Low-dose azathioprine is effective and safe for maintenance of remission in patients with ulcerative colitis

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Background. 6-Mercaptopurine (6-MP) and azathioprine (AZA) have been used in patients with Crohn’s disease (CD) and ulcerative colitis (UC) for reducing the dose of steroids and maintaining remission. However, some patients treated with 6-MP/AZA develop bone marrow suppression, one of the most serious side effects. The aim of this study was to evaluate the efficacy and safety of low-dose AZA (0.6–1.2 mg/kg per day) for maintaining remission in patients with UC. We also investigated the relationship between bone marrow suppression and thiopurine methyltransferase (TPMT) mutation in the Japanese population.

Methods. Study 1. To investigate the frequency of TPMT mutation, findings for 82 patients among 141 patients with UC or CD who were treated with AZA or 6-MP were analyzed retrospectively. Polymerase chain reaction (PCR) methods were used to analyze allele mutations of the TPMT gene. Study 2. A multicenter prospective trial was performed. The subjects were 22 patients with UC with presence of remission for 3 months or more. They were treated with 50 mg/day of AZA, and we evaluated the remission rate at 6 months, adverse side effects, and changes in prednisone doses after the initiation of AZA.

Results. Study 1. Seventy-four (91%) of the 82 patients analyzed had no TPMT mutation, 7 (8%) had one mutant allele, and 1 (1%) had two mutant alleles. Of the total of 141 patients, 4 (44%) of the 9 patients who were treated with 50 mg/day of 6-MP or 100 mg/day of AZA developed bone marrow suppression, although no mutation of TPMT was seen in any of these patients. On the other hand, 8 (6%) of the 132 patients who were treated with 30 mg/day of 6-MP or 50 mg/day of AZA developed bone marrow suppression. Seven of 8 patients (88%) who developed bone marrow suppression with a low dose of AZA had a mutant TPMT allele.

Study 2. In the 17 patients who could continue taking low-dose AZA for 6 months, 15 (88%) maintained remission. Of 8 patients treated with low-dose prednisone (5–10 mg/day), 3 patients (38%) could discontinue oral prednisone and 4 (50%) could reduce its dose. Six of the 22 patients (27%) had some adverse side effects. These side effects were ameliorated, or disappeared spontaneously, or disappeared with the discontinuation of AZA.

Conclusions. A dose of 50 mg/day of AZA is effective and safe for maintenance of remission in the Japanese population. Investigation of the TPMT allele may be useful for predicting the appearance of bone marrow suppression, when low-dose 6-MP or AZA is given.

Key words: ulcerative colitis, azathioprine, maintenance of remission, thiopurine methyltransferase

Introduction

In ulcerative colitis (UC), no fundamental therapy has been established, because the etiology of this disease remains unknown. The initial therapy for UC in patients with mild to moderate activity is a combination of oral 5-aminosalicylate/sulfasalazine and corticosteroids. Before 1990, the efficacy of immunosuppressive agents in patients with inflammatory bowel disease (IBD) was controversial. More recently, many clinicians appear to favor 6-mercaptopurine (6-MP)/azathioprine (AZA) as immunosuppressants, mainly because of their familiarity and abundant experience with these agents. In Crohn’s disease (CD), it has been established that these immunosuppressive agents are effective for inducing remission, for a steroid-sparing effect, for ameliorating fistulas, and for maintaining remission. Furthermore, controlled trials with these agents in CD patients have been scrutinized in two
metaanalyses.5,10 The efficacy of these agents in UC has also been demonstrated by some studies,11–14 but it is less well established in comparison to the CD studies.2 Doses of 1.0 to 1.5 mg/kg per day of 6-MP, 1.5 to 2.5 mg/kg per day of AZA were used in most of these studies. However, there have been very few reports defining the optimal dose of these agents for the maintenance of remission in patients with UC, and the efficacy and safety of lower-dose AZA (50 mg/day; 0.6–1.2 mg/kg per day) have not been investigated.

However, almost 10% of patients treated with 6-MP/AZA develop side effects during treatment.15 Bone marrow suppression is one of the serious side effects in 6-MP/AZA therapy. In the previous reports, it was found that 2%–10% of patients developed bone marrow toxicity.15–19 Both the response to the thiopurine agents and the bone marrow suppression are regulated in part by the genetic composition of the patient.20 Metabolism of 6-MP/AZA is accomplished by thiopurine methyltransferase (TPMT). In recent studies, many investigators demonstrated relationships among TPMT polymorphism, the activity of the TPMT enzyme,21 the level of 6-thioguanine (6-TG),22 and bone marrow suppression.23 An analysis of TPMT polymorphism may be useful for predicting a high risk of toxicity, thereby improving the safety of 6-MP/AZA.24 It has been reported that the TPMT mutation in the Japanese population is 1*/3C*,24 and this distribution is different from that in Caucasians.

The aim of this study was to evaluate the efficacy and safety of low-dose AZA for maintaining remission in patients with UC. We also investigated the relationships between bone marrow suppression and TPMT mutation, and tried to describe an appropriate dose of 6-MP/AZA in the Japanese population.

Patients and methods

Study 1: analysis of the relationship between TPMT mutation and bone marrow suppression

Among 141 patients who were treated with AZA or 6-MP for UC or CD at Keio University Hospital from 1985 to 1999, we obtained blood samples from 82 patients with UC (n = 47) or CD (n = 35). Fifty-six patients were treated with 6-MP (30 mg/day, 50 patients; 50 mg/day, 6 patients), and 26 were treated with AZA (25 mg/day, 2 patients; 50 mg/day, 21 patients; 100 mg/day, 3 patients). The mean duration of 6-MP/AZA administration was 4.1 years (range, 9 months–13 years). Informed consent had been obtained from all patients. Five milliliters of blood was drawn from each patient and sent in ice for analysis.

To characterize the patients’ TPMT alleles, we extracted genomic DNA from total blood cells, using the Wizard genomic DNA purification kit (Promega, Madison, WI, USA) with 500 ng placental genomic DNA being used as a template. The final volume for all polymerase chain reaction (PCR) assays was 50 µl, containing 1.5 µl of each primer, 5 µl of 10 × PCR Buffer, 1 µl of 10 mM-dNTP, 1.5 µl of 50 mM-MgCl2 (GibcoBRL, Gaithersburg, MD, USA) and 0.5 µl of platinum Taq DNA polymerase (GibcoBRL). Amplification was conducted for 30 cycles with the GeneAmp PCR system 9600 (Perkin Elmer, Norwalk, CT, USA), and consisted of denaturation at 94°C for 1 min, annealing at 55°C for 2 min, and extension at 72°C for 1 min.21 A final extension step at 72°C for 7 min was also performed. To detect the G238C, G460A, and A719G mutations, we used the following specific primers, as described in previous reports:21,24 G238C wild-type sense, 5′-GTA TGA TTT TAT TAT GCA GGT TTG-3′; G238C mutant-type sense, 5′-GTA TGA TTT TAT GCA GGT TTC-3′; G238C antisense, 5′-TAA ATA GGA ACC ATC GGA GCA CAC-3′; G460A sense, 5′-ATA ACA GAG TGG GGA GGC TGC-3′; G460A antisense, 5′-CTA GAA CCC AGA AAA AGT ATAG-3′; A719G sense, 5′-GAG ACA GAG TTT CAT CAC CAT GTT GG-3′; and A719G antisense, 5′-CAG GCT TTA GCA TAA TTT TCA ATT CCT CCT C-3′. The G460A PCR product was digested with MWO (New England Biolabs, Beverly, MA, USA) for 1 h at 60°C. The A719G PCR product was digested with ACC (Toyobo, Osaka, Japan) for 2 h at 37°C. The digested products were analyzed by gel electrophoresis.

We investigated the relationships among the incidence of bone marrow suppression, the TPMT genotype, and the dose of 6-MP/AZA. The TPMT genotype was classified according to a previous reference.21 Bone marrow suppression was defined as a white blood cell count of 3 × 10^9/l or less. In this study, the platelet count did not fall below 150 × 10^9/l.

Study 2: a multicenter trial of low-dose AZA for UC patients

Twenty-two UC patients in remission were enrolled in the present study (Keio University Hospital, National Okura Hospital, Ryukyu University Hospital, and Hyogo Medical College Hospital). The inclusion criteria were a radiological and/or endoscopic diagnosis of UC and the presence of remission for a period of at least 3 months before entry. Remission was defined as absence of symptoms of active disease (no bloody stool, and diarrhea of four times or less/day) with no or low doses of prednisone (10 mg/day or less). Patients with previous surgery for UC were excluded. Patients whose total white blood cell count was below 3 × 10^9/l or whose platelet count was below 150 × 10^9/l before the study were also excluded. Patients with recent