CASE REPORT

Azathioprine-induced fatal myelosuppression in systemic lupus erythematosus patient carrying TPMT*3C polymorphism

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Azathioprine (AZA) is a commonly used immunosuppressant for systemic lupus erythematosus (SLE). Myelosuppression is a serious adverse reaction due to AZA and its metabolites. Thiopurine S-methyltransferase (TPMT) is the rate-limiting enzyme. Variations of TPMT enzyme activity may be responsible for myelosuppression. However, a correlation between certain mutant alleles of low TPMT enzyme activity and myelotoxicity has also been suggested as a factor. We describe herein a case of AZA-induced severe myelosuppression associated with TPMT*3C heterozygous mutant allele in a SLE patient. The patient presented with pancytopenia, sepsis, typhlitis and disseminated intravascular coagulopathy after a short period of AZA therapy. The patient had low TPMT activity and TPMT*3C genotype. Measurement of TPMT activity and determination of TPMT variant allele may identify patients at risk for AZA-induced myelosuppression.

Key words: genetic polymorphism; myelosuppression; 6-mercaptopurine; pharmacogenetics; systemic lupus erythematosus; thiopurine S-methyltransferase

Introduction

Azathioprine (AZA) is a commonly used immunosuppressant in systemic lupus erythematosus (SLE) and other autoimmune diseases. It can induce myelosuppression by interfering with purine metabolism and DNA synthesis. Leucopenia is the most common adverse hematological side-effect with an incidence of 5–25%. The incidence of myelosuppression differed among different ethnic groups, largely due to TPMT polymorphism.1 Thai patients have been found to have a high frequency of TPMT*3C ranging up to 10%.2 Identification of genotyping or measuring the TPMT activity may predict patients at risk. We report a SLE patient who carried the TPMT*3C heterozygous mutant and developed fatal myelosuppression from AZA immediately after initiation of the treatment. There was a temporal relationship between RBC TPMT activity, leucopenia and clinical manifestations.

Case report

A 20-year old woman was diagnosed as SLE at the age of 10 years. She had clinical manifestations of polyarthritis, autoimmune hemolytic anemia and nephritis. Her serum showed high-titers of antinuclear (ANA) and anti-DNA antibodies. She was treated with monthly intravenous cyclophosphamide (IVCY) for seven months then continued on quarterly IVCY for three years. The dosages of IVCY ranged from 0.5 to 0.8 gram per 1.73 m² of body surface area. Prednisolone and ACE-inhibitor was also administered. She then developed cryptococcal meningitis at the age of 15, which was successfully treated with amphotericin B. Cyclophosphamide was stopped and hydroxychloroquine was administered with low-dose prednisolone achieving a clinical remission. Four years later, she developed edema of legs, hypertension and a nephrito-nephrotic syndrome. Kidney biopsy showed diffuse proliferative lupus nephritis. Mycophenolate mofetil 1 g/day and prednisolone 30 mg/day were administered for six months with partial remission. She developed recurrent upper urinary tract infections. A kidney biopsy after mycophenolate mofetil treatment showed diffuse glomerulosclerosis with severe
Lupus

tubulointerstitial infiltration. Two weeks later, AZA (Imuran®) was administered at 50 mg twice daily due to persistent proteinuria. Her body weight was 47 kg. A complete blood count taken before starting AZA showed a hemoglobin level of 7.7 g/dL, white blood cell count of 11 340 cells/mm³ with 87.4% of neutrophils and 10% of lymphocytes and a platelet count of 500 000/mm³ (Figure 1). Anemia of chronic disease was diagnosed according to the hematological studies. Neither myelosuppressive drugs nor allopurinol was used. The patient denied any use of traditional medicines. Two weeks later, she developed fever with petechial hemorrhages. A complete blood count showed severe pancytopenia. The hemoglobin level was 4.8 g/dl, white blood cell count was 520 cell/mm³, with 2 % of neutrophils, 73% of lymphocytes and a platelet count of 32 000 cells/mm³. Renal and liver function tests were normal. After admission, she developed severe abdominal pain, pneumonia and septic shock. The abdominal CT-scan showed generalized bowel wall dilatation most marked at the ileum and minimal ascites, which led to the diagnosis of typhlitis. Treatment included administration of broad-spectrum antibiotics plus granulocyte colony-stimulating factor and total parenteral nutrition. The bone marrow study showed marked hypocellularity with maturation arrest of the myeloid series (Figure 2). We excluded parvovirus infection as an alternate cause of marrow suppression. TPMT activity and the genotype was investigated. TPMT activity was at an intermediate low level of 25.40 nmol 6-MTG/g Hb/hr determined using the HPLC method. Genotype analysis by real time PCR showed TPMT*1/*3C. She died from Acinetobacter baumannii septicemia.

Figure 1  Temporal relationship between AZA administration and complete blood count. The patient had fever with petechial hemorrhages two weeks after initiation of AZA. A complete blood count showed severe pancytopenia. Hb, hemoglobin; Wbc, white blood cell; Plt, platelet.

Figure 2  Bone marrow aspiration at day 14 after initiation of AZA. Figure 2A depicts severe hypoplastic marrow (×100 magnification) and Figure 2B immature myelocytes (arrows) were mostly found which suggests myeloid maturation arrest (×400 magnification).

Discussion

AZA is a commonly used immunosuppressant in SLE and other autoimmune diseases. The recommended dosage of AZA is 1–3 mg/kg/day for 6–12 months with the aim to control disease activity and prevent disease flare-ups. AZA-induced myelosuppression is an occasional complication. In SLE patients, it is important to distinguish between active disease and drug-induced myelotoxicity. Active disease often has concomitant lymphopenia as well as neurological, renal or musculoskeletal involvement. Other causative factors include immunosuppressants and viral infection. Frequent blood monitoring must be performed but drug-induced myelosuppression remains a major threat. We describe herein a fatal case of AZA-induced pancytopenia in an SLE patient who had the TPMT heterozygous mutant. Samples drawn confirmed that the patient had a marginal level of TPMT activity of red blood cells.
AZA is rapidly converted to 6-mercaptopurine (6-MP) and is subsequently metabolized by three competing routes.4 6-MP is first metabolized by hypoxanthine-guanine phosphoribosyl transferase into thioguanine nucleotides (TGN) which exert immunosuppressive activity. A second catabolic route, which has little inter-individual variation, is oxidation by xanthine oxidase (XO). Lastly, a major catabolic pathway, TPMT enzyme converts AZA into the inactive form. Inter-individual variation in TPMT activity is significant largely because of genetic polymorphism. Currently, more than 20 allele mutants have been identified. TPMT*2 (G238C), TPMT*3A (G460A and A719G) and TPMT*3C (A719G) are the majority mutant alleles that account for 80–95% of intermediate and low enzyme activity. The TPMT mutant alleles were found in 10–14% of Caucasians and TPMT*3A is the common mutant allele found in this population whereas TPMT*2 and TPMT*3C are also found. TPMT*3C is the predominant mutant allele that is found in Asian and African populations.5 Prevalence of TPMT mutant alleles in Asians varied from below 5% in Southwest Asia, Japan and China. It is up to 9% in Thai.6

Lennard et al. first reported the association between TPMT polymorphism and AZA-induced toxicity and this was confirmed by other investigators.1,7 Alternatively, Naughton et al.8 studied genotyping in Caucasian patients and only one in seven SLE patients carried mutant alleles that had been associated with myelosuppression. Another study in Japanese SLE patients showed lower TPMT activity in TPMT*/1/*3C. One of two SLE patients with heterozygous TPMT*3C had developed AZA-induced leucopenia. The study did not find a significant association between TPMT*3C and AZA toxicity because of low frequency of TPMT*3C in Japanese.9 Although evidences supported the potential use of TMPT genotyping and enzymatic activity in inflammatory bowel disease or other rheumatologic diseases,10 application to SLE patients awaits further study.

In this report, the patient had fatal myelosuppression within two weeks after AZA therapy. The onset of toxicity characteristically occurs within one month. The clinical manifestations were similar to those of a severe form of neutropenia which we were unable to rescue resulting in death from septicemia. Although AZA-induced leucopenia is not uncommon, fatal outcomes have been rare. Our patient had low TPMT activity due to TPMT mutant allele and received a high dose of AZA (2 mg/kg/day). Both conditions led to severe immunosuppression. It is common practice to perform frequent blood monitoring early after AZA initiation. Lower doses (25–50 mg per day) are usually recommended although a high dose was prescribed in this case due to the persistent proteinuria and the severity of the renal pathology. Identification of high-risk genotypes and avoidance of AZA prescription is the best approach. Considering the high incidence of TPMT mutant alleles in Thais, the impact of various pharmacogenetic approaches is important. This issue needs further study.

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References

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