

Azathioprine-Induced Severe Bone Marrow Toxicity - A Report of 3 Cases

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Dermatologists have been using azathioprine as a “steroid sparing agent” for the treatment of various dermatoses including photodermatoses, immunobullous diseases, psoriasis and eczematous diseases. However, in Taiwan, the currently approved indications of azathioprine include only adjunct therapy of renal transplant, systemic lupus erythematosus, severe rheumatoid arthritis, and leukemia. Owing to the off-label nature of azathioprine use in most dermatological practice, clinical vigilance must be taken. We herein report 3 cases of severe bone marrow toxicity after the use of azathioprine and discuss the pathogenesis and suggestive management of this rare complication. The first patient had prolonged pancytopenia lasting for 3 months despite immediate azathioprine withdrawal and 2 consecutive doses of granulocyte colony-stimulating factor. The full blood count of the other two patients had returned to normal one week after treatment. The result of genotyping of three patients to detect most prevalent mutant allele (TPMT*3C) was negative. (*Dermatol Sinica* 27: 44-51, 2009)

Key words: Azathioprine, Bone marrow toxicity, Pancytopenia, Granulocyte colony-stimulating factor

INTRODUCTION

Azathioprine is a cytotoxic drug used frequently as a steroid-sparing agent in dermatology and most adverse effects were considered to be the results of the co-administered corticosteroids.¹ However, a wide range of adverse reactions of azathioprine manifesting from trivial gastrointestinal discomfort to life-threatening myelosuppression or anaphylactic shock has been reported in different medical specialties. We herein report 3 cases of azathioprine-induced myelosuppression defined by grading system of the National

cancer institute, United States.² None of them had impaired liver and renal function at the commencement of azathioprine.

CASE REPORT

Patient 1

A 40-year-old man had suffered from disseminated prurigo for 2 years. He had been treated with topical corticosteroid and oral antihistamine but the effects were unsatisfactory. Azathioprine (Imuran®; Glaxo-SmithKline) was initially given with a daily dosage of 50 mg and then was increased to

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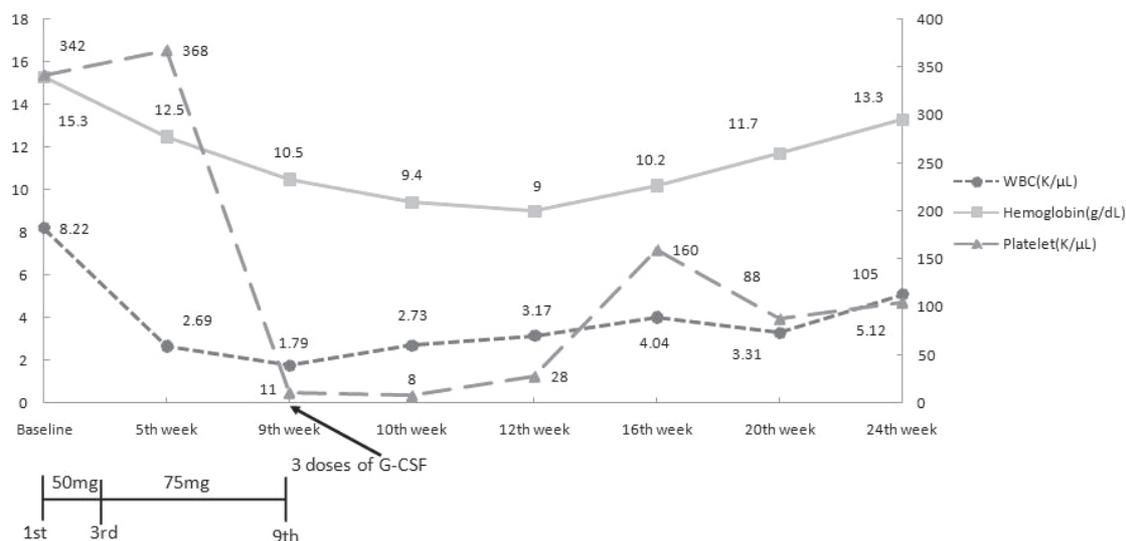


Fig. 1

Clinical course with relevant laboratory data of patient 1. (note that the withdrawal of azathioprine was done at week 9 and G-CSF was given for 2 doses)

75mg per day 2 weeks later. After receiving 5 weeks of azathioprine, the skin condition improved. However, he later presented with fever, oral ulcer, angular cheilitis, constipation and perianal abscess. Grade III myelosuppression (hemoglobin 10.5g/dL, leukocyte count 1790/ μ L, absolute neutrophil count 145/ μ L, and thrombocyte count 11×10^3 / μ L) but normal liver function (AST/ALT: 15/11 U/L) were identified, hence azathioprine was discontinued on week 9. He was treated at out-patient clinic with granulocyte colony stimulating factor (G-CSF) (300 mcg/vial/day) in 2 consecutive days. The leukocytes count increased a week later but his hemoglobin and thrombocytes count continued to decline. During the follow-up, the whole blood count recovered gradually, but a low thrombocytes count was still present after 12 weeks (Fig. 1).

Patient 2

A 26-year-old man had suffered from successive eruption of tense, grouped bullae for 2 months. The lesions were mildly pain-

ful and accompanied with intractable itching. He was referred to our outpatient clinic and the diagnosis of bullous pemphigoid was then established after immunohistopathological examination. The initial management of oral prednisolone with a daily dosage of 60 mg was commenced. One month later, oral azathioprine, 100 mg per day was added to the original regimen. The complete blood count (CBC) and the liver function including total bilirubin were all within normal limits prior to the commencement of azathioprine. The bullous eruption improved gradually. However, progressively tarry stool, hematuria, hair loss, oral pain, painful defecation and diarrhea developed 2 weeks later, and when he came back to the clinic 4 weeks after the commencement of azathioprine. He presented with the symptoms of jaundice, shortness of breath and high fever. The leukocyte count was markedly decreased (from baseline 10670/ μ L to 8910/ μ L at week 2 to 500/ μ L at week 4) with elevated total bilirubin (2.87 mg/dL). Grade I thrombocytopenia (126×10^3 / μ L) was also noticed.

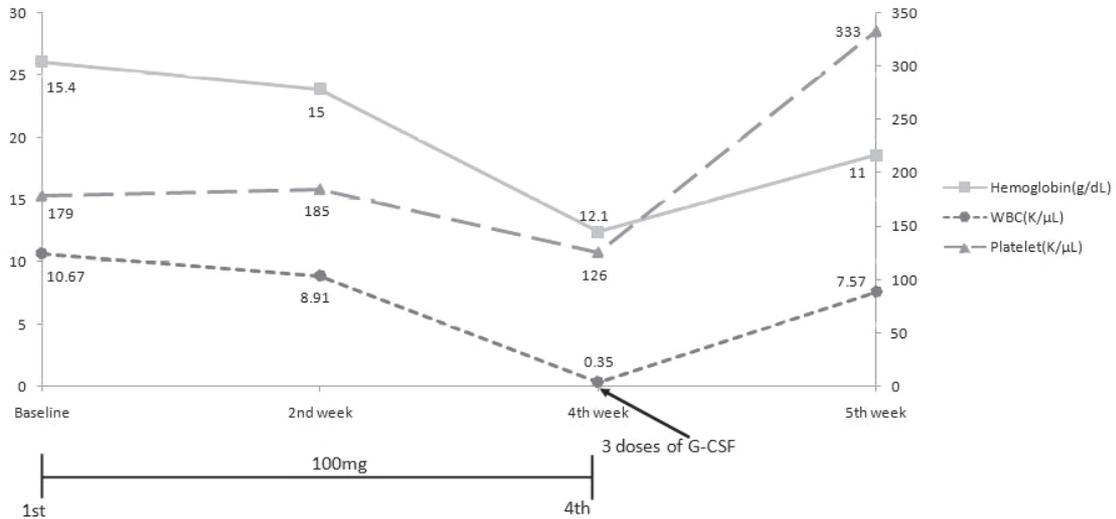


Fig. 2

Clinical course with relevant laboratory data of patient 2, neutropenic fever occurred at week 4.

Under the impression of azathioprine induced hepatotoxicity and neutropenic fever, he was then admitted to our ward. The leukocyte count decreased to a nadir of 350/ μL 1 day later (absolute neutrophil count: 112/ μL). Bone marrow biopsy demonstrated a marked suppression of myeloid cells and maturation arrest of myeloid cells. After the administration of antibiotics, granulocyte colony stimulating factor (300mcg for 3 consecutive days) and oral antiviral agents (valaciclovir) under the specialist's suggestion, patient's blood count returned to normal 1 week later (Fig. 2).

Patient 3

An 81-year-old man was referred to our outpatient clinic due to recalcitrant atopic dermatitis. On physical examination, marked erythematous and lichenified eruptions with or without scales distributed on his flexural areas. The CBC and liver function on the same day were all within normal limits (hemoglobin: 14g/dL, leukocyte count: 5890/ μL , absolute neutrophil count: 3799/ μL , thrombocytes: 182x10³/ μL , AST/

ALT: 25/18 U/L). Thus, we initiated the azathioprine with a daily dosage of 50 mg. Three weeks later, the dose was increased to 75 mg. In week 8, routine blood test revealed a mild decrease in the whole blood file (hemoglobin: 13.2g/dL, leukocyte count: 3900/ μL , neutrophils: 3120/ μL , thrombocytes: 155x10³/ μL) with a normal liver function, (AST/ALT: 23/18 U/L). Azathioprine had been used for one month. Nevertheless, he went abroad without regular laboratory monitoring for the next three months and came back to our outpatient clinic at week 20 with the complaint of generalized malaise since week 12. CBC showed leucopenia (1750/ μL , absolute neutrophil count: 735/ μL). He also noticed stomatitis for a few weeks without other associated symptoms. Azathioprine was discontinued and G-CSF (300 mcg/vial/day) was given for 3 consecutive days, the complete blood count recovery 1 week later (Fig. 3).

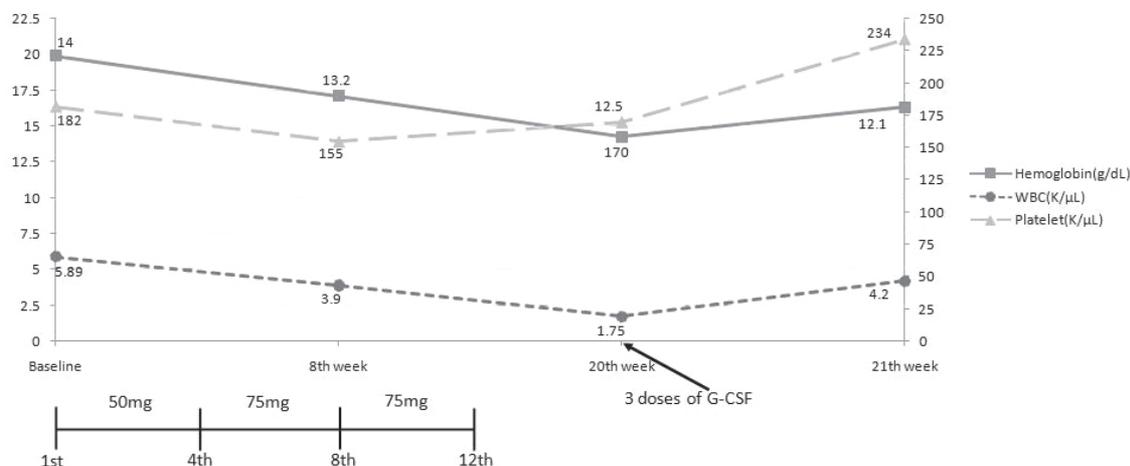


Fig. 3

Clinical course with relevant laboratory data of patient 3. Loss of regular laboratory monitoring since the 8th week led to the subsequent leucopenic event.

DISCUSSION

Azathioprine is prodrug of 6-mercaptopurine (6-MP) synthesized by attaching an imidazole ring to the sulfur atom at the 6th position of the 6-MP molecule. The success in the field of kidney transplantation in 1962 broadened its clinical applications thereafter.³ This immunosuppressant is now widely used in dermatology, oncology, gastroenterology and rheumatology for its antileukemic, anti-inflammatory, and immunosuppressive properties.

After oral administration, azathioprine is absorbed almost completely by the gut. It is then converted non-enzymatically to 6-MP and then undergoes complex metabolism by three competitive enzymes. 6-MP is either oxidized by xanthine oxidase (XO) to 6-thiouric acid, methylated by thiopurine methyl transferase (TPMT) to form 6-methyl-mercaptopurine, or converted to 6-thioguanine nucleotides (6-TGN) by hypoxanthine guanine phosphoribosyl transferase (HGPRT). The relative activities of XO, HGPRT and TPMT determine the net concentration of the active 6-TGN which is responsible for the majority of azathioprine's efficacy and ad-

verse effects.

Despite its reported safety in pemphigus vulgaris, 15-28% of patients with inflammatory bowel disease treated with azathioprine have adverse drug reactions, which lead to withdrawal of the therapy or dose reduction.^{4,6} A formal statistics of the adverse effects in dermatological patients remains unclear. In a retrospective survey conducted in England, dermatologists reported a 29.1% incidence of adverse effects in their patients.⁷

The adverse effects of thiopurines can be categorized into the dose-dependent and dose-independent.⁸ The former comprises general malaise, nausea, vomiting, infectious complications, hepatitis and myelosuppression. The later includes rash, fever, arthralgia, pancreatitis and anaphylactic shock. Nausea and general malaise are the most frequently observed dose-dependent reactions. 5% of patients with inflammatory bowel disease in a retrospective study by Connell *et al.*⁹ experienced myelosuppression (3.8% with leukopenia, 0.4% with pancytopenia, 2% with thrombocytopenia). The survey in English dermatologists by questionnaires revealed a 4% incidence of neutropenia.⁷

Table. 1 Comparison of Three Patients Profiles and Performed Genotyping

	Age	Gender	Onset of myelosuppression	Nadir white cell count	Associated symptoms	G-CSF administration	Prognosis	TPMT* 3C(A719G)	ITPase C94A
Patient 1	40	male	5 th week	WBC:1790/ μ L ANC:145/ μ L 9 th week	Fever, oral ulcer, angular cheilitis, constipation, perianal abscess	2 doses	recovered 4 months later after G-CSF was given	AA	CC
Patient 2	26	male	3 rd week	WBC:350/ μ L ANC:112/ μ L 4 th week	Tarry stool, hematuria, hair loss, oral pain, painful defecation, diarrhea shortness of breath, fever, jaundice	3 doses	recovered 1 week later after G-CSF was given	AA	CC
Patient 3	81	male	12 th week	WBC:1750/ μ L ANC:142/ μ L 20 th week	Stomatitis, general malaise	3 doses	recovered 1 week later after G-CSF was given	AA	CA

ANC: absolute neutrophil count; G-CSF: granulocyte colony-stimulating factor; 1 dose= 300mcg/vial/day; TPMT: thiopurine methyl transferase; ITPase: inosine triphosphate pyrophosphatase; A: adenine; C: cytosine

It has been well recognized that those with low or absent TPMT enzyme activity are prone to developing rapid onset and severe myelotoxicity caused by intracellular 6-TGN accumulation.¹⁰ In contrast, Dubinsky *et al.*¹¹ proposed that patients with high enzyme activity tend to metabolize 6-MP into 6-methyl mercaptopurine, which leads to hepatotoxicity. The remaining adverse effects such as influenza-like illness, intense myalgia and pancreatitis are thought to be related to the inosine triphosphate pyrophosphatase (ITPase) deficiency.¹² Currently, two major methods, i.e. TPMT enzyme activity (phenotyping) and genotyping, are used to identify those at risk of developing rapid onset bone marrow suppression. Both methods have advantages and disadvantages. In general, the phenotyping is more commonly used but genotyping is more accessible and technically easy.¹⁵

In Caucasian population, about 1 in 300 individuals are of very low or undetectable TPMT enzyme activity and are homozygous

for variant alleles (TPMT^L/TPMT^L), whereas 6-11% of individuals are of intermediate activity (TPMT^H/TPMT^L).¹⁶ Roughly 89 to 94% of study group show the high methylator phenotype (TPMT^H/TPMT^H, wild type).¹⁶ Another study suggested the ratio of undetectable TPMT activity reach 1 in 220 and furthermore there exists a population of high enzyme activity (9%).¹⁷ To date, more than 20 alleles have been identified and contribute to the polymorphism of TPMT activity. The alleles vary in the different ethnic groups and TPMT*3C(A719G) is the most common variant allele in Taiwanese with a prevalence rate of 0.12-1.28%.¹⁸

In England, E.A. Fargher *et al.*⁷ found that 94% of dermatologists requested the test before prescribing, higher than the gastroenterologists and rheumatologists. Unfortunately, both methods are not routinely available in Taiwan. We had performed genotyping of TPMT and ITPase for our three patients but none of them had mutant TPMT*3C alleles as shown in Table 1. The results were not

too surprising because low TPMT enzyme activity is not the sole mechanism resulting in bone marrow suppression. Colombel *et al.*¹³ demonstrated that only 29% of leukopenic patients had mutant polymorphism and indicated that only one-third of myelosuppression episodes can be attributed to genetic polymorphism. Patients with TPMT wild type may also develop myelosuppression by other reasons such as viral infection or coadministration of benzoic acid derivatives which act as TPMT inhibitors.^{13,14}

Azathioprine-induced myelosuppression usually manifest with unexplained infection, ulceration of oral cavity, bruising and bleeding.¹⁹ The earliest hematological abnormality is leukopenia followed by thrombocytopenia.¹⁹ In Connell's study, the timing of bone marrow toxicity can be as soon as 2 weeks but may be delayed until 11 years and the median time of onset was 9 months.⁹ In the same study, the full blood counts returned to normal within one month in all patients who had myelotoxicity. A single report of azathioprine-induced pancytopenia showed a prolonged recovery course of 3 months after appropriate treatment.²⁰

Regular monitoring of complete blood count and liver function plays an important role in prescribing this cytotoxic drug. The suggested monitoring method by British association of dermatologists¹⁹ are as follows: 1. weekly monitoring of CBC and liver function for the first 4 weeks of therapy, or until the maintenance dose is achieved; then reduce the frequency to the minimum of once every 3 months during the therapy. 2. those with hepatic and renal impairment, elderly and patients treated with high dosage need more frequent monitoring of CBC and liver function. 3. any increase in dosage should be accompanied by return to weekly monitoring for 4 weeks and then reduce to a minimum of once monthly or every two months during the therapy. A white blood cell count below

4000³/μL or a platelet count below 150x10³/μL should prompt dose reduction and if the WBC is below 2500/μL or the platelet count is below 100x10³/μL, azathioprine should be withheld.²¹

Currently, there are no standard guidelines in treating the azathioprine-induced myelosuppression. The major principles of managements include 1. hospitalization of patients with febrile neutropenia and early commencement of empirical broad-spectrum antibiotics. 2. prophylactic or therapeutic blood and platelet transfusion to maintain the physiological demand. 3. G-CSF administration is suggested in patients who show no recovery of leukocyte count after withdrawal of offending agent and in patients with febrile neutropenia who are at high risk for infection-associated complications.^{19, 22, 23} The dosage of G-CSF for adult is 5μg/kg/day subcutaneously until the absolute neutrophil count reaches 2-3x10³/μL.²³

In conclusion, the use of azathioprine is usually safe in dermatological practice but still runs a small but significant risk of developing lethal adverse effects. Clinical vigilance and close laboratory follow-up are needed during azathioprine treatment. The use of TPMT and ITPase assays as predictive methods for myelosuppression is not confirmed in our patients. The exact value of testing for TPMT and ITPase in Taiwanese patients needs further confirmation from future large-scale prospective studies.

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Azathioprine 引發之嚴重骨髓毒性

- 三例臨床報告

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皮膚科醫師常將azathioprine當成減少類固醇使用的藥劑，並使用在許多的皮膚疾病，諸如日光性或水疱性皮膚疾患、乾癬、濕疹性疾患已行之有年。然而，在台灣，目前azathioprine所核准的適應症只有腎臟移植之輔助治療，系統型紅斑性狼瘡，嚴重的類風濕性關節炎以及白血病。由於在皮膚科大部分不是目前核可的適應症，臨床使用時更應提高警覺。我們於此報告三個因此藥物引起之嚴重骨髓毒性之病例。第一位患者的全血球下降於停藥以及給予顆粒性白血球刺激因子輸注之後仍持續了三個月之久。另外兩位患者的全血球則在治療後於一個禮拜回復至正常範圍。於三位患者針對最盛行的變異對子(TPMT*3C)所作的檢測皆無基因變異。（中華皮誌：27: 44-51, 2009）