Analysis of Thiopurine S-Methyltransferase Genotypes in Japanese Patients with Inflammatory Bowel Disease

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Abstract

Background and Aims Myelosuppression observed in patients with inflammatory bowel disease (IBD) treated with azathioprine (AZA) has been attributed to low thiopurine S-methyltransferase (TPMT) activity. TPMT activity is dependent on the genetic polymorphism of high-versus low-metabolizing alleles. We investigated the association between TPMT genotypes and myelosuppression in Japanese IBD patients.

Methods Forty-one healthy volunteers and 70 IBD patients (UC, n = 50; CD, n = 20) were recruited. All IBD patients were treated with AZA. The TPMT genotypes were determined by polymerase-chain reaction-restriction fragment length polymorphism (PCR-RFLP) analyses.

Results One healthy volunteer showed a heterozygous mutation of TPMT*1/*3C. All other volunteers and the 70 IBD patients were of the wild allele type (TPMT*1/*1). In the IBD patients, 7 patients developed leucopenia (<3,000/μL). One of them developed severe leucopenia (<1,000 μL) with agranulocytosis on day 14 after drug initiation.

Conclusion TPMT mutations are not associated with myelosuppression in Japanese IBD patients. Even in IBD patients with a wild TPMT genotype, clinicians should pay attention for the possible development of myelosuppression.

Key words: ulcerative colitis, Crohn’s disease, leucopenia, thrombocytopenia

Introduction

The purine analogs azathioprine (AZA) and 6-mercaptopurine (6-MP) are the most common drugs used to maintain clinical remission in Crohn’s disease (CD) and ulcerative colitis (UC) (1-3). These drugs are also important as steroid-sparing agents in steroid-dependent and in chronic active inflammatory bowel diseases (IBD). However, there remain concerns regarding drug-induced toxicity, such as bone marrow suppression, hepatotoxicity, pancreatitis, fever, rash and gastrointestinal intolerance (4).

AZA and 6-MP are metabolized to 6-thiouric acids (6-TUAs), 6-methylmercaptopurine (6-MMP), and 6-thioguanine nucleotide (6-TGN) (5, 6). 6-TUA is an inactive metabolite, and 6-MMP is associated with hepatotoxicity but therapeutically inactive (7). The cytotoxic and immunosuppressive properties of AZA/6-MP are mediated by 6-TGNs, which are incorporated into the DNA, thus leading to DNA breakage and the inhibition of immune cell proliferation (5). Some patients are more susceptible to bone marrow suppression while on AZA/6-MP therapy. This susceptibility is genetically dependent on inter-individual variations in thiopurine S-methyl transferase (TPMT) enzyme activity based on the genetic polymorphism of high- versus low-metabolizing alleles (5, 6). TPMT*1 is the wild type, and TPMT*2 (G238 C), TPMT*3A (G460A and A719 G) and TPMT*3C (A719 G) are the major mutant alleles that account for 80-95% of intermediate and low enzyme activity. Patients with low enzyme activity have an increased risk of bone marrow suppression, since 6-MP metabolism is shunted towards high 6-TGN concentrations.

In this study, we analyzed the TPMT genotypes in 41 healthy volunteers and 70 IBD patients, including 7 patients...
Patients and Methods

We enrolled 41 healthy volunteers (female/male 18/23; average age 51.1) and 70 IBD patients (UC, n = 50; CD, n = 20) attending the gastroenterology outpatient clinic at the Hospital of Shiga University of Medical Science. All subjects were Japanese. Demographic and clinical characteristics of IBD patients enrolled in this study are shown in Table 1. All patients were treated with 5-aminosalicylic acid and AZA/6MP. The disease activity was evaluated using established indices, the Crohn’s disease activity index (CDAI) (8) and the clinical activity indices (CAIs) described by Rachmilewitz et al for UC (9). A CDAI <150 and Rachmilewitz’s CAIs <4 were regarded as remission of CD and UC, respectively.

Results

Of the 41 healthy volunteers and 70 IBD patients analyzed, only one healthy volunteer showed a heterozygous mutation of TPMT*1/*3C (Fig. 1). All other healthy volunteers and the 70 IBD patients were of wild allelotpe type (TPMT*1/*1). The allelic frequency of TPMT*1/*3C was

Table 1. Baseline Clinical Characteristics

<table>
<thead>
<tr>
<th></th>
<th>UC (n=50)</th>
<th>CD (n=20)</th>
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<tbody>
<tr>
<td>Gender (F/M)</td>
<td>15/35</td>
<td>8/12</td>
</tr>
<tr>
<td>Age (year)(mean + range)</td>
<td>40.0 (17-79)</td>
<td>36.6 (26-60)</td>
</tr>
<tr>
<td>Disease duration (mean + range)</td>
<td>5.8 (2-10.5)</td>
<td>6.2 (3.5-11)</td>
</tr>
<tr>
<td>5-ASA treatment</td>
<td>50</td>
<td>20</td>
</tr>
<tr>
<td>AZA or 6MP treatment</td>
<td>50</td>
<td>20</td>
</tr>
</tbody>
</table>

| Type of UC            |           |           |
|                       | distal colitis | 25        |
|                       | extensive colitis | 25        |
|                       | active (CAI>5) | 22        |
|                       | remission (CAI<4) | 28        |

| Location of CD       |           |           |
|                      | ileal       | 3         |
|                      | ileocolonic | 10        |
|                      | colonic     | 7         |
|                      | active (CDAI>150) | 8         |
|                      | inactive (CDAI<150) | 12        |

CD, Crohn’s disease; UC, ulcerative colitis; CAI, clinical activity index of UC reported by Rachmilewitz et al (9); CDAI, Crohn’s disease activity index reported by Best et al (8); AZA, azathioprine; 6MP, 6-mercaptopurine.
Myelosuppression with AZA/6-MP treatment has been attributed to low TPMT activity. TPMT activity is reported to depend on the genetic polymorphism of high- versus low-metabolizing alleles (5, 6). In this study, we evaluated TPMT genotypes in healthy volunteers and IBD patients including 7 patients with AZA-induced leukopenia.

Several previous studies have reported TPMT genotypes in the Japanese population. Kubota et al showed that the frequency of the mutation TPMT*3C, which is associated with low or intermediate TPMT activity, is 1.1% (95% CI 0.6-2.3) in Japanese subjects (n = 308) (11). Kumagai et al showed that the TPMT*3C mutant allele was found in 15/522 (2.9%) Japanese people (12). Hibi et al observed that TPMT mutant alleles were found in 8/82 (9%) Japanese IBD patients (13). In this study, we found TPMT*3C mutation in 0.9% of the Japanese population, and this is similar to the observations by Kubota et al (11, 14). On the other hand, we observed myelosuppression in 7 IBD patients with a wild TPMT genotype. This is supported by previous reports demonstrating that in IBD patients, bone-marrow suppression is not solely dependent on TPMT activity, but is also associated with other factors (15, 16). Kubota et al also showed that the TPMT activities in 157 healthy Japanese subjects revealed large inter-individual variations, and varied by approximately 4-fold (11). Based on these notions, an analysis of TPMT genotypes may be useful to predict severe myelosuppression in IBD patients with TPMT gene mutations. However, it also became clear that a wild TPMT genotype does not guarantee safe treatment with AZA/6-MP. To avoid myelosuppression, other modalities such as measurements of the TPMT activity and/or 6-TGNs concentration may be advisable.

One UC patient was characterized by rapidly developing (approximately 14 days), severe myelosuppression (WBC < 1,000/µL and agranulocytosis) with a normal TPMT genotype. This rapid development of severe myelosuppression is a typical clinical feature of the homozygote of the mutant allele, but is rare in patients with a normal genotype (15). Colombel et al showed that a patient developed rapid and severe myelosuppression among 30 CD patients with a wild type genotype (15). In the report by Hibi et al, 12 out of a total of 141 IBD patients developed myelosuppression (13), but a rapidly progressing, severe case was not included. The present case indicates the possibility of rapid and severe myelosuppression, even in patients with a wild TPMT genotype. For monitoring the side-effects of AZA/6-MP, the American Gastroenterological Association (AGA) recommends obtaining a complete blood count weekly for 4 weeks, biweekly for 4 weeks, and then every 1-2 months for the duration a patient is treated with AZA or 6-MP (17).

In conclusion, myelosuppression due to AZA treatment is not always associated with TMPT gene mutations, and the determination of the TPMT genotype may be useful for predicting myelosuppression in patients with TPMT homozygotes and heterozygotes. Furthermore, clinicians should be aware that rapid and severe myelotoxicity can develop in IBD patients with a wild TPMT genotype.

References

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