Thiopurine Methyltransferase Genotype and the Toxicity of Azathioprine in Japanese

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Objective Thiopurine S-methyltransferase (TPMT) is a cytosolic enzyme that preferentially catalyzes the S-methylation of aromatic and heterocyclic sulfhydryl compounds, including Azathioprine (AZA). It has been reported that the level of AZA toxicity is dependent on the TPMT genotypes in Caucasian individuals; we thus investigated this relationship in Japanese. Methods The TPMT genotype was determined using peripheral blood cell DNA obtained from 36 Japanese patients with rheumatic diseases who were treated with AZA, by polymerase chain reaction (PCR) technique. Duration of AZA administration, white blood cell counts before and after AZA administration, and side effects were investigated in each subject, and were compared between the patients with or without TPMT mutation. Results The TPMT allelotype was TPMT*1/TPMT*1 in 33 (91.7%) and TPMT*1/TPMT*3C in 3 (8.3%) individuals. All 3 patients (100%) with the mutant TPMT allele (TPMT*3C) discontinued AZA treatment due to leucopenia while only 4 patients (12%) without mutant TPMT alleles showed leucopenia (p=0.0049, Fisher's exact test). However, leucopenia developed relatively late in patients with mutant TPMT. Conclusion The TPMT mutant allele, TPMT*3C, also exists in Japanese individuals, and the bone marrow toxicity of AZA is likely stronger in patients with this mutant allele.

Key words: allele, bone marrow toxicity, rheumatic disease

Introduction

Azathioprine (AZA) is one of the most commonly used immunosuppressants in the treatment of rheumatic diseases and other immune-associated systemic disorders. Some patients suffer from severe bone marrow suppression without allergic reaction after a short period of AZA administration, while most patients do not. Recently it was proposed that this variability in the sensitivity to AZA is largely due to the thiopurine S-methyltransferase (TPMT; EC2.1.1.67) activity; TPMT is a cytosolic enzyme that preferentially catalyzes the S-methylation of aromatic and heterocyclic sulfhydryl compounds, including the anticancer agents mercaptopurine and thioguanine and the immunosuppressant AZA (1). TPMT activity shows codominant genetic polymorphism (2, 3). To date 5 common alleles have been distinguished by the presence or absence of a single nucleotide substitution at 3 loci: TPMT*1 (wild type), TPMT*2 (G238C; G238→C at codon 80), TPMT*3A (G460A; G460→A at codon 154 and A719G; A719→G at codon 240), TPMT*3B (G460A), and TPMT*3C (A719G) (4–6). By an in vitro yeast heterologous expression system, these mutations lead to a reduction in the TPMT protein level as well as catalytic activity. In vivo, it was reported that 88.6% of healthy individuals show high TPMT activity in erythrocytes, 11.1% show intermediate activity, and 0.3% show TPMT deficiency (2), representing wild homozygotes, heterozygotes, and mutant homozygotes, respectively (6). Black et al (7) reported that all patients heterozygous for mutant TPMT discontinued AZA therapy within one month, mainly due to leukopenia. Stolk et al (8) reported that gastrointestinal intolerance as a side effect of AZA might also be related to the reduced TPMT activities. To determine this relationship in Japanese, TPMT genotypes in patients with rheumatic diseases treated with AZA were investigated.
Materials and Methods

Patients and blood samples
Peripheral blood samples were obtained from 36 Japanese patients with rheumatic diseases, including 15 of rheumatoid arthritis and 16 of systemic lupus erythematosus or Sjögren syndrome, who had been treated with AZA from 1996 to 1999. All patients were treated with 50 mg/day of AZA. Blood was drawn for clinical examination and the remaining portion was used for this study under informed consent.

DNA preparation and PCR
Genomic DNA was extracted from total blood cells using DNA Extractor WB Kit™ (Wako, Osaka). One μl of DNA was subjected to PCR amplification in a 50 μl reaction mixture containing 0.5 μM of each primer, 0.2 mM of each deoxynucleotide, 10 mM Tris-HCl (pH 8.5), 50 mM KCl, 1.5 mM MgCl₂, 0.001% gelatin, and 2.5 units of Taq DNA polymerase (AmpliTaq Gold™, Perkin Elmer, Foster City, CA). The PCR conditions were denaturation at 95°C for 1 minute, annealing at 58°C (G238C and A719G) or 55°C (G460A) for 1 minute, and extension at 72°C for 1.5 minutes for 35 cycles using a Program Temp Control System PC-800™ (ASTEC, Fukuoka). Specific primers were synthesized according to the previous reports (6, 9) as follows: G238C wild specific sense, 5'-GTA TGA TTT TAT GCA GGT TTG-3'; G238C mutant specific sense, 5'-GTA TGA TTT TAT GCA GGT TTC-3'; G238C common antisense, 5'-TAA ATA GGA ACC ATC GGA CAC-3'. G460A sense, 5'-AGG CAG CTA GGG AAG AAA AAG GGT TTG-3'; G460A antisense, 5'-CAA GCC TTA TAG CCT TAC ACC CAG G-3'; A719G sense, 5'-GAG ACA GAG TTT CAC CAT TTT CCT CTA G-3'; A719G antisense, 5'-CAG GTG TTA GCA TAA TTT CTA TTT CCT C-3'. For the detection of G460A and A719G, each PCR product was digested to completion with restriction enzyme MwoI or AccI (New England Biolabs, Beverly, MA, USA) respectively. After digestion, wild-type G460A is expected to yield 443 and 251 bp fragments, while mutant G460A remains an uncleaved 694 bp fragment. On the contrary, mutant A719G is expected to yield 283 and 90 bp fragments, while wild-type A719G remains an uncleaved 373 bp fragment. To determine the genotype, PCR products were subjected to electrophoresis on 2% agarose gels and stained with ethidium bromide.

Relationship between TPMT genotype and AZA toxicity
Dose and duration of AZA administration, white blood cell counts before and after AZA administration, and side effects were investigated in each subject, and compared between the patients with or without TPMT mutation.

Results
Among the 72 alleles (36 individuals), frequency of the mutant allele for codons 238, 460, and 719 of the TPMT gene was 0 (0%), 0 (0%), and 3 (4.2%), respectively. Thus, the distribution of the TPMT genotype was considered to be 91.7% of TPMT*1/TPMT*1 and 8.3% of TPMT*1/TPMT*3C.

Dose of AZA administered was 50 mg/day. Duration of AZA administration was from 2 weeks (discontinued due to allergic reaction) to 106 weeks (median 36 weeks). Among them, 11 cases discontinued AZA treatment due to side effects: leucopenia (WBC<4,000/mm³ & <75% before administration) in 7 cases (Table 1), abnormal liver function and/or gastrointestinal intolerance in 3 cases, and allergic reaction (fever elevation and eruptions) in 1 case.

All 3 patients (100%) with the mutant TPMT allele (TPMT*1/TPMT*3C), two patients with Sjögren's syndrome under treatment with 5-6 mg of prednisolone and a patient with systemic lupus erythematosus under treatment with 7-20 mg of prednisolone, discontinued AZA treatment due to leucopenia after 32, 106, or 40 weeks from commencement, respectively. On the other hand, only 4 patients (12%) without mutant TPMT alleles (TPMT*1/TPMT*1) showed leucopenia (p=0.0049, Fisher's exact test). However, since the patients with TPMT mutation developed leucopenia relatively late compared with those without TPMT mutation (Table 1), it was not statistically significant when it was evaluated at 40 weeks from the commencement of AZA treatment (p=0.1842, Fisher's exact test).

Discussion
We found 3 individuals (8.3%) with the TPMT*1/TPMT*3C genotype among 36 Japanese individuals and the others (91.7%) were considered to be the TPMT*1/TPMT*1 genotype. Although the total number analyzed in the present study is small and it was restricted to patients with rheumatic diseases, this distribution is different from Caucasians, in which TPMT*2 or TPMT*3A alleles are more common (p<0.01, χ² test), but similar to African and Chinese populations (9, 10).

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treatment due to leucopenia. However, in all 3 cases, bone marrow toxicity developed after 32 weeks or more from commencement. In the previous reports in Caucasians, severe bone marrow toxicity and gastrointestinal intolerance developed more rapidly (7, 8). We considered two explanations for this discrepancy: First, the reduction in TPMT activity level examined by in vitro yeast heterologous expression system was reportedly 100 fold in TPMT*2, >200 fold in TPMT*3A, nine fold in TPMT*3B, and about 30% in TPMT*3C (5, 11). Furthermore, TPMT*3C protein was revealed to have a degradation half-life comparable to the wild-type protein, while enhanced proteolysis of mutant TPMT proteins encoded by TPMT*2 and TPMT*3A alleles was observed, when heterologously expressed in yeast (12). Thus, the reduction of the TPMT activity in patients with TPMT*3C allele might not be so critical as in patients with TPMT*2 or TPMT*3A allele, which is more dominant in Caucasians. Second explanation may be the relatively low dose of AZA administration in Japan (commonly 1 mg/kg/day), compared to the dose commonly prescribed for the treatment of rheumatoid arthritis in Caucasians (2–3 mg/kg/day). However, recently it was reported that erythrocytes obtained from patients with TPMT*3A/TPMT*3C genotype showed no TPMT function and patients with TPMT*1/TPMT*3C showed intermediate TPMT activity, indicating that TPMT*3C allele is non-functional in vivo (6), and when expressed in humans, TPMT*3C is likely associated with lower TPMT protein levels and catalytic activity in erythrocytes (13). Considering these, the second explanation, that the relatively low dose of AZA administration in Japan is the reason for the mildness of side effects, is considered to be more plausible. Nevertheless, the fact that all 3 patients with TPMT*1/TPMT*3C genotype discontinued AZA treatment due to leucopenia indicates that this AZA dosage, 50 mg/body/day, is still high for patients with a TPMT mutant allele. Moreover, since the leucopenia due to the reduced TPMT activity is likely to occur relatively late with this dosage, we must continue close monitoring of hemato logical parameters for patients with a mutant TPMT allele under low-dose AZA treatment.

In conclusion, knowing the TPMT genotype before commencement of AZA treatment may provide useful information to determine the proper dosage for each patient, inducing the therapeutic efficacy as well as to decrease severe toxicity also in Japanese patients with rheumatic diseases.

Acknowledgements: We are grateful to Dr. S. Yamana (Higashi-Hiroshima Memorial Hospital) for his generous supports to this study. This work was supported in part by a Grant-in-Aid from the Japanese Ministry of Education, Science and Culture, and from the Ministry of Health and Welfare. A part of this study was carried out at the Research Center for Molecular Medicine, Hiroshima University School of Medicine.

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