits XO but has no direct inhibiting effect on TPMT *(8)*. An open-label prospective study of allopurinol combined with low-dose thiopurine therapy showed increased HPRT activity *(9)*. This increase in HPRT activity, which catalyzes the first step in 6-TGN production, is consistent with the 6-TGN increase, but it does not fully explain the simultaneous dramatic decrease in 6-MMPR, which leads to an improved metabolite ratio. Finally, an in vitro study *(10)* suggested an alternative biochemical mechanism: XO inhibition by allopurinol may lead to preferential 6-MP oxidation via aldehyde oxidase, with the production of dihydroxy-6-MP, a compound responsible for feedback inhibition of TPMT activity.

Another approach for increasing blood 6-TGN concentrations is the combination of 5-aminosalicylic acid (5-ASA) and thiopurine. An alteration in thiopurine metabolism caused by the addition of 5-ASA has been described *(2)*. Adding 5-ASA led to an increase in the 6-TGN concentrations, probably caused by TPMT inhibition *(2)*.

Determining the TPMT phenotype or genotype before initiating thiopurine therapy ensures the identification of TPMT-deficient patients, thereby enabling the prevention of serious adverse hematologic events. Determining the TPMT phenotype also allows identification of patients with a very high TPMT activity. Such patients are prone to thiopurine resistance and may therefore require an increased thiopurine dosage or alternative forms of treatment. A switch to 6-MP is useless in these patients. Metabolite assays are useful for dosage adjustments during thiopurine therapy. Moreover, the combination of low-dose thiopurine therapy and allopurinol may be helpful in patients with an unfavorable metabolite profile and pharmacologic resistance or hepatotoxicity. This therapeutic strategy requires close monitoring of blood cell counts and metabolite assays.

In conclusion, early analysis of thiopurine metabolism in patients with thiopurine resistance can help physicians optimize therapy and avoid an early switch to another drug from the limited panel of therapies available for treating inflammatory bowel disease. Close cooperation between the pharmacologist and the physician is crucial to optimize patient care and decrease the time to remission.

POINTS TO REMEMBER

- Determining the TPMT phenotype or genotype is recommended to help in selecting the initial thiopurine dosage for patients scheduled for thiopurine therapy.
- Early assessment of metabolite concentrations is useful for validating the initial thiopurine dosage in patients with a very high or low TPMT activity.
- Metabolite assays can help identify underdosing, poor compliance, or an unfavorable metabolic profile in patients with thiopurine resistance.
- Thiopurine therapy can be optimized in patients with an unfavorable metabolic profile by adding 100 mg allopurinol per day to the therapy. This strategy requires lowering the thiopurine dosage to 25%–50% of baseline and monitoring blood cell counts at short intervals.

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References

1. Dervieux T, Boulieu R. Simultaneous determination of 6-thioguanine and methyl 6-mercaptopurine nucleotides of azathioprine in red blood cells by HPLC. Clin Chem 1998;44:551–5.
2. Chouchana L, Narjoz C, Beaune P, Loriot M-A, Roblin X. Review article: the benefits of pharmacogenetics for improving thiopurine therapy in inflammatory bowel disease. Aliment Pharmacol Ther 2012;35:15–36.
3. Dubinsky MC, Yang H, Hassard PV, Seidman EG, Kam LY, Abreu MT, et al. 6-MP metabolite profiles provide a biochemical explanation for 6-MP resistance in patients with inflammatory bowel disease. Gastroenterology 2002;122:904–15.
4. Relling MV, Gardner EE, Sandborn WJ, Schmiegelow K, Pui C-H, Yee SW, et al. Clinical Pharmacogenetics Implementation Consortium guidelines for thiopurine methyltransferase genotype and thiopurine dosing. Clin Pharmacol Ther 2011;89:387–91.
5. Hoentjen F, Hanauer SB, de Boer NK, Rubin DT. Two brothers with skewed thiopurine metabolism in ulcerative colitis treated successfully with allopurinol and mercaptopurine dose reduction. Dig Dis Sci 2012;57:250–3.
6. van Egmond R, Chin P, Zhang M, Sies CW, Barclay ML. High TPMT enzyme activity does not explain drug resistance due to preferential 6-methylmercaptopurine production in patients on thiopurine treatment. Aliment Pharmacol Ther 2012;35:1181–9.
7. Sparrow MP, Hande SA, Friedman S, Cao D, Hanauer SB. Effect of allopurinol on clinical outcomes in inflammatory bowel disease nonresponders to azathioprine or 6-mercaptopurine.

Clin Gastroenterol Hepatol 2007;5:209–14.
8. Oselin K, Anier K. Inhibition of human thiopurine *S*-methyltransferase by various nonsteroidal anti-inflammatory drugs in vitro: a mechanism for possible drug interactions. Drug Metab Dispos 2007;35:1452–4.
9. Seinen ML, de Boer NKH, Smid K, van Asseldonk DP, Bouma G, van Bodegraven AA, et al. Allopurinol enhances the activity of hypoxanthine-guanine phosphoribosyltransferase in inflammatory bowel disease patients during low-dose thiopurine therapy: preliminary data of an ongoing series. Nucleosides Nucleotides Nucleic Acids 2011;30:1085–90.
10. Blaker PA, Arenas M, Fairbanks L, Irving PM, Marinaki AM, Sanderson JI. A biochemical mechanism for the role of allopurinol in TPMT inhibition. Gastroenterology 2011;140:S769.

Commentary

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Azathioprine and 6-mercatopurine (6-MP) are effective treatments for inflammatory bowel disease. Multiple clinical trials have documented the effectiveness of azathioprine (2–3 $mg \cdot kg^{-1} \cdot day^{-1}$) and 6-MP (1.5 $mg \cdot kg^{-1} \cdot day^{-1}$) for inducing and maintaining remission in moderate-to-severe inflammatory bowel disease. The mean time to full effect is 3 months, although clinical improvement may be apparent in 4 to 8 weeks. Azathioprine/6-MP may be underused because of the delayed onset of action and because of a fear of toxicity, especially leukopenia, infection, and hepatotoxicity.

6-MP is converted nonenzymatically to 6-thioguanine (6-TGN), the biologically active metabolite responsible for the therapeutic response. 6-MP is also converted by thiopurine-*S*-methyltransferase (TPMT) to 6-methylmercaptopurine (6-MMP), high concentrations of which can contribute to hepatotoxicity. Eighty-nine percent of the population has normal to high activities of TPMT. When TPMT activity is negligible (0.3% of population) or intermediate (10%–11%), 6-TGN concentrations are higher than normal, substantially increasing the risk of myelosuppression. When TPMT activity is very high, the clinical response may be limited because of low 6-TGN concentrations. Some professional societies recommend pretreatment *TPMT* genotyping or phenotyping; however, compliance with this recommendation is unknown. When wild-type TPMT is documented, azathioprine/6-MP therapy can be started at or near the full therapeutic dose. In patients with TPMT heterozygosity, the starting dose of azathioprine/6-MP is one-third to one-half the standard dose and should be increased slowly. In both cases, the complete blood count must be monitored regularly for myelosuppression. Patients with negligible TPMT activity should not be treated with thiopurines. There are minimal data on very high TPMT activity and its effect on clinical response. Monitoring of 6-TGN is not recommended generally, but it can be helpful when the clinical response is muted. Low 6-TGN concentrations may indicate medication noncompliance or hyperactive TPMT activity. If the latter is documented (associated with high 6-MMP concentrations), escalated doses of azathioprine/6-MP or treatment with biologic agents may be appropriate.

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