Successful Azathioprine Treatment with Metabolite Monitoring in a Pediatric Inflammatory Bowel Disease Patient Homozygous for TPMT*3C

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Thiopurine S-methyltransferase (TPMT) methylates purine analogues, showing TPMT activity in inverse relation to concentrations of active metabolites such as 6-thioguanine nucleotide (6-TGN). With conventional dosing of thiopurines, patients with homozygous variant TPMT alleles consistently suffer from severe myelosuppression. Here, we report a patient with TPMT*3C/*3C who managed successfully with monitoring of thiopurine metabolites. The patient was an 18-year-old male diagnosed with Crohn’s disease. The standard dose of azathioprine (AZA) (1.8 mg/kg/day) with mesalazine (55.6 mg/kg/day) was prescribed. Two weeks after starting AZA treatment, the patient developed leukopenia. The DNA sequence analysis of TPMT identified a homozygous missense variation (NM_000367.2: c.719A>G; p.Tyr240Cys), TPMT*3C/*3C. He was treated with adjusted doses of azathioprine (0.1-0.2 mg/kg/day) and his metabolites were closely monitored. Leukopenia did not reoccur during the follow-up period of 24 months. To our knowledge, this is the first case of a patient homozygous for TPMT*3C successfully treated with azathioprine in Korea. While a TPMT genotyping test may be helpful to determine a safe starting dose, it may not completely prevent myelosuppression. Monitoring metabolites as well as routine laboratory tests can contribute to assessing drug metabolism and optimizing drug dosing with minimized drug-induced toxicity.

Key Words: Thiopurine methyltransferase, azathioprine, inflammatory bowel disease, metabolite levels

INTRODUCTION

Azathioprine (AZA) plays a pivotal role in the treatment of inflammatory bowel disease (IBD). AZA rapidly changes to 6-mercaptopurine (6-MP) and consequently turns into 6-thioguanine nucleotides (6-TGN) by hypoxanthine-guanine phosphoribosyltransferase to exercise its immunosuppressive action. Alternately, AZA is converted to inactive metabolites, 6-methylmercaptopurine (6-MMPN), by thiopurine S-methyltransferase (TPMT).
To date, about 30 allelic variants of TPMT with affecting protein stability or enzymatic activity have been identified. In Whites and Asians, TPMT*3A and TPMT*3C are the most important variants with low enzyme activity, respectively. TPMT*3A exhibits deficient activity, while TPMT*3C has moderate activity in vitro. In patients with deficient TPMT, 6-TGN is rapidly accumulated, causing potentially fatal myelotoxicity.

Adverse effects, including myelosuppression, are found in 9-34% of IBD patients. Consequently, AZA should be decreased or stopped in up to 30% of patients. Considering the chronic course of IBD, it is important to optimize AZA dosing before treatment failure. In cases with deficient TPMT, laboratory tests are applicable to measure thiopurine metabolites. These tests can confirm a TPMT phenotype and optimize personalized dosing to prevent myelosuppression. Here, we report the first case of a Korean IBD patient homozygous for TPMT*3C, successfully treated with AZA by monitoring metabolite levels.

**CASE REPORT**

An 18 year-old male was referred to our hospital for abdominal pain and loose stool. On physical examination, he had tenderness on the lower left quadrant of the abdomen. Routine laboratory tests, including complete blood cell counts (CBC) and liver function were all within normal limits except mild anemia and an elevated erythrocyte sediment rate of 82 mm/hr (reference interval, 0-22 mm/hr).

The patient was diagnosed with Crohn’s disease according to standard clinical, endoscopic, and histologic criteria. Oral treatment with AZA (1.8 mg/kg/day) and mesalazine (55.6 mg/kg/day) was started. Two weeks later, the absolute neutrophil count (ANC) and white blood cell count (WBC) decreased from 5140/μL to 1010/μL, and 6270/μL to 2810/μL, respectively. The AZA dosage was reduced from 1.8 to 0.9 mg/kg/day. Three weeks later, ANC and WBC continued to decline further to 190/μL and 1910/μL, respectively. The AZA was discontinued. After leukopenia was recovered, the patient was restarted on AZA (0.8 mg/kg/day) with the discontinuation of mesalazine. The patient’s daily dose of AZA was cautiously increased to 1.2 mg/kg while monitoring CBC levels.

Peripheral blood samples were taken from the patient for TPMT genotyping. After obtaining written informed consent, sequence analysis of all coding exons with their flanking intron regions of TPMT gene were performed, and we identified a homozygous variant (c.719A>G; p.Tyr240Cys), TPMT*3C/*3C.

Simultaneously, 6-TGN and 6-MMPN concentrations were measured by the Waters 2795 Alliance HPLC system and a Quatro Micro API tandem mass spectrometer (Waters, Manchester, UK). The thresholds indicating increased likelihood efficacy (6-TGN >235 pmole/8×10⁸ red blood cells, RBC), increased risk of leukopenia (6-TGN >450 pmole/8×10⁸ RBC), and increased risk for hepatotoxicity (6-MMPN >5700 pmole/8×10⁸ RBC) were suggested. The patient’s 6-TGN concentration (7206 pmole/8×10⁸ RBC) corresponded to a higher risk of leukopenia, although the daily dose had already been reduced from 1.2 mg/kg to 0.8 mg/kg because of neutropenia. The daily dosage was readily decreased to 0.2 mg/kg, and 6-TGN declined to therapeutic levels (437 pmole/8×10⁸ RBC). Daily AZA dose was reduced further to 0.1 mg/kg as 6-TGN concentration increased again to 745 pmole/8×10⁸ RBC (Fig. 1). 6-MMPN concentrations were detected at less than the limit of quantitation. The patient’s laboratory parameters, including CBC and liver function, were within normal limits during the follow-up period of 24 months.

**DISCUSSION**

The effect of extremely low or absent TPMT activity can be fatal due to severe myelosuppression. The most significant variants with low TPMT enzyme activity in Western and Asian countries are TPMT*3A and TPMT*3C. Compared with Whites, East Asians showed different allele frequencies of TPMT*3A (Whites vs. East Asians: 3.2-5.7% vs. 0.0%) and TPMT*3C (0.2-0.8% vs. 0.3-2.3%). Although TPMT*3C is a predominant variant in Asian populations, this is the first case of an IBD patient homozygous for TPMT*3C in Korea.

Myelosuppression in IBD patients treated with AZA/6-MP was reported as more common in Koreans (31.0-56.4%) than in Western countries (2.0-16.7%). The mechanism contributing to a higher incidence of leukopenia in Koreans remains unclear. According to some reports, TPMT polymorphisms affected myelotoxicity in only a small portion of patients, in which large numbers of patients with leukopenia also had wild type TPMT. As the TPMT genotype could not exclusively elucidate myelosuppression during AZA/6-MP therapy, one possible hy-
Thiopurine Monitoring in a Patient with TPMT*3C/*3C

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mg/kg). This suggests the importance of metabolite monitoring for individualized-dose adjustment. To determine a safe starting dose, evaluation of the TPMT genotype or phenotype (TPMT activity) prior to beginning AZA treatment is recommended. Slow metabolizers typically respond to much lower doses of medication. Patients treated with AZA require periodic monitoring of CBC and liver function to prevent AZA-induced toxicities. The monitoring of thiopurine metabolites may be helpful in assessing drug metabolism and optimizing drug dosing, as well as drug-interaction, dosing compliance, and intraindividual variability of TPMT activity during AZA treatment, which cannot be explained exclusively by the TPMT genotype. The onset of toxicity distinctively develops within one month. In cases with a TPMT variant, long-term thiopurine therapy is likely to fail because of significant toxicity or an inadequate response during treatment. In this report, our patient had experienced myelosuppression only two weeks after starting AZA therapy. Since dose adjustment based on TPMT genotype followed by metabolites monitoring was applied, the patient’s disease activity was successfully controlled without relapse of neutropenia.

In conclusion, this is the first reported case of an IBD patient homozygous for TPMT*3C in Korea. The patient received successful AZA treatment without recurrent leukopenia after dose optimization based on the presence of the TPMT genotype and metabolite monitoring. Our report sug-
gests that AZA dosage should be determined based on the presence of a TPMT genotype and with careful metabolite monitoring as this may provide safe and efficient dosing.

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